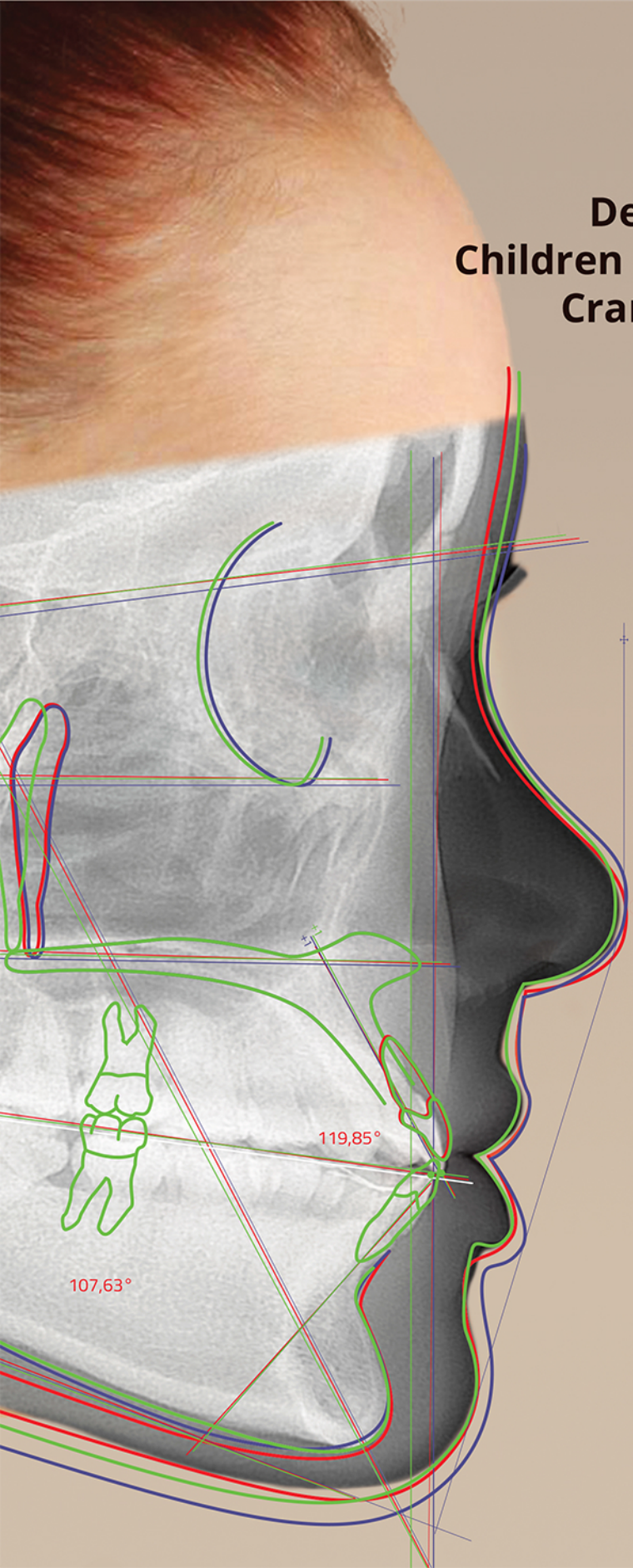
Dental Development in Children and Its Influences on Craniofacial Morphology

Strahinja Vučić



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Tandontwikkeling bij kinderen en de invloed ervan op craniofaciale morfologie

Thesis
to obtain the degree of Doctor from the Erasmus University Rotterdam
by command of the
Rector Magnificus

Prof.dr. H.A.P. Pols

and in accordance with the decision of the Doctorate Board
The public defense shall be held on
Wednesday 6th of June 2018 at 09:30 hours

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born in Niš, Serbia

Erasmus University Rotterdam

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Grgic O*, Vucic S*, Medina-Gomez C, Trajanoska K, van Wijnen AJ, Dhamo B, Monnereau C, Felix JF, Uitterlinden AG, Jarvelin MR, Timpson NJ, Evans DM, Jaddoe VWV, Ongkosuwito EM, Wolvius EB, Rivadeneira F. Genome-wide association study identifies three novel genetic determinants of dental maturation. Manuscript in preparation. (Chapter 3)

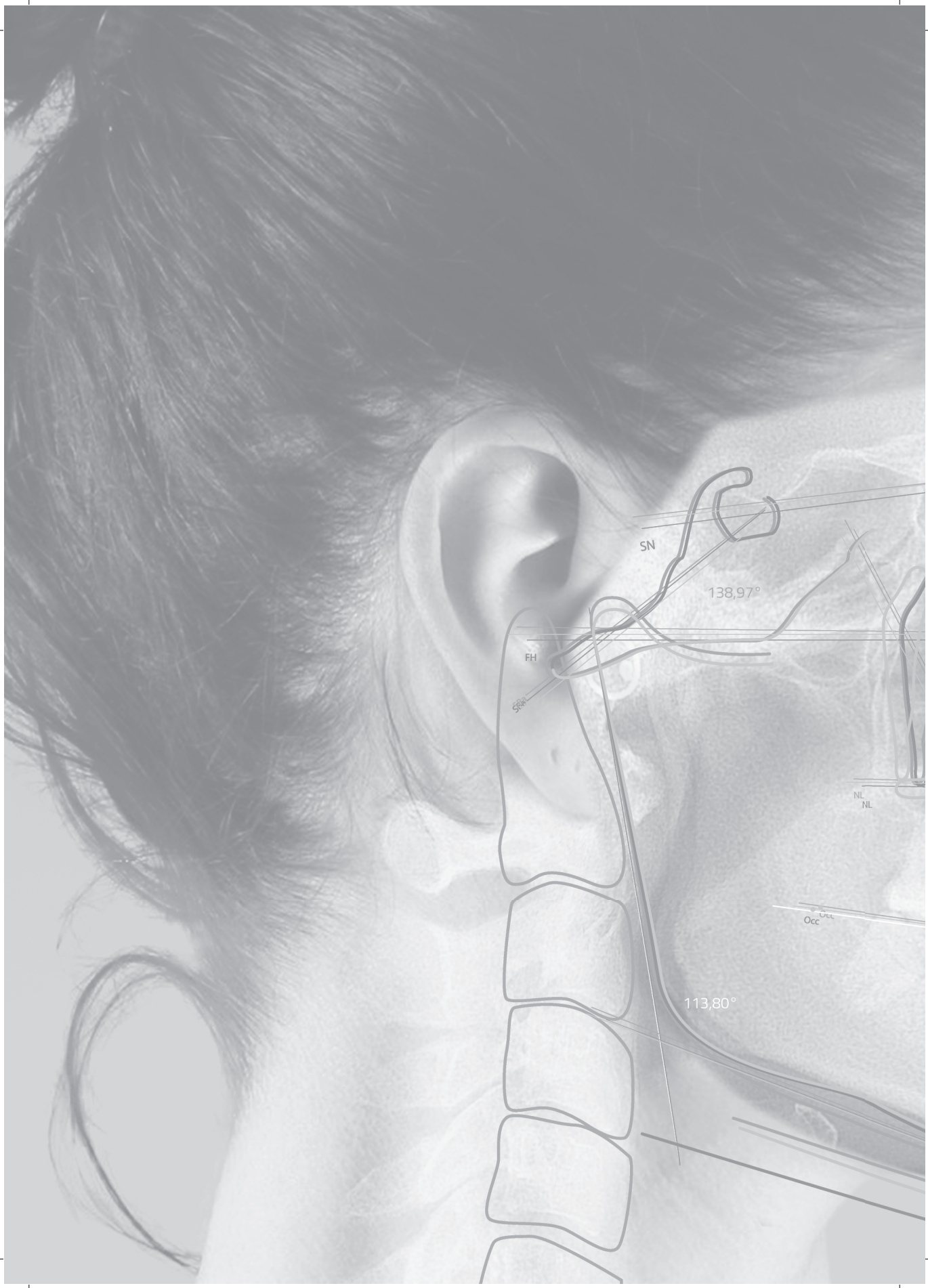
Vucic S, Korevaar TIM, Dhamo B, Jaddoe VWV, Peeters RP, Wolvius EB, Ongkosuwito EM. Thyroid function during early life and dental development. J Dent Res. 2017, 2017 Aug;96(9):1020-6. (Chapter 4)

Dhamo B, Vucic S, Kuijpers MA, Jaddoe VW, Hofman A, Wolvius EB, Ongkosuwito EM. The association between hypodontia and dental development. Clin Oral Investig. 2016 Jul;20(6):1347-54. (Chapter 5)

Vucic S, Dhamo B, Kuijpers MA, Jaddoe VW, Hofman A, Wolvius EB, Ongkosuwito EM. Craniofacial Characteristics of Children with Mild Hypodontia. Am J Orthod Dentofacial Orthop. 2016 Oct;150(4):611-9. (Chapter 6)

Vucic S, Dhamo B, Jaddoe VWV, Wolvius EB, Ongkosuwito EM. The association of dental development and craniofacial morphology in school-age children: The Generation R study. Am J Orthod Dentofacial Orthop. Manuscript submitted for publication. (Chapter 7)

CONTENTS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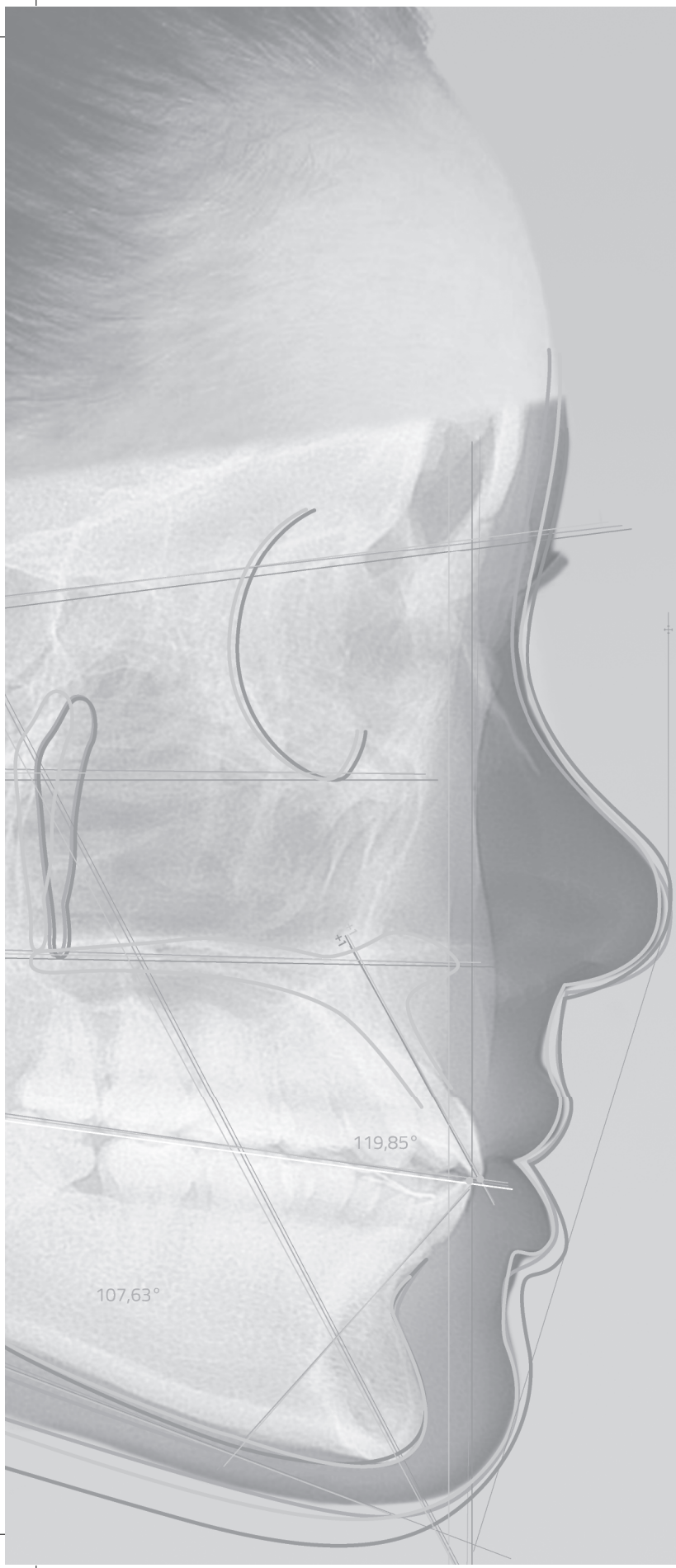
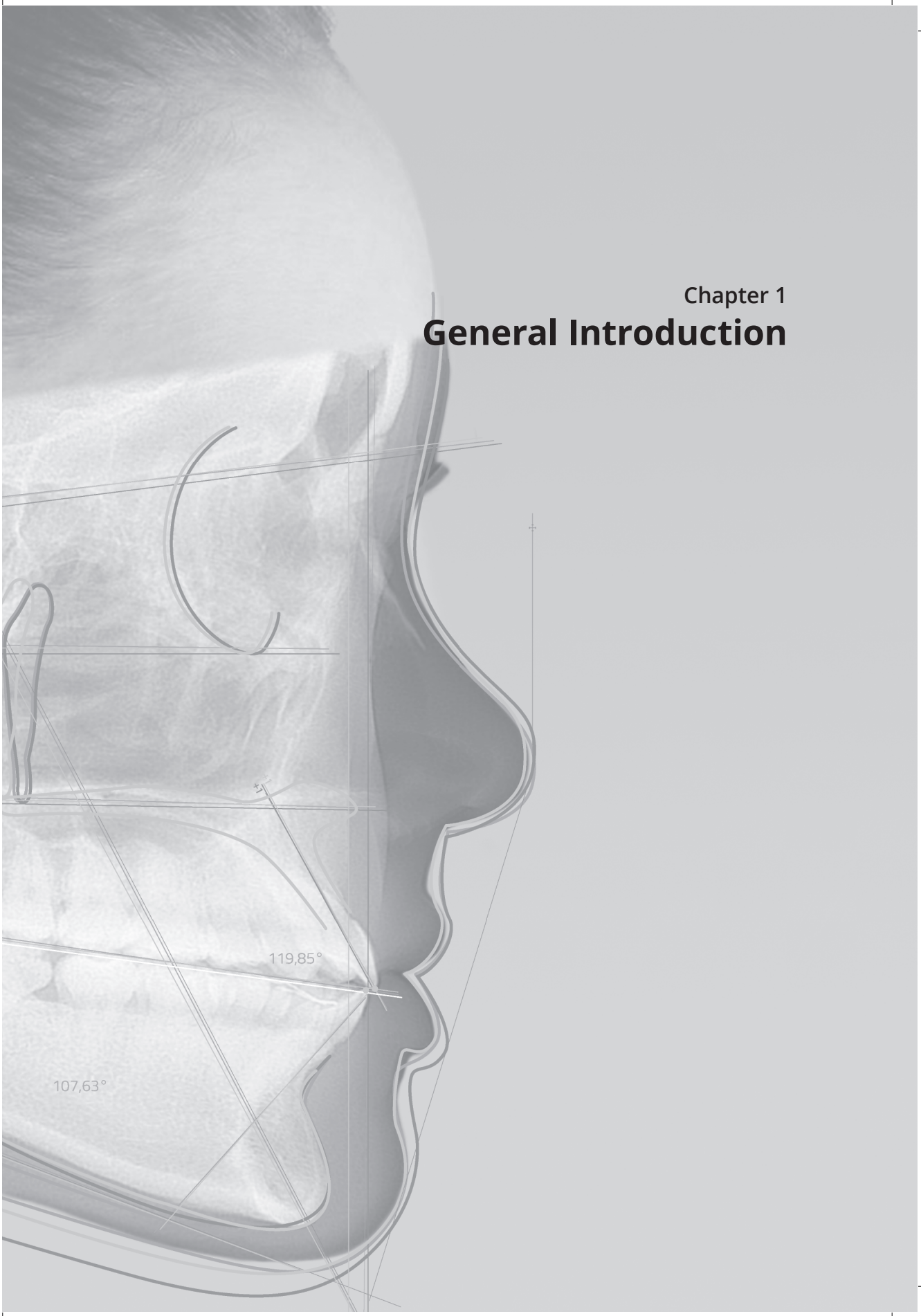


Part I



Chapter 1

General Introduction

INTRODUCTION

The relation between dental development and facial morphology has been a major point of interest for dental care professionals. An evident example of this relation is the facial change that occurs during a specific process of dental development known as eruption, or the axial tooth movement towards the opposing jaw. During typical dental development, once contact is established between the teeth of the opposing jaws, the eruption process is complete. Teeth that have fully erupted form an occlusion, or 'bite' which determines the vertical and sagittal relationship between the jaws. In the event of disturbances in dental development/eruption, a malocclusion, or incorrect alignment between the teeth of opposing jaws, can occur. Additionally, the teeth and surrounding bones are subject to continuous static and dynamic loading due to skeletal muscle activity (Moss 1997). The bones of the viscerocranium react to this mechanical loading through the process of bone resorption and bone formation, which together are referred to as bone remodeling (Proffit et al. 2014a). Therefore, the developing teeth and the simultaneous reaction of the surrounding tissues play an important role in craniofacial morphology. However, the interaction between dental development and craniofacial morphology is a complex process. This interaction begins during the intrauterine development and is influenced by genetic, epigenetics and environmental factors (Dixon et al. 1997).

Embryological development of the face, oral cavity, and teeth

The human head and neck develop from the cells of the neural crest and brachial arch system at the third week of gestation (Figure 1). The derivatives of the first, second, and third branchial arch participate in the formation of the face, mouth, and tongue (Nanci 2017). By the 24th day of gestation, the primitive mouth, the stomatodeum, is limited cranially by the frontal prominence, and laterally and ventrally by the maxillary and mandibular processes, respectively, all of which are derived from the first branchial arch. The frontal prominence participates in the development of the lateral and medial nasal processes. Merging of the left and right medial nasal processes forms the middle portion of the maxilla, which carries the upper incisors. The remaining maxillary teeth are located in the maxillary processes, which, after merging with the medial nasal process, form the upper jaw. The lower jaw is formed by the fusion of the left and right mandibular processes. At roughly the 37th day of gestation, the fused surfaces of the medial nasal process, maxillary processes, and the mandibular arch facing towards the stomatodeum will start to thicken, forming an odontogenic epithelium (dental lamina).

From the first thickening of the odontogenic epithelium, teeth go through several morphological stages of development: the bud, cap, and bell stages, as well as

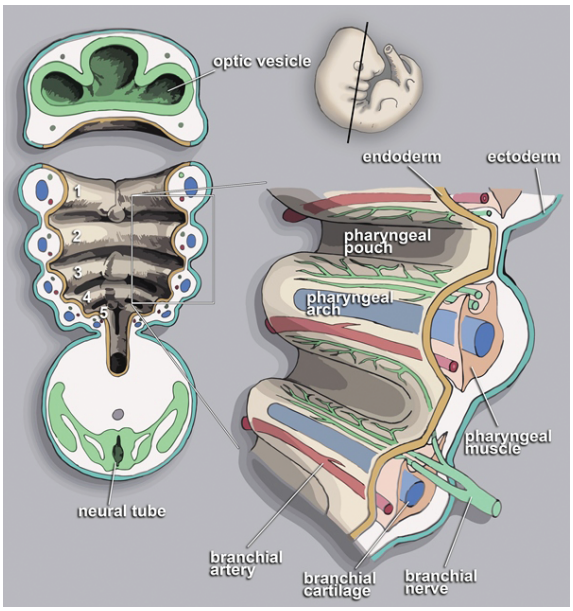


Figure 1. The pharyngeal arch system. (Reprinted with permission from https://commons.wikimedia.org/wiki/ File:PharyngealArchHuman.jpg by Loki austanfell)

hard tissue formation (Figure 2). During the bud stage, the epithelium of the dental lamina begins to move towards the inside of the jaw, causing the surrounding ectomesenchymal cells to condense. During the cap stage, cells from a tooth bud will grow in a concave formation, called an enamel organ, which encapsulates the condensed ectomesenchymal cells known as dental papilla. The enamel organ, dental papilla, and the surrounding cells of a dental follicle form a dental organ. During the bell stage, the dental organ has six layers, each performing specific functions. The outermost layers are the 1) outer enamel epithelium, 2) stellate reticulum and 3) stratum intermedium, all of which function in a supportive fashion. Next, the 4) inner enamel epithelium (which will eventually differentiate into ameloblasts), followed by the 5) dental papilla (which will eventually differentiate into odontoblasts). The innermost layer, where the outer enamel epithelium meets the inner enamel epithelium, a group of cells called the 6) cervical loop play an important role in the formation of cementoblasts. The final stage of tooth development is characterized by the formation of the mineralized matrices enamel, dentin, and cement.

At birth, all primary teeth have entered the stage of hard tissue formation while in the permanent dentition only first molars have entered this stage. This underlines

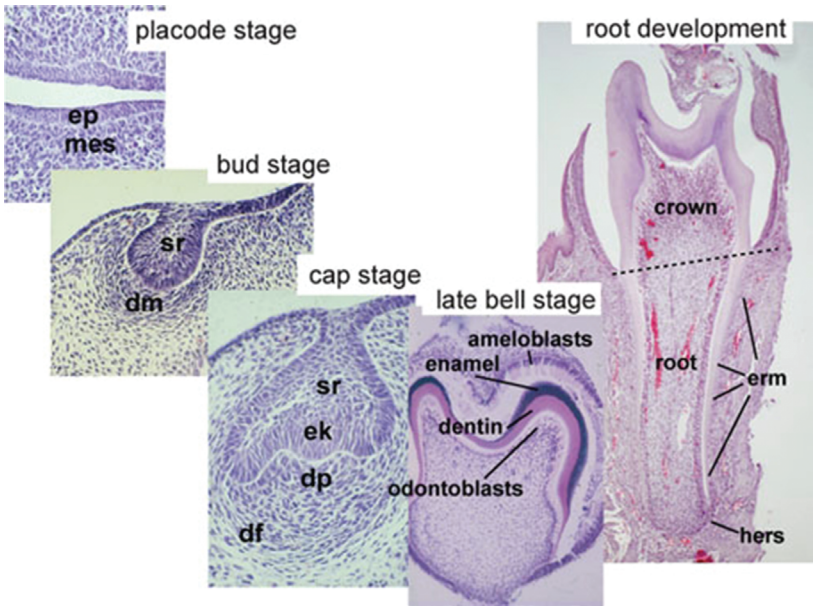


Figure 2. Stages of tooth development. Abbreviations: Ep, epithelium; mes, mesenchyme; sr, stellate reticulum; dm; dental mesenchyme; dp, dental papilla; df, dental follicle; ek, enamel knot; erm, epithelial cell rests of malassez; hers, hertwig's epithelial root sheath. (Reprinted with permission from Tooth organogenesis and regeneration by Thesleff, I. and Tummers, M., January 31, 2009, StemBook, ed. The Stem Cell Research Community, StemBook, doi/10.3824/stembook.1.37.1, http://www.stembook.org).

the importance of the early postnatal period for the development of the permanent dentition.

Disturbances of dental development

The most common disturbance in dental development is a congenitally missing tooth (tooth agenesis). Based on the number of teeth missing, tooth agenesis can be classified as hypodontia (up to six missing teeth), oligodontia (six or more missing teeth) and anodontia (absence of all teeth). The most common form of tooth agenesis for the permanent dentition is hypodontia, with a prevalence of 6% in the general population (Khalaf et al. 2014; Polder et al. 2004; Rakhshan and Rakhshan 2015), though in the primary dentition it is less common (Larmour et al. 2005; Rakhshan 2015). The prevalence of tooth agenesis is considerably higher in genetic syndromes such as Down, ectodermal dysplasia, Witkop, Rieger, Van der Woude, Crouzon, and Ehlers-Danlos (De Coster et al. 2009; Lucas 2000). Clinically, hypodontia often re-

quires orthodontic treatment because it disrupts the continuity of the dental arch, affecting function and esthetics (Aasheim and Ogaard 1993; Laing et al. 2010). A better understanding of the etiology of this congenital malformation and its relation to dental and facial development might provide more insight into treatment planning and clinical care.

Postnatal dentofacial changes until adolescence

At birth, the facial bones comprise only a small proportion of the craniofacial complex (Figure 3). However, facial bones will develop rapidly from birth until 18 years of age. The growth of the facial skeleton and the relationship between the jaws are predominantly determined by the development and eruption of the teeth and establishment of the occlusion (Bjork and Skieller 1972). Therefore, the most crucial features for the formation of the occlusion are the eruption and spatial relation of the teeth in the primary, mixed, and permanent dentition.

The development of the primary dentition begins around eight to ten months of age with the eruption of the first primary incisors. The development of the primary dentition is complete with the eruption of the primary second molars at roughly two years of age. Between the age of five-to-six years, the first permanent molars begin erupting (Table 1). The major facial changes that occur during this phase are forward growth of the lower jaw (Bhat et al. 2012; Hegde et al. 2012) and transversal growth of the jaws (to accommodate the anterior teeth) (Bjork and Skieller 1974; Smartt et al. 2005). The major transversal growth potential of the lower jaw ends with the ossification of mandibular symphysis at six months of age while the palatal suture in the upper jaw will hold its growth capacity during the whole phase of dental development. Therefore, with the eruption of primary teeth, the upper jaw will adapt to the lower jaw.

The mixed dentition phase starts with the eruption of the first permanent molars at the age of six (Table 2, Figure 4). As the sagittal growth potential of the jaws decreases with age, especially in the lower jaw, the proper positioning of the permanent teeth is crucial for the stability of normal occlusion during the transition from primary to permanent dentition. To accommodate larger permanent teeth, the mandibular dental arch becomes wider, partly due to the buccal positioning of the teeth as well as bone remodeling. Furthermore, growth of the mandibular ramus in the vertical direction helps to compensate the vertically larger crown size of the permanent successors (Nanda and Taneja 1972). On the other hand, the greater growth potential of the upper jaw in the sagittal direction results in a more convex facial profile in the mixed dentition period. Importantly, the considerable variation in the growth of the teeth and jaws, and the occurrence of malocclusions, is one im-

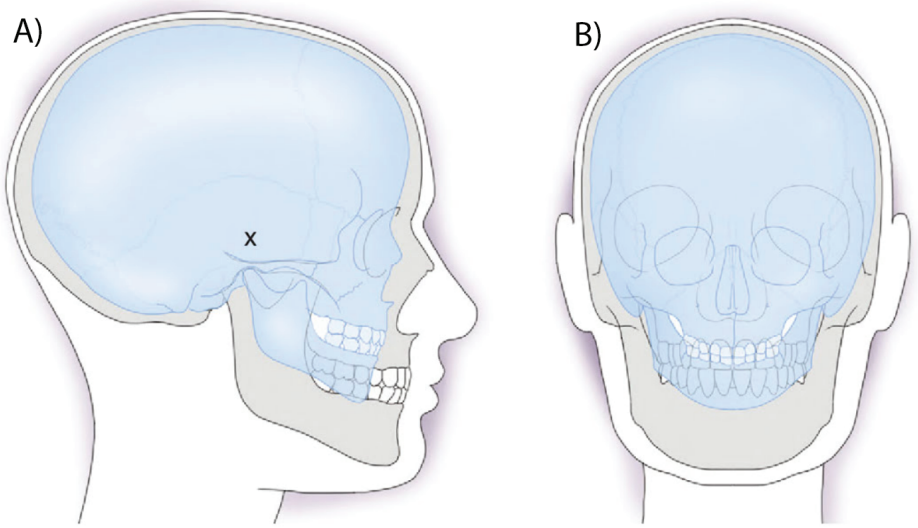


Figure 3. Summary of postnatal craniofacial growth and development from 3 to 18 years of age in a lateral and frontal view. A is a lateral view, and B is a frontal view. The location of the sella turcica is denoted by x. (Reprinted with permission from Ten Cate's Oral Histology: Development, Structure, and Function. Nanci A. Facial Growth and Development. 336. Copyright Elsevier Health Sciences; 2014)

portant factor for the majority of orthodontic treatments starting at this early stage (Crawford and Aldred 2012).

The permanent dentition phase coincides with the onset of puberty, beginning at the age of 12 years when the last primary tooth has been replaced with a permanent successor (Table 2). The accelerated maturation and physical growth elsewhere in the body during this phase are also observed in the growth of the jaws, particularly for the mandible; its height and width increase, the chin becomes more prominent and the mandible can rotate forward depending on the growth pattern (Bjork and Skieller 1983; Subramaniam and Naidu 2010). For this reason, mandibular anterior teeth are positioned more lingually, and posterior teeth move more mesially, which decreases the perimeter of the dental arch. Regarding the nasomaxillary complex, the most notable increases occur in the forward and downward direction (Proffit et al. 2014b). After puberty, the growth process substantially declines, although small dentofacial changes continue throughout life (Proffit et al. 2014b).

The described dentofacial growth pattern is the most common growth pattern and describes the development to a neutro-occlusion or "normal occlusion" (Andrews 1972). However, due to substantial variation of dentofacial growth patterns

Table 1. Dental development and eruption of primary teeth (Linden 2010)

	Central incisor	Lateral incisor	Canine	First molar	Second molar
Initial calcification	5 mo I.U.	5 mo I.U.	6 mo I.U.	6 mo I.U.	7 mo I.U.
Crown completed	2-3 mo	2-3 mo	9 mo	6 mo	11 mo
Root completed	2.5 yr	2.5 yr	3.5 yr	3 yr	3.5 yr
Eruption	6-10 mo	9-13 mo	16-20 mo	15-18 mo	23-29 mo

I.U.- Intrauterine, mo-months, yr- years

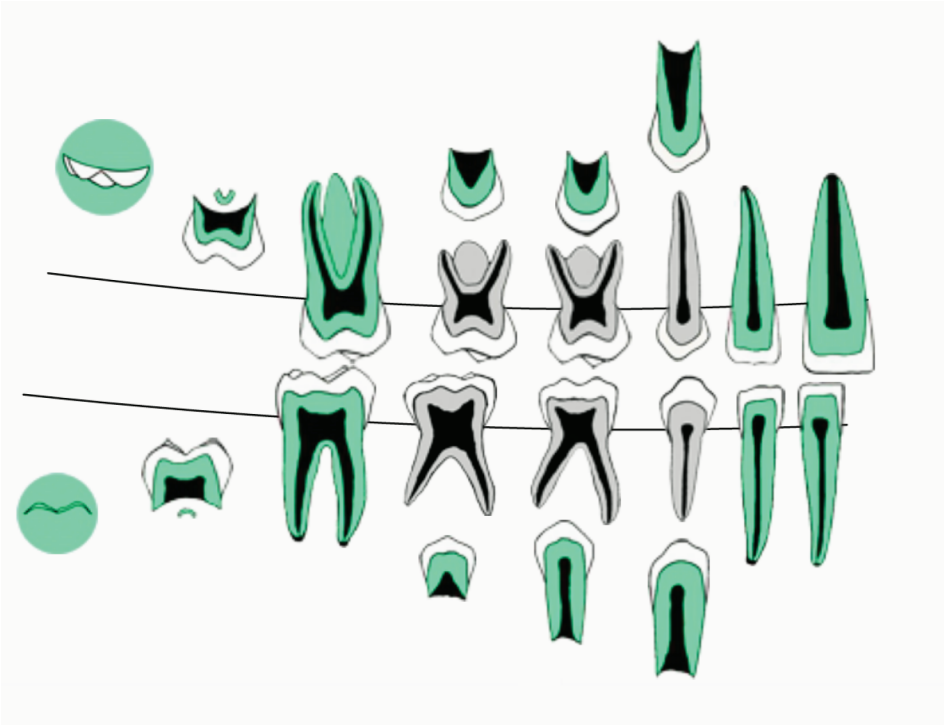


Figure 4. Scheme of the stages of tooth development and eruption during the mixed dentition period (9.5 years) (Reprinted with permission from Atlas of tooth development and eruption by Sakher Jaber AlQahtani, https://www.atlas.dentistry.qmul.ac.uk/).

Table 2. Dental development and eruption of permanent teeth excluding third molars (Linden 2010)

Permanent teeth	Central incisor	Lateral incisor	Canine	First premolar	Second premolar	First molar	Second molar
Initial calcification	6 mo	6-9 mo	12 mo	2.5 yr	3 yr	at birth	3.5 yr
Crown completed	4 yr	4 yr	6.5 yr	6.5 yr	7 yr	3 yr	6.5 yr
Root completed	9 yr	10 yr	14 yr	13 yr	14 yr	9 yr	15 yr
Eruption	6-7 yr	7-8 yr	10-11 yr	10-11 yr	11 yr	6 yr	11-12 yr

mo- months, yr- years

and relatively high prevalence of disto-occlusion and mesio-occlusion, there is an ongoing debate whether such a term, "normal occlusion", is appropriate.

Dental development assessment

From the initial mineralization phase of the hard dental tissues until the roots and crowns of the teeth are fully developed, teeth experience several calcification stages. Investigators have developed several methods for measuring the stage of tooth calcification (Garn et al. 1959; Haavikko 1974; Moorrees et al. 1963). The most widely used method was developed by Demirjian et al. (1973) and later modified by Willems et al. (2001). In this approach, seven left mandibular teeth, excluding the third molars, are scored according to eight developmental stages (A–H) of crown and root mineralization. The method utilizes only mandibular teeth from one side due to the high symmetry of development. Further, relatively small differences were observed when using all 14 mandibular teeth when comparing results from 7 vs 14 mandibular teeth (Demirjian et al. 1973). Taking into account that initial calcification of molars occurs around birth, and that the mandibular dentition (excluding third molars) is fully developed with the formation of second molar roots at the age of 15 years, we can use the Demirjian method, in theory, to quantify the dental development of children from birth until adolescence. However, the radiation dose involved when taking a panoramic radiograph limits the use of this method at younger ages.

Craniofacial morphology assessment

From the introduction of the cephalogram, a lateral radiograph of the head, by H. Broadbent in 1931, cephalometric analysis has remained the most widely used method for assessing the relationships between dental, skeletal, and soft tissue landmarks in practice and research (Broadbent 1981). Over time, authors have developed new, or improved previously defined, landmarks and measurements (Downs 1956; Jacobson 1995; Pancherz 1982a; 1982b; Ricketts 1960; Steiner 1959). Although investigators appreciate the information gathered when using numerous cephalometric parameters, handling large datasets may be challenging from a statistical

perspective (Farrar and Glauber 2014; Miller 2012). To address this problem, several authors have proposed using a principal component analysis to efficiently reduce the number of cephalometric parameters by combining those with high correlation (Al-Moraissi and Ellis 2014; Halazonetis 2004). As a result, craniofacial morphology is more efficiently depicted by principal components, each representing a distinct dental or skeletal craniofacial pattern.

Factors influencing dentofacial growth

Dental and craniofacial growth is a complex process regulated by the interaction between genetic and environmental factors (Carlson 2014; Cameron and Bogin 2012).

Genetic Factors Influencing the Development of the Craniofacial Region

The Genetic influences on craniofacial parameters are particularly prominent in the early development of dentofacial structures. Very early in development, *HOX* gene expression will participate in determining the pattern of the branchial regions of the developing head. Next, *EDN1* homeobox expresses its effect mainly through the *DLX* genes, which are active primarily in the first pharyngeal arch from which maxillary (*DLX1*, *DLX2*) and mandibular processes (*DLX5*, *DLX6*) are derived (Jeong et al. 2008; Wu et al. 2015). *EDN1* also promotes the expression of *BARX1*, a determining factor in the formation of the mandibular joint. *BARX1* also encodes a protein involved in the differentiation of Meckel's cartilage, the area of future maxillary bone, masseter muscles, and tongue (Tissier-Seta et al. 1995). The homeodomain transcription factor *PITX2* is expressed during the intrauterine stage of the formation of the ectoderm in the oropharyngeal membrane (Mitsiadis et al. 1998).

The *FGF8* gene plays a key role in the development of the facial skeleton from the facial ectoderm, whereas *WNTs* promote lateral growth of maxillary and mandibular processes (Carlson 2014). *MSX1* is expressed in mesenchyme growth of five facial primordia, the frontonasal prominence, and the paired maxillary and mandibular prominences (Blin-Wakkach et al. 2001). The interaction between *BMP2*, *BMP4*, and *MSX1* stimulates mesenchymal cells from the palatal shelves to later form a secondary palate. Also, *TGFβ3* plays an important role in the apoptosis of ectodermal cells from palatine shelves at the fusion seam.

Genetic Factors Influencing the Formation of Teeth

PITX2 initiates the formation of an ectodermal layer from which tooth germs will develop (Mitsiadis et al. 1998). *DLX1* and *DLX2* are involved in amelogenesis, and they are particularly important for the development of maxillary molars. In contrast, *PITX1* is expressed in the mesenchyme of mandibular molars (Carlson 2014; Zhang et al. 2015). Similarly, *BARX1*, activated through the *FGF8* gene, regulates mo-

lar development (Nanci 2007; Thesleff and Sharpe 1997). On the other hand, *MSX1* and *MSX2* are involved in the development of the incisors (Carlson 2014), with *MSX1* particularly being involved in root formation (Li et al. 2017; Yamashiro et al. 2003).

Genetic Disturbances in Dentofacial Region

Due to the common genetic background, it is not an uncommon manifestation that craniofacial and dental disturbances co-occur. Mutations in the *PITX2* gene are associated with Axenfeld-Rieger syndrome, characterized by eye, facial and teeth developmental disturbances (Mucchielli et al. 1997). *MSX1* and *MSX2* genes are associated with cleft lip and palate (Jagomagi et al. 2010; Vieira et al. 2005), as well as tooth agenesis (Vastardis et al. 1996). Knock out of both *DLX1* and *DLX2* in mice causes the upper jaw to develop without molars (Carlson 2014). Mutations in this *DLX* genes cause a tricho-dento-osseous syndrome, a human autosomal disease associated with hair, teeth and bone defects (Jain et al. 2017). Furthermore, this gene is associated with cleft palate and abnormal jaw development (Wu et al. 2015).

Further studies are necessary to explore which craniofacial traits are influenced by delayed or advanced dental development. Also, large-scale genetic studies might reveal potential novel genes and biological pathways that regulate the development of the dentofacial complex.

Environmental factors

Previous studies have reported that, maternal exposures during pregnancy and birthweight (Paulsson et al. 2004; Seow 1997), chronic diseases (Cistulli 1996; Dahllof 1998; El-Bialy et al. 2000; Selimoglu et al. 2013), endocrine regulation (Garn et al. 1965; Pirinen 1995), ethnicity (Chaillet et al. 2004; Wen et al. 2015; Zhuang et al. 2010), and nutrition (Guerrero et al. 1973; Moynihan and Petersen 2004) are associated with the growth of dental and craniofacial tissues. However, the effects of the environmental factors that influence dentofacial growth still remain largely unknown.

AIMS

The aim of this thesis is to investigate the patterns of dental and craniofacial development by analyzing data from healthy children, and children with tooth agenesis.

Part I of this thesis examines the secular variation of dental development in Dutch children between periods 1961 and 2004 (Chapter 2). Furthermore, we examined the genetic, endocrine and dental determinants of dental development. Chapter 3 examines the genetic loci associated with dental development using a genome-wide association study (GWAS) meta-analysis. Chapter 4 studies the relationship be-

tween thyroid function from the fetal period until early childhood and dental development at school age. Lastly, in Chapter 5 we investigated the association between hypodontia and dental development.

In part II, we explored the dental influences on the craniofacial morphology. We examined cephalometric characteristics of children with mild hypodontia (Chapter 6), as well as the association between dental development and craniofacial morphology (Chapter 7). Finally, in Chapter 8 we elaborate on the impact of our findings in a broader context, consider methodological limitations and discuss recommendations for the future studies.

SETTING

The majority of the studies presented in this thesis were embedded in the Generation R Study, a multi-ethnic population-based prospective cohort study from fetal life onwards, which was initiated to identify early environmental and genetic determinants of growth, development, and health (Kruithof et al. 2014). All mothers who resided in the Rotterdam area and had a delivery date between April 2002 and January 2006 were eligible. Initially, 9,778 pregnant women were enrolled, of whom 8880 were included in the study. Dentofacial assessments were performed in their children at the mean age of nine years (school-age period). In total, 8548 were enrolled at school-age, of whom 4475 had a panoramic radiograph from which dental development and tooth agenesis was assessed, and 4156 had craniofacial measurements assessed from cephalometric radiographs.

Studies in Chapter 2 and 5 were done in the Nijmegen Growth Study, a mixed-longitudinal, interdisciplinary population-based cohort study in healthy Dutch children born between 1961 and 1968 This study was conducted from 1971 to 1976 at the Radboud University Medical Centre in Nijmegen, the Netherlands. The design of this cohort has described in previously (Prahl-Andersen and Kowalski 1973). Children were enrolled at 4, 7, and 9 years of age and followed until 9, 12, and 14 years, respectively.

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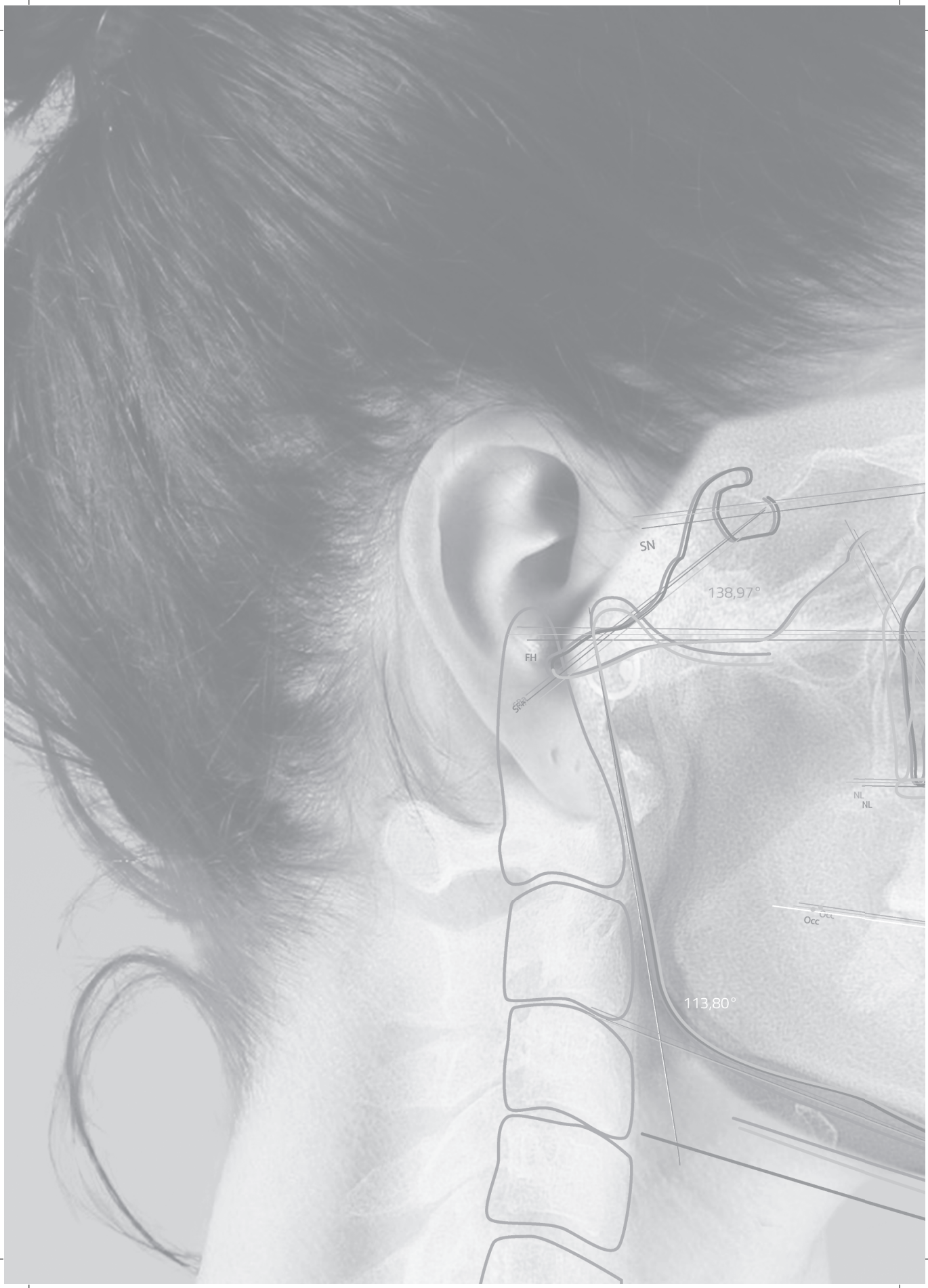
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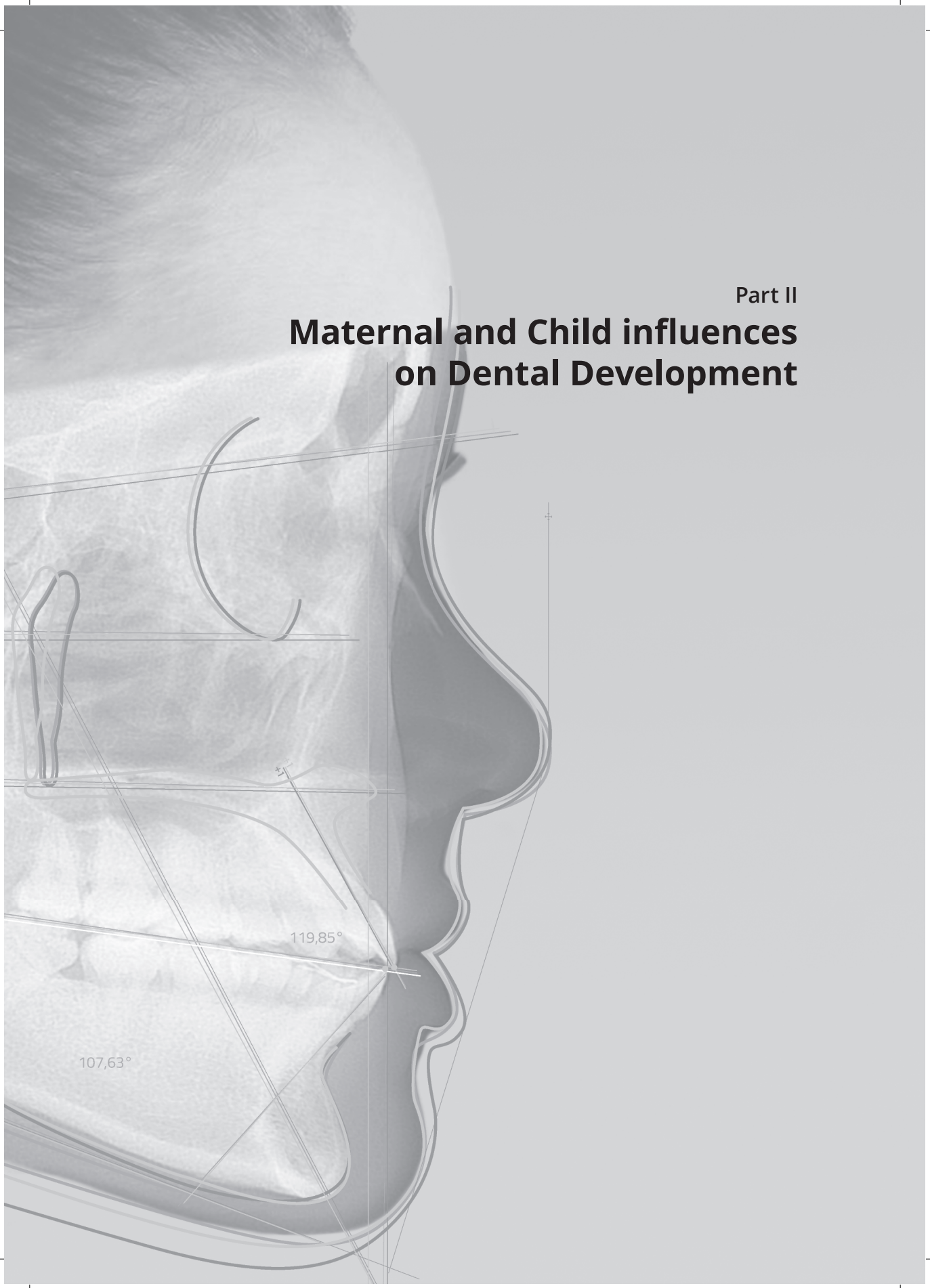
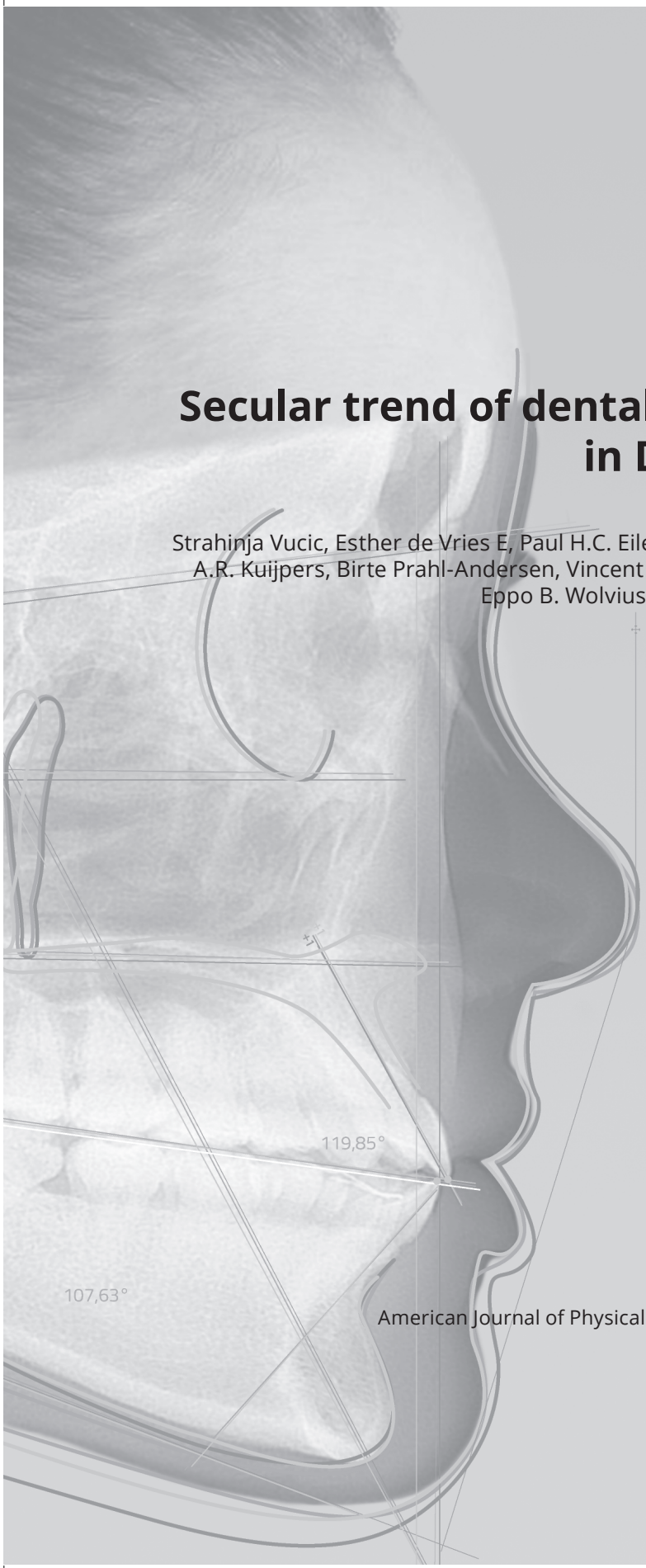


Part II

Maternal and Child influences on Dental Development



Chapter 2

Secular trend of dental development in Dutch children

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ABSTRACT

Many studies have established dental age standards for different populations; however, very few studies have investigated whether dental development is stable over time on a population level. Therefore, the aim of this study was to analyze changes in dental maturity in Dutch children born between 1961 and 2004. We used 2,655 dental panoramic radiographs of 2- to 16-year-old Dutch children from studies performed in three major cities in the Netherlands. Based on a trend in children born between 1961 and 1994, we predicted that a child of a certain age and gender born in 1963 achieved the same dental maturity on average, 1.5 years later than a child of the same age born 40 years later. After adjusting for the birth year of a child in the analysis, the regression coefficient of the city variable was reduced by 56.6%, and it remained statistically significant. The observed trend from 1961 to 1994 was extrapolated to 9- to 10-year-old children born in 2002–2004, and validation with the other samples of children with the same characteristics showed that 95.9%–96.8% of the children had dental maturity within the 95% of the predicted range. Dental maturity score was significantly and positively associated with the year of birth, gender, and age in Dutch children, indicating a trend in earlier dental development during the observation period, 1961–2004. These findings highlight the necessity of taking the year of birth into account when assessing dental development within a population with a wider time span.

INTRODUCTION

In the study of paleoanthropology, dental development has been used as a key factor for understanding the growth of juvenile extinct species for several reasons (Garrod et al. 1928; Mann 1968; 1975; Smith 1986; 1994). Firstly, teeth are in the most cases the best-preserved part of a skeletal sample, mainly due to the mineral composition of enamel. Unlike other parts of the skeleton, they are more influenced by environmental factors such as an eating habit, which can reflect the dietary pattern of a specific population. Moreover, teeth are observable in both extinct and extant human groups, thus making them suitable for a long- and short-term secular trend assessment. Anthropologists rely on determining dental age by identifying a stage of the crown and root formation, the degree of calcification, or timing of teeth eruption (Al-Tuwirqi et al. 2011).

According to Mann and Smith, dental development in extant great apes and humans proved to have important inferences on the aging process in juvenile fossil hominids (Mann 1968; 1975; Smith 1986; 1994). Pioneer studies that compared teeth development observed varying differences in dental growth patterns between extinct and extant species (Grine 1987; Mann 1968; Smith 1994). Smith (1986) was the first investigator who used central tendency discrimination analysis to classify dental development of juvenile fossil hominids as being "more like apes" or "more like humans." However, some authors questioned the accuracy of this method. Lampl et al. (1993) showed that by applying this technique to the sample of modern humans, 98% of those subjects were classified as having an ape-like pattern of dental development. Nevertheless, most investigators reached consensus that dental growth patterns in Neanderthals and Homo Erectus are more similar to modern humans (Dean et al. 1986; Smith 1994), than growth patterns in early Homo, Australopithecus, and Paranthropus, which had more rapid dental development, distinctive for African apes (Mann 1968; Dean et al. 1986; Beynon and Wood 1987; Bromage 1987; Beynon and Dean 1988; Smith 1994). In spite of similarities between modern humans and late Homo, fairly recently have studies pointed to the problem of using modern dental age standards in the genus Homo (Dean et al. 2001; Fernando and José Maria Bermudez de 2004; Smith et al. 2007). Even in regard to mentioned differences, investigating dental development in fossil remains puts human and primate biology into an evolutionary context, essential to understanding growth patterns of living primates (Dean 2000).

Although many techniques for assessing tooth development have been developed, the most widely used is Demirjian's method (Demirjian et al. 1973; Jayaraman et al. 2013b) based on quantifying the stage of tooth formation in seven mandibular teeth from panoramic radiographs. The first standardized tables were established

in French–Canadian children (Demirjian et al. 1973). However, using Demirjian's method with French–Canadian weighted standards is less accurate when it is implemented in other geographical regions or ethnic groups (Garn et al. 1973; 1972; Chaillet et al. 2005; Al-Tuwirqi et al. 2011). Consequently, many authors have used Demirjian's method to create databases representative of other populations (Leurs et al. 2005; Roberts et al. 2008; Bagherian and Sadeghi, 2011).

Secular variations have been observed in sexual development and physical growth due to continuous changes in genetic, epigenetic, and environmental factors (Gohlke and Woelfle 2009; Moonz 2011; Rigon et al. 2010; Silventoinen et al. 2011). However, few authors investigated whether dental development is stable over time on a population-level. Sasso et al. (2013) recently observed a positive secular trend in accelerated dental development over a 30-year period. Jayaraman et al. (2013a) showed that significantly accelerated development was only present in the maxillary dentition in 5- to 6-year-old Chinese children. Other authors also compared the dental development of children's skeletons obtained from archeological funeraries with children living at the time they conducted their study to gain a wider time span (Heuze and Cardoso 2008; Cardoso et al. 2010). However, the limitations of these previous studies were small sample size, lack of radiographs, short time-span, or the use of basic statistical methods.

The aim of this study was to analyze secular changes in dental development in a large group of Dutch children born between 1961 and 1994. Additionally, we extrapolated the observed trend for the year 2003 and validated this prediction with data from 9- to 10-year-old children born between 2002 and 2004 from the Generation R study.

MATERIALS AND METHODS

Study sample

We used 2,338 dental panoramic radiographs (DPRs) of 753 children living in two major cities in the Netherlands, namely, Nijmegen and Amsterdam (Table 1). Additionally, DPRs of 317 children (Figure 1) from the city of Rotterdam (Generation R study) were included in the study to perform an external validation of the predictive model assessed from the Nijmegen and Amsterdam samples.

We used the DPRs of 141 boys and 161 girls from the Nijmegen Growth Study born between 1961 and 1968. The Nijmegen Growth Study is a population-based cohort study conducted from 1971 to 1976 as a mixed-longitudinal, interdisciplinary study of growth and development of healthy Dutch children 4–14 years of age, and was conducted at the University of Nijmegen in the Netherlands. Only Caucasian

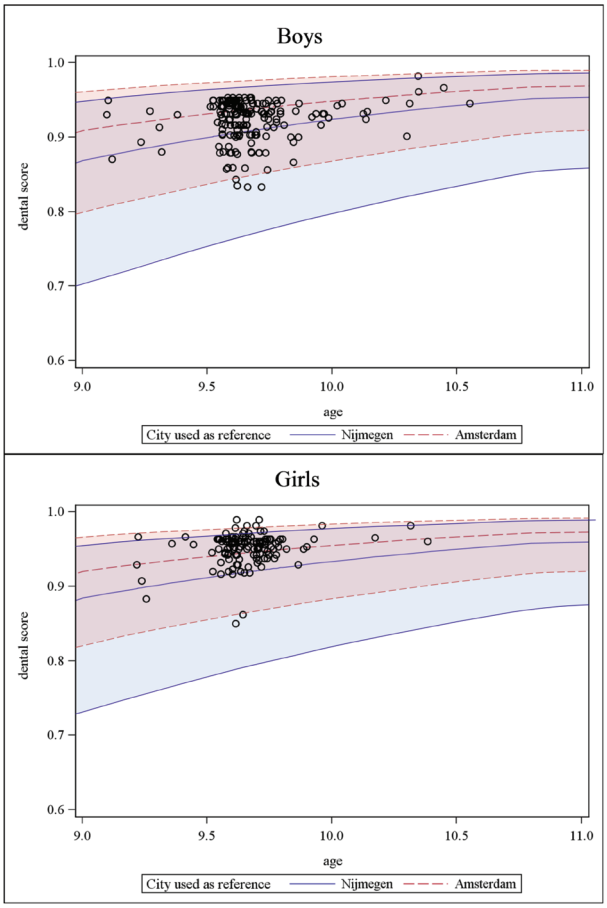

Figure 1. External validation was performed separately for boys and girls. Predicted dental maturity score (DMS) with 95% confidence range based on regression model II for children born in 2003. The solid line and blue area indicate predicted DMS and 95% range for Nijmegen children. The dotted line with the read area indicate predicted DMS and 95% range for Nijmegen children. Black circles are over-plotted observed DMS scores of Generation R children (180 boys and 137 girls).

children born in the Netherlands were included in the study. The inclusion of other ethnicities that fall under Caucasians but were non-Dutch is very unlikely due to the predominance of Dutch people in Nijmegen at the time when the study was conducted. Socioeconomic background of children's families was categorized as low (54%), middle (31%), and high (15%), based on the occupational status of fathers, since only 2% of mothers had full-time employment at the time of inquiry. Examination sessions of the children took place every 6 months; therefore, 1–10 DPRs for

Table 1. Dental Maturity Score by Age and Gender in the Nijmegen and Amsterdam Samples

	Dental maturity score							
	Nijmegen			Amsterdam				
Age, years	N	Mean	SD	N	Mean	SD	Difference	P- value
Boys								
2				1	0.151	0		
3				16	0.238	0.049		
4	3	0.238	0.009	33	0.354	0.076	−0.116	0,013
5	17	0.292	0.046	33	0.506	0.106	−0.214	≤0.001
6	19	0.43	0.064	24	0.616	0.093	−0.186	≤0.001
7	58	0.563	0.064	17	0.726	0.086	−0.163	≤0.001
8	85	0.637	0.079	13	0.778	0.068	−0.141	≤0.001
9	139	0.768	0.087	15	0.869	0.066	−0.101	≤0.001
10	200	0.859	0.059	17	0.912	0.058	−0.053	≤0.001
11	145	0.908	0.035	15	0.951	0.031	−0.043	≤0.001
12	85	0.934	0.018	6	0.968	0.021	−0.034	≤0.001
13	73	0.949	0.027	13	0.98	0.027	−0.031	≤0.001
14	18	0.96	0.015	11	0.999	0.002	−0.039	≤0.001
15				7	0.998	0.004		
16				4	0.996	0.008		
Girls								
3				8	0.195	0.065		
4				33	0.348	0.102		
5	13	0.26	0.048	24	0.512	0.093	−0.252	≤0.001
6	20	0.445	0.066	23	0.621	0.096	−0.177	≤0.001
7	55	0.591	0.073	26	0.765	0.068	−0.174	≤0.001
8	89	0.689	0.091	18	0.837	0.089	−0.148	≤0.001
9	180	0.806	0.083	14	0.9	0.059	−0.094	≤0.001
10	239	0.898	0.048	14	0.947	0.039	−0.049	≤0.001
11	201	0.929	0.035	10	0.964	0.037	−0.035	0,002
12	117	0.956	0.03	13	0.99	0.006	−0.034	≤0.001
13	104	0.975	0.013	10	0.993	0.007	−0.018	0.022
14	27	0.98	0.015	9	0.998	0.004	−0.018	≤0.001
15				14	1	0.001		
16				10	1	0		

each child were available. In total, 1,887 DPRs were used in this study (Prahl-Andersen and Kowalski 1973).

The second sample was obtained from the records of patients attending the general dental clinic at the Academic Centre for Dentistry Amsterdam (ACTA), the Netherlands. We used 451 DPRs (225 boys, 226 girls) of 2- to 16-year-old children. The children were born between 1972 and 1994. Although no exact data on socioeconomic background of children from this sample were collected, most of the patients visiting ACTA were from low-to-middle class families considering the district of the hospital and more affordable dental care. Ethnicity was determined retrospectively, and based on surnames suggesting a Dutch or non-Dutch background (Leurs et al. 2005). This method has been previously validated by Bouwhuis and Moll (2003). Results showed that using surnames to determine the ethnicity of a child was a reliable method to differentiate major ethnic groups in the Netherlands; differentiating Turkish and Moroccan from the Dutch accurately, and for distinguishing Surinamese from Dutch surnames less accurately. Only children with surnames indicating Dutch origin were included in the study. Of these children, DPRs had been made during systematic oral examinations by final year dentistry students, and the oral treatment was not a part of this examination. One DPR was available for each child. The third sample consisted of DPRs from 317 children (180 boys, 137 girls) from the Generation R study, a population-based cohort study from fetal life onward in Rotterdam (Jaddoe et al. 2012). The study was approved by the Medical Ethics Committee of the Erasmus Medical Centre in Rotterdam, the Netherlands. The DPRs used in this study were from 9- to 10-year-old children born between 2002 and 2004. Only children of Dutch origin were included. This Generation R sample was used to assess external validation of the DMS predictive regression model based on the trend observed in children born between 1961 and 1994.

Dental development assessment

A dental maturity score (DMS) for each sample was calculated using the protocol described by Demirjian (Demirjian et al. 1973). Scores for the Nijmegen and Amsterdam samples were collected retrospectively. The Generation R sample was scored by one experienced examiner. After scoring was completed, two investigators independently scored the same subsample of 20 randomly selected DPRs from the Generation R study to determine the inter-observer reliability. For each sample, the French–Canadian weighted standards were used.

Selection criteria

Only DPRs of children without any disorders that could affect dental development were included. If a child had a missing tooth due to extraction, agenesis, etc., a score

was obtained from the matching contralateral mandibular tooth. DPRs with missing teeth on both sides of the mandible were excluded. Children were not subjected to orthodontic treatment at the time when the OPG was taken, however previous history of orthodontic treatment could not be rejected completely. However, in the Rotterdam region, early orthodontic intervention is rare, and the change that early orthodontic treatment would interfere with our results seems small.

Statistical analysis

Inter-observer reliability and the absolute agreement for both the Nijmegen and Amsterdam samples were tested elsewhere and showed no significant differences (Prahl-Andersen and Kowalski 1973; Leurs et al. 2005). We calculated the inter-class correlation coefficient and Cohen's kappa coefficient to determine inter-examiner agreement for the Generation R subsample between two independent researchers who assessed developmental stages (A–H) for each of the seven observed teeth.

Prior to the regression analysis, the DMS was logit-transformed for the Amsterdam and Nijmegen samples to obtain a more linearly distributed outcome variable. A logit of value 1 yields to $+\infty$; therefore, to avoid transformation error, we corrected the values of 72 DPRs from DMS = 1 to DMS = 0.997. Birth year was used as a continuous variable and counted from the year 1961 (e.g., a child born on 17 May 1982 had a year of birth variable 1982.37 − 1961 = 21.37).

We investigated the association between the birth year and the DMS with linear mixed-effects models, which account for repeated measurements, irregular intervals between measurements, and within-person correlation of the Nijmegen cohort data. For a given structure, the intercept and age were modeled as random effects. We used the full model for predicting dental development curves given the birth year of the Generation R sample. We validated the accuracy of the predictive regression model for the birth year 2003 by plotting the DMS of the Generation R children over the predicted 95% interval range. All statistical analyses were performed using the statistical software, SAS version 9.2 (SAS Institute, Cary, NC).

RESULTS

Analysis of Nijmegen and Amsterdam children

The mean DMS scores were significantly lower ($P \leq 0.005$) in the Nijmegen children compared with the Amsterdam children (Table 1). The greatest DMS difference be-

Table 2. Association Between Dental Maturity and Adjusting Determinants Estimated from Two Linear Mixed-effect Models.

			Model I			Model II[a]		
Variables	**N-p [b]**	**N-dpr [c]**	**β1**	**95% CI**	**P-value**	**β_2**	**95% CI**	**P-value**
Intercept	753	2338	−4.348	(−4.450, −4.247)	≤0.001	−4.516	(−4.654, −4.377)	≤0.001
Age	753	2338	0.593	(0.583, 0.604)	≤0.001	0,605	(0.593, 0.618)	≤0.001
Gender								
Boys (ref.)	366	1067	0	–	–	–	–	–
Girls	387	1271	0.132	(0.070, 0.194)	≤0.001	0.142	(0.080, 0.203)	≤0.001
City								
Nijmegen (ref.)	302	1887	0	–	–	–	–	–
Amsterdam	451	451	0.952	(0.889, 1.014)	≤0.001	0.413	(0.104, 0.723)	0.009
Year of Birth	753	2338	–	–	–	0.023	(0.010, 0.036)	≤0.001

a The likelihood ratio test showed a statistically significant better fit of the Model II compared to Model I (P-value ≤0.001).

b Number of participants.

c Number of dental panoramic radiographs.

Table 3. The Percentage of Inter-examiner Agreement in Determining the Developmental Stages for Each of the Seven Observed Teeth on a Generation R Subsample of 20 Dental Panoramic Radiographs.

	Scored left-side mandibular teeth						
	m2	**m1**	**pm2**	**pm1**	**c**	**i2**	**i1**
Cohen's Kappa coefficient	0.846	1	0.917	0.924	0.895	1	1
Inter-class correlation coefficient	0.913	1	0.947	0.959	0.919	1	1

tween the two samples was at the age of 5, both for boys (21.4) and girls (25.2). From the age of 5 onward, DMS differences between genders steadily decreased.

The results of the mixed multivariate regression analysis are presented in Table 2. Estimates from the first model showed that children from Amsterdam had 0.952 (95% CI: 0.889, 1.014; $P \leq 0.001$) higher logit-DMS compared with children from Nijmegen. Due to this difference, we estimated from the model that a child from Amsterdam would reach, on average 1.605 years, the same dental maturity earlier compared with a child of the same age and gender from Nijmegen. After adding the birth year into model II, the results showed that for every year of increase, the birth year effect caused a 0.023 (95% CI: 0.010, 0.036; $P \leq 0.001$) increase in logit-DMS. Therefore, children of a certain age and gender in a given year are estimated to reach the same dental maturity on average 0.038 years (~14 days) earlier than children of the same age born 1 year earlier. The city variable remained statistically significant in model II ($P = 0.009$); however, the regression coefficient of the city variable was 56.6% lower than in the previous model ($\beta 2 = 0.413$; 95% CI: 0.104, 0.723), indicating a strong confounding by a year of birth variable. Consequently, the 1.605 years in city difference estimated from model I, decreased to a value of 0.683 years in model II.

Gender was a significant variable in both models ($P \leq 0.001$). Based on the model I, we estimated that girls attained on average 0.223 years (~81 days) earlier the same DMS as boys from the same city. After adjusting for the birth year in model II, the difference in dental development between boys and girls was 0.235 years (~86 days), under the assumption that they were born in the same year and in the same city.

Inter-examiner agreement with the generation R sample

Table 3 shows the results of the inter-examiner reliability, which was determined on a subsample of 20 DPRs. There were no differences between the two examiners in the scoring of incisors and first molars. Cohen's Kappa coefficient varied between

Table 4. Characteristics of the Generation R Children

	Boys	Girls	P-value [a]
Generation R			
N	180	137	0,016
Age (years ±SD)	9.679±0.204	9.659±0.150	0,303
Year of Birth (year ±SD)	2002.933±0.250	2002.971±0.169	0,132
Dental Maturity Score	0.923±0.028	0.951±0.020	≤0.001
Nijmegen			
Dental Maturity Score [b]	0,909	0,919	
Amsterdam			
Dental Maturity Score [c]	0,938	0,945	

a) Difference between boys and girls is based on Chi-square -test for categorical variables and T-test for continuous variables. b) Predicted dental maturity score for a Nijmegen children, based on regression Model II, given the average age and year of birth of the Generation R sample among boys and girls, based on regression Model II. c) Predicted dental maturity score for Amsterdam children given the average age and year of birth of the Generation R sample among boys and girls, based on regression Model II

84.6% and 92.4%, and the interclass correlation coefficient varied between 91.3% and 95.9% for canines, premolars, and second molars.

Validation of the secular trend with generation R children

The characteristics of the Generation R sample are given in Table 4. The girls had a significantly higher DMS than the boys ($P \leq 0.001$), but no significant differences were found in age ($P = 0.303$) and year of birth ($P = 0.113$). The average DMS scores of the Generation R children (Boys = 0.923; Girls = 0.951) resembled the estimated dental development of Amsterdam children (Boys = 0.938; Girls = 0.945), and both of these samples had higher DMS compared with Nijmegen children (Boys = 0.909; Girls = 0.919).

Figure 1 shows the results of modeling of the 95% confidence range of dental development for the birth year 2003 by gender and study population. We found that predicting DMS in Generation R children using model II was 96.8% accurate (97.8% for boys, 95.6% for girls) when Amsterdam data were used as the referent sample and 95.9% accurate (99.4% for boys, 91.9% for girls) when the Nijmegen cohort was used as an underlying referent sample.

Table 5. Characteristics of Studies on Secular Changes in Dental Development

Nr.	Lead author, Year	Total Sample Size (Historical Sample Size)	Birth Year Range	Age Range of Children	Population	Secular Change in Dental Development
1	Cardoso, 2010	N=635 (114)	1887-1997	6-18 years	Portuguese	Positive secular trend of 1.22 years (range: 0.19- 1.98) in boys and 1.47 years (range: 0.59- 2.14) in girls, in over 50-year period.
2	Heuze, 2008	N=2426 (40)	Not available	4- 15years in the historic sample	Portuguese (historic sample) and Ivory Coast, Iran, Morocco and France (modern sample)	Positive secular trend of 1year over the 50-year period.
3	Jayaraman, 2013	N=400 (200)	1981-2001	5-6 years	Chinese	Positive secular trend in maxillary dentition, odds ratio= 1.29 ($P \leq 0.001$).
4	Sasso, 2013	N=1000 (500)	Not available	6-16 years	Croatian	Positive secular trend of 0.72 years during 30-year period ($P \leq 0.001$).

DISCUSSION

The study showed a positive secular trend in accelerated dental development in Dutch children born between 1961 and 1994. This trend continued beyond the observation period for children born between 2002 and 2004. Our findings suggest that children born in 2003 reach the same dental maturity on average about 1.5 years earlier than children who were born 40 years earlier.

Summarized conclusions from previous studies on secular changes in dental development are presented in Table 5. A similar positive trend for dental development was observed in a study in Croatian children, where children examined between 2007 and 2009 had 0.72 years higher dental age scores than children examined 30 years earlier (Sasso et al. 2013). This is in contradiction with the results of the investigation on the secular trend in the maturation of permanent teeth in Chinese children. Jayaraman et al. (2013a) demonstrated a positive secular trend only in the maxillary

dentition, whereas changes were not significant in the mandibular dentition. However, only a specific age group (5–6-year-olds) was investigated in this study; secular differences could possibly be more distinctive at later stages when children enter a more active growth spurt. In another investigation on secular changes in root formation, the authors compared developmental stages in Portuguese children with the skeletons of children living a half-century earlier (Cardoso et al. 2010). Root formation was more advanced in the modern sample, but the duration of root formation did not differ. These findings were also confirmed when a Bayesian dental age assessment method was used to compare the Portuguese skeletons with children from the Ivory Coast, Iran, Morocco, and France (Heuze and Cardoso 2008). These studies used skeletal samples because radiographs or other sources of information on dental development were unavailable at the time. The authors took precautions to avoid bias due to the children's cause of mortality; however, health conditions that caused the child's death may also have influenced dental development.

Trends in the earlier dental development coincide with positive secular changes of other attributes in Dutch children that were investigated during the observation period. Mean final height has increased an average of 8 cm during the period between 1955 and 1997 (Fredriks et al. 2000a), the body mass index of 52%–60% children older than 3 years in 1997 exceeded the 50th percentile of 1980 (Fredriks et al. 2000b), and a positive secular change toward earlier puberty was observed until 1980 (Mul et al. 2001). Although the exact mechanism of these associations is still debated, the most frequently acknowledged factors reported in the literature are the rapid increase in economic status and education in the Netherlands during the 1960s and 1980s (Boelhouwer and Stoop 1999; Fredriks et al. 2000a). Consequently, food became more readily accessible and a shift toward increased protein and fat contents in foods changed the children's nutrition habits (Fredriks et al. 2000a). Improved infrastructure and transportation in the Netherlands has lowered daily calorie expenditure (Groote et al. 1999). Additionally, disease control and prevention contributed to positive secular changes in average height (Hatton and Bray 2010).

Although the mentioned studies showed a significant effect of environmental factors on the skeleton and general somatic development, the effects on dental development are still questionable. Studies thatfollowed malnourished children showed that dental development is a biologically stable process and independent of nutritional habits (Bagherian and Sadeghi 2011; Elamin and Liversidge 2013). Elamin and Liversidge (2013) performed extensive stratified analysis, resulting in a total of 44 comparisons between malnourished and normal children. Although in 35 comparisons (80%) a group of malnourished children attained a certain developing stage of the tooth at a later age, compared with a normal group of children, none of

the results was statistically significant (0.980 > P > 0.052). For socioeconomic class, non-significant differences (P = 0.0705 and P = 0.085) were reported of delayed dental development in children from lower socioeconomic class families compared with children from higher socioeconomic class families (Cardoso 2007). In this article, we have demonstrated significant secular changes in dental development and a difference in dental development between Nijmegen and Amsterdam children, but in order to ascertain true causality, further investigations are necessary, exploring new and know determinants on larger datasets.

Validation of our predictive model showed that the accuracy varied between 95.9% and 96.8% depending on which city was used as the reference (Figure 1). This indicates that when establishing standard tables and percentile curves for dental development over the long term for current and future generations of children, studies should take the year of birth into account. Still, when making predictions based on the trend determined retrospectively, it is assumed that changes in the causal factors are fixed, which is very unlikely. Current positive trends in dental development may decline or stabilize in the future due to changes in the previously mentioned causal factors. Nevertheless, with the pace observed in our study showing that for every year increase in the child's birth year there is an additional 13.9 day increased effect on DMS, our prediction model was still a useful tool when assessing the DMS of children born in 2002–2004 based on the trend observed from 1961 to 1994. Figure 1 also illustrates that a positive trend was more evident in girls than in boys, with the majority of the Generation R girls attaining a higher DMS than the predicted Nijmegen referent curve. Assuming the continuation of a positive secular trend in dental development, a recommendation for future investigations could be the inclusion of children of younger ages (e.g., <7-year-olds) to avoid the comparison of children with fully or almost fully developed dentition.

Potential limitations of this study are the use of the Demirjian method and French–Canadian standards to calculate the DMS. We did not convert the DMS to dental age as these standards cannot be accurate when used in a Dutch population (Leurs et al. 2005). French–Canadian standards were used for the measurement of dental maturity in the historical sample of Nijmegen children due to the absence of representative Dutch standards during that time period. Therefore, the DMS of Amsterdam and Generation R samples were calculated using the same standards from the DPRs. Using different standards is unlikely to significantly change the results, as DMS reflects the developmental stage of teeth and not their age. Blinding of the investigators was not done since the DMS was calculated before the aim of this study was defined. Another possible limitation of this study is that we used a proxy

value for DMS of 72 DPRs (3%) with a value of 1 because the logit transformations yield to +∞.

Our findings have an impact on multiple disciplines. In orthodontics, the optimal age to start treatment in patients is of great importance (Yang and Kiyak 1998). The common recommendation for starting orthodontic treatment is when the growth spurt occurs (Prasad et al. 2011). The results of this article show secular and intra-population variations that support the notion that clinicians should not be rigid itn interpreting these recommendations, but rather use them as a guide in making the final decision on a case-by-case basis. From a paleoanthropological perspective, a topic of extensive investigation was the relationship between skeletal and dental maturation. This topic could shed light on questions such as whether Neanderthals or Homo erectus had adolescent growth spurts, or whether the timing of a growth spurt was similar to those of modern Homo sapiens adolescents (Dean et al. 1986; Smith 1994). We have known since the work of Tanner in the mid-20th century that skeletal and dental maturation display considerable independence in development (Tanner 1952). Although the application of a dental age assessment technique could provide a more unbiased estimate of chronological age in historical specimens than using techniques based on skeletal development, the results of our study showed the significant variability of dental growth patterns during the observed 42-year period. As a result, these findings implicate a more precise applicability of dental age in forensics, due to a lower time span between human remains and their comparison group. However, it is important to identify to what extent possible unmeasured secular changes in dental development on longer interval terms, play a role in prehistoric samples. Since in those cases it is not always possible to measure secular variations, often due to limited samples of fossils, conclusions about age estimation in anthropological studies should be formulated preferably based on several other criterions as well, and not only based on dental development.

CONCLUSION

DMS was significantly positively associated with the year of birth, gender, and age in Dutch children, indicating a trend toward earlier dental development in the period between 1961 and 2004 due to unknown causes. These findings suggest a greater susceptibility of dental development to secular changes than it was previously thought, and thus the necessity of taking the year of birth into account when assessing dental development within a population with a wider time span.

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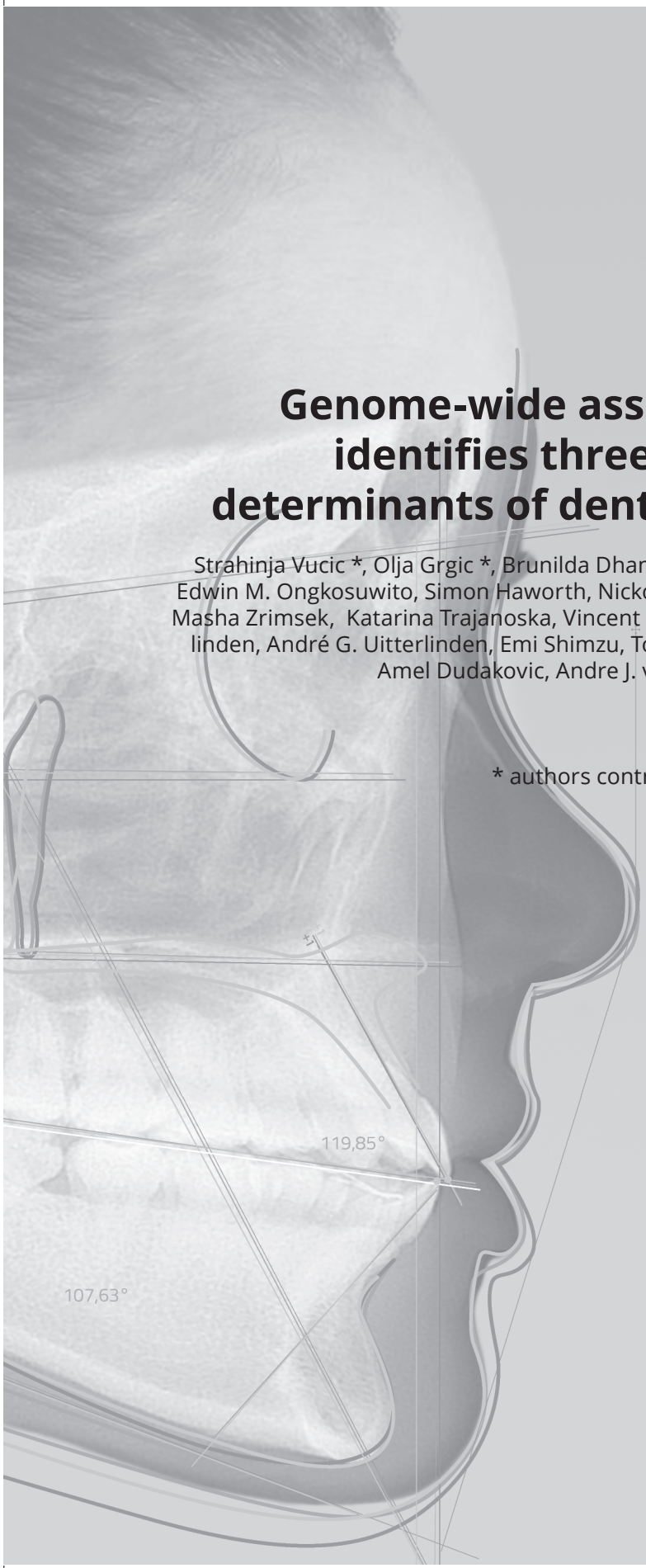

Chapter 3

Genome-wide association study identifies three novel genetic determinants of dental maturation

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ABSTRACT

Objectives: Previous genome-wide association study (GWAS) meta-analyses studying dental developmental traits as "Number of Teeth" (NT) and "Age at First Teeth Eruption" (AFTE) have identified 15 loci. We performed a GWAS meta-analysis on radiographic dental development (DD) in children of school age to identify genetic determinants of dental maturity.

Methods: Discovery GWAS of DD was performed in the Generation R study, a multiethnic pregnancy cohort in Rotterdam, The Netherlands. We included 2,793 children with mean age 9.82 (SD 0.34) years. DD was determined from dental panoramic radiographs using the Demirjian method. Participants were genotyped with the HumanHap 610K platform, imputed to the 1000GP reference panel. The analysis was adjusted for age, sex, BMI, and 20 genomic principal components; genome-wide significance (GWS) was set at $P<5x10^{-8}$. Replication of signals associated with DD was pursued using summary level results from the published GWAS meta-analysis of the ALSPAC and NFBC1966 studies (n=12,012) studying NT and AFTE. Fisher's combined probability test weighted by sample size was used for the combined meta-analysis. DEPICT is used to identify the most likely causal gene at a given associated locus, reconstituted gene sets enriched for DD associations, and tissues and cell types in which genes from associated loci are highly expressed. The heritability of NT and genetic correlation with the other phenotypes were estimated. A two-step Mendelian Randomization approach was used to assess a potential causal effect of body weight-related measurements (BMI, lean mass fraction, and fat percent) and serum lipids on dental development.

Results: Top signals associated with greater DD mapped to 16q12.2 (*IRX5*; rs12444195-C, $P=2.8x10^{-7}$) , 17p11.2 (*SREBF1*; rs2955382-C, $P=2.8x10^{-7}$) and 3q22.1 (*TMCC1*; rs73206256-T, $P=1.5x10^{-7}$) loci. Significant evidence for replication of two GWS signals was observed in the previous NT meta-analysis (*IRX5*: $P=2.7x10^{-5}$, *SREBF1*: P=0.008). Alleles associated with greater DD were nominally associated with earlier teeth eruption in the AFTE meta-analysis (*IRX5*: $P=1.5x10^{-5}$ and *SREBF1*: P=0.01). After genome-wide meta-analysis variants in three loci were associated at genome-wide significant level: 16q12.2 (*IRX5*; $P=3.4x10^{-9}$), 7p15.3 (*IGF2BP3*; $P=2.7x10^{-8}$) and 14q13.3 (*PAX9*; $P=6.6x10^{-9}$).

Conclusion: We describe here three novel loci associated with dental development on top of replicating nine previously reported. These findings provide further insight into the process of dental maturation in children from early infancy to late school age.

INTRODUCTION

Dental development (DD) is a complex process which begins with the development of primary teeth in the eighth week of prenatal development and completes postnatally around the age of 18-25 years with the formation of third molar roots (1). Throughout their development, teeth go through several stages: initiation, the bud, cap, bell and root development stage. Gene families *AXIN, BARX, BMP, EDA, FGF, MSX, PAX, SHH* and *WNT* are most frequently reported in the literature to be associated with the process of dental development (1–3). Dental anomalies such as tooth agenesis result from arrested growth in earlier stages of development affecting the initiation, bud, and cap stages (4). On the other hand, genetic factors which regulate dental development at later stages (late bell stage and root development) and are radiographically seen as various mineralization stages of crown and root (5), might be more relevant for the regulation of the rate of dental development (6). The process of mineralization and its genetic regulation have been extensively described for the model of bone development (7). Simultaneously to the process of development, the teeth move in an axial direction during the process of tooth eruption. The moment when the tooth breaches the gingiva and becomes visible in the mouth is called emergence. This process usually occurs when the root has reached around ¾ of its final length (8). Therefore, the formation of tooth structures, eruption and emergence are intertwined processes and integral components related to the human tooth development (1) which are likely under genetic control.

Studies of the genetic background of tooth development and eruption comprise:

- transgenic mice and other animal models (9–11):

- human congenital disorders in which dental abnormalities are a feature, such as Down syndrome (trisomy 21), cleft lip and palate, ectodermal dysplasia, etc. (12,13);

- family-based and twin studies (14) to estimate the heritability of dental development;

- genome-wide association studies (GWAS)/ meta-analysis (17–19).

The latter has proven to be a powerful and successful tool for investigating and identifying the genetic basis of complex traits (20). A GWAS meta-analysis studying the age of the first erupted tooth and number of teeth at one year of age identi-

fied 10 and 11 loci associated with those traits on a genome-wide significant level, respectively (17). Demirjian et al. described a method which estimates DD on dental panoramic radiographs, through estimating dental mineralization, shape, and proportions (21). In contrast to tooth eruption, which is influenced by different environmental factors (ankyloses, extraction of primary teeth), those criteria are considered to be less prone to environmental influences, giving a better insight into the genetic architecture of DD (22). To our knowledge, there have been no GWAS studies investigating the developmental criteria of the tooth, based on Demirjian method.

Therefore, we aimed in this study to (a) pursue the first GWAS on radiographic DD, (b) to examine its underlying pathways using a Mendelian randomization approach, and (c) assess the relationship with the other correlated traits, using LD-Score regression.

METHODS

Study Population

The discovery genome-wide association study (GWAS) on the radiographic assessment of human tooth development was performed in The Generation R Study. Replication was pursued using a proxy phenotype from a published GWAS meta-analysis studying dental development defined as "the number of teeth at 15 months" (17) from the Avon Longitudinal Study of Parents and Children (ALSPAC) and "the number of teeth at 12 months" from 1966 Northern Finland Birth Cohort (NFBC1966). A meta-analysis of the discovery and replication sets was then performed.

The Generation R Study is a population-based cohort study from fetal life until adulthood, established in Rotterdam, the Netherlands, at the Erasmus University Medical Center. Mothers of children born between April 2002 and January 2006 were invited to participate since pregnancy. The Generation R Study was designed to identify early environmental and genetic determinants of growth, development, and health as described previously (23). The Medical Ethics Committee of the Erasmus Medical Centre (MEC-2012-165) in Rotterdam, the Netherlands approved the study. At the start of each phase, children and their parent(s) were asked to provide written informed consent.

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a population-based birth cohort study consisting of 14,541 women and their children recruited in the county of Avon, UK, in the early 1990s (24). Both mothers and children have been extensively followed from the eighth gestational week onwards using a combination of self-reported questionnaires, medical records, and physical examinations.

Biological samples including DNA have been collected from the participants. Ethical approval was obtained from the ALSPAC Law and Ethics Committee and relevant local ethics committees and written informed consent provided by all parents.

The 1966 Northern Finland Birth Cohort Study (NFBC1966) followed pregnancies with an expected delivery date in the year 1966 in the Oulu and Lapland provinces of Finland (25). A total of 5,403 samples were available for analysis from NFBC1966. All aspects of the study were reviewed and approved by the Ethics Committee of the University of Oulu and by the respective local research committees. Participants gave written informed consent to be included in the study.

A total of 14,805 children with complete genetic and phenotypic information were included in the meta-analysis.

Dental Development Assessment

In the Generation R Study, tooth development was quantified at a mean age of 9.82 (SD 0.34) years on panoramic radiographs using the method described by Demirjian (21). Following this approach, seven teeth (excluding third molars) located on the left side of the mandible were scored with one of the eight developmental stages (A-H), depending on the calcification of the crown and root. Child's overall dental development was established by calculating the mean value of the standard deviation scores for the seven teeth. The overall dental development score was normalized using rank-based transformation due to non-normal distribution (26). The inter-observer agreement between two raters was performed on a random subsample of 100 subjects for each of the seven teeth using the intraclass correlation statistic. Correlation coefficients ranged between 0.65-0.80 which is considered substantial agreement according to conventional criteria (27). First incisors were not considered due to the absence of variation in the stage of tooth development.

In ALSPAC, tooth eruption phenotypes of the children were derived from questionnaires completed by the mothers and included items regarding the 'age at first tooth' (AFTE) assessed at 15 months and the 'number of teeth' (NT) in the child's mouth at 15 months (17).

In NFBC1966, AFTE and NT were gathered by public health professionals during the children's monthly visits to child welfare centers. AFTE was recorded as the month of visit at which the first tooth was observed (so that the first tooth could have erupted at any time between the end of the previous month and the recorded month, i.e. 'interval censoring'). The number of teeth was recorded at 12 months (17).

Covariates (The Generation R study)

Body composition and serum lipids

Height and weight of the children were measured by trained personnel at the research center following a previously described protocol (as described previously). Body mass index (BMI) was calculated from directly measured height, and weight of the children and logarithmically transformed [log(BMI)] to reduce skewness of its distribution. Further, we calculated lean mass fraction (lean mass/weight) and fat mass fraction (fat mass/weight). Thirty-minutes fasting, blood samples were collected in children at the age of six years to measure total-, HDL-, and LDL-cholesterol and triglycerides, using Cobas 8000 analyser (Roche, Almere, The Netherlands).

Genotyping

In the Generation R Study, individuals were genotyped using Illumina HumanHap 610 or 660 Quad chips (Illumina Inc., San Diego, USA). The GWAS datasets underwent a stringent QC process, which has been described in detail previously (28). Briefly, exclusions of single nucleotide polymorphisms (SNP) were made if departure from Hardy-Weinburg equilibrium (HWE) was greater than $P<10^{-7}$, minor allele frequency (MAF) was <1% or if more than 2.5% of SNPs were not successfully genotyped (SNP call rate <97.5%). Imputations were performed using MACH and minimac software and 1000G Phase l (version 3) as the reference panel. Only SNPs exceeding an rsq imputation quality (r2) of 0.3 and a MAF of >1% were included in subsequent analyses. In ALSPAC, participants were genotyped using the Illumina HumanHap550 Quad genotyping platform (23andMe, Cambridge, UK; Burlington, NC, USA). A stringent QC process performed is described elsewhere (17). Individuals of non-European ancestry were removed from further analysis. SNPs with a final call rate of <95%, MAF <1% and evidence of departure from HWE ($P < 5 \times 10^{-7}$) were also excluded from analyses. Individuals were imputed to HapMap Phase II (Build 36, release 22) using the Markov Chain Haplotyping software (MACH v.1.0.16) (29). Only SNPs exceeding r 2 of 0.3 and an MAF of >1% were included in subsequent analyses. In NFBC1966 participants were genotyped using the Illumina HumanCNV370-Duo DNA Analysis BeadChip. More details of genotyping and quality control procedures can be found in Sabatti et al. (25). SNPs were excluded from the analysis if the call rate in the final sample was <95%, if there was a lack of HWE ($P < 5 \times 10^{-4}$) or if the MAF was <1%; imputation was carried out using IMPUTEv1 with CEU haplotypes from HapMap Phase

II (release 21) as the reference panel. Only SNPs exceeding r2 of 0.3 and an MAF of >1% were included in subsequent analyses.

Enrichment and gene prioritization analyses

We used DEPICT to identify the most likely causal gene at a given associated locus, reconstituted gene sets enriched for DD associations, and tissues and cell types in which genes from associated loci are highly expressed (30). To accomplish this, the method relies on publicly available gene sets (including molecular pathways) and uses gene expression data from 77,840 gene expression arrays (31) to predict which other genes are likely to be part of these gene sets, thus combining known annotations with predicted annotations. Association cutoff in DEPICT was set to $P=1x10^{-5}$.

Statistical Analysis

In the Generation R study association between DD and genetic markers was carried out using a simple linear regression model adjusted for age, sex, BMI and 20 PC. Standardized residuals were utilized in all further association analysis using mach2qtl software package as implemented in GRIMP (32). Genetic data was filtered by MAF<0.01 and r2 lower than 0.3. Genome-wide significance was set at $P<5x10^{-8}$. Replication of top hits was pursued in the meta-analysis of the ALSPAC and NFBC1966 (n=12012) published earlier (17) for proxy dental maturity traits (NT and AFTE). Fisher's combined probability test weighted by sample size and implemented in METAL was used to meta-analyze the discovery and replication sets. Genetic correlation between dental development (using the largest meta-analysis of NT as a proxy) with other phenotypes (metabolites, anthropometric traits, blood lipids, hormones, glycemic traits) was estimated using LD score regression as implemented in the LD Hub database (33).

Mendelian Randomization Analysis (The Generation R study)

We applied a two-step Mendelian Randomization approach in which we used allelic scores for BMI and lipids as instrumental variables to assess a potential causal effect of body weight-related measurements (BMI, lean mass fraction, and fat percent) and serum lipids on dental development, using a two-stage least squares method. There was a high correlation (r2>0.7) between BMI, lean mass fraction (LMF) and fat percent (Fat%) in the Generation R study. Therefore, we applied Mendelian Random-

ization to all three body weight measurements pursuing better refinement of the phenotype. A similar principle was also applied for lipid measurements.

Allelic score for BMI

The BMI allelic score was created using 15 SNPs known to be associated with BMI in children identified in a previous GWAS (34) by summing the number of BMI-increasing risk alleles from the dosage data. Allelic scores were weighted using effect estimates from the original GWAS, as described previously (35) and after excluding Generation R data

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Allelic score for serum lipids

Allelic score for total cholesterol, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL) and triglycerides were extracted from a previous GWAS, which identified 102 SNPs associated with at least one of the mentioned traits (36). Of the 102 SNPs, 6 were not available in our sample, and only 1 was replaced with a proxy (D'>0.99, R2>0.98). A total of 97 SNPs were used for the scores including rs12916 (*HMGCR*) and rs10401969 (*CILP2*) SNPs for total cholesterol, rs629301 (*SORT1*), rs4420638 (*APOE*), rs6511720 (*LDLR*) and rs1367117 (*APOB*) SNPs for LDL, rs1532085 (*LIPC*) and rs7241918 (*LIPG*) SNPs for HDL and rs964184 (*APOA1*), rs1260326 (*GCKR*) and rs12678919 (*LPL*) SNPs for triglycerides. Subsequently, allelic scores were created using the same procedure described for a BMI-allelic score, by summing the number of lipid-increasing risk alleles from the GWA dosage data, weighted using effect estimates from the original GWAS (35).

Statistical Analysis

In the first stage, we examined the robustness of the genetic instrument variables (e.g. BMI-allelic score) and the exposure variable (e.g. BMI) in the Generation R study. In the second stage, we examined the significance level and effect size of the observed and casual relationship between the exposure variable (e.g. BMI) and the outcome variable (e.g. DD). The casual relationship was analyzed using Two-Stage Least Squares regression analysis. Sensitivity analyses for the Mendelian randomization approach were performed with both allelic scores, using different GWAS sources and weighting methods, and by using single variants. All regression analyses were adjusted for covariates selected according to biological plausibility, change in effect size for the variable of interest or the residual variability of the model. The analysis was performed using SPSS V.21.0 (IBM Corp, Armonk, New York, USA) and R

Table 1. Descriptive characteristics of the study sample

Child's characteristics at 6 years	**n**	**Value**
Age (years±SD)	2606	6.1±0.4
Girls (%)	1392	50
Serum lipids levels (mmol/l±SD)		
Total Cholesterol	1837	4.2±0.6
High-density lipoproteins	1839	1.3±0.3
Low-density lipoproteins	1838	2.4±0.6
Triglycerides	1833	1.1±0.5
Child's characteristics at 9 years		
Age (years±SD)	2793	9.8±0.3
Height (cm±SD)	2793	141.8±6.7
Body mass index (kg/m2±SD)	2793	17.5±2.7
Dental development stage by tooth [median, (IQR)])	2793	
Central incisor		8 (8-8)
Lateral incisor		8 (8-8)
Canine		6 (6-7)
First premolar		6 (6-7)
Second premolar		6 (5-6)
First molar		8 (8-8)
Second molar		6 (5-6)

Abbreviations: n- number of subjects in the non-imputed dataset, SD- standard deviation, IQR- interquartile range

statistical software V.3.3.2 (http://www.r-project.org). Results were considered statistically significant if the p-value was less than 0.05.

RESULTS

Baseline Characteristics

The characteristics of the discovery sample, the Generation R study are presented in Table 1. At the age of 6.1±0.4 years, serum lipids measurements were available for 1833-1837 children. The mean serum total cholesterol was 4.2±0.6 mmol/l. At 9.8±0.3 years, growth measurements were available in 2793 children. The mean value of child's BMI and height was 17.5±2.7 kg/m2 and 141.8±6.7 cm, respectively. The median stage of development for mandibular canines, first premolars, second

Table 2. Replicated loci suggestively associated with DD in the discovery cohort; SNP- single nucleotide polymorphism, CHR-chromosome, EA-effect allele, EAF- frequency of the effect allele, N-sample size, B- beta, SE- standard error

							Generation R, N=2793			ALSPAC+NFBC1966, N=12012			Meta, N=14805	
SNP	**CHR**	**BP**	**GENE**	**EA**	**OA**	**EAF**	**B**	**SE**	**P-value**	**B**	**SE**	**P-value**	**Z**	**P-value**
rs12444195	16	55044333	*IRX5*	A	C	0,57	-0,14	0,028	$2.8x10^{-7}$	-0,1	0,02	$2.7x10^{-5}$	-5,9	$3.4x10^{-9}$
rs2955382	17	17947710	*SREBF1*	T	C	0,5	-0,14	0,03	$2.8x10^{-7}$	-0,06	0,02	0,008	-4,5	$5.4x10^{-6}$
rs73206256	3	129512168	*TMCC1*	T	C	0,92	0,3	0,06	$1.5x10^{-7}$	NA	NA	NA	NA	NA

Table 3. Novel loci associated with DD obtained from combined meta-analysis; SNP- single nucleotide polymorphism, CHR-chromosome, EA-effect allele, EAF- frequency of the effect allele, N-sample size, B- beta, SE- standard error

						Generation R, N=2793			ALSPAC+NFBC1966, N=12012			Meta, N=14805	
SNP	**CHR**	**BP**	**GENE**	**EA**	**EAF**	**B**	**SE**	**P-value**	**B**	**SE**	**P-value**	**Z**	**P-value**
rs12444195	16	55044333	*IRX5*	A	0,57	-0,148	0,028	$1.1x10^{-7}$	-0,104	0,02	$2.75x10^{-5}$	-5,98	$3.4x10^{-9}$
rs6967145	7	23517468	*IGF2BP3*	C	0,4	-0,07	0,02	0,007	-0,12	0,02	$5.8x10^{-7}$	-5,5	$2.87x10^{-8}$
rs7156439	14	37100738	*PAX9*	T	0,62	-0,11	0,03	$6.9\ x10^{-5}$	-0,11	0,02	$4.6x10^{-6}$	-5,8	$6.6x10^{-9}$

premolars, and second molars was six (out of eight), and for the rest of the teeth, it was eight.

Discovery GWAS, Meta-analysis, and Genetic Correlation

A total number of 30,072,738 SNPs were tested for association with DD in the discovery setting (n=2,793), while 2,216,656 SNPs common to all three studies were included in the meta-analyses (n=14,805). The QQ plots indicate no early inflation of the test statistics due to genotyping biases, stratification or cryptic family relatedness in either the Generation R discovery setting (λ=0.98) or the meta-analysis (λ=1.007). The GWAS of DD in the discovery cohort identified variants in the 16q12.2 (*IRX5*; rs12444195-C, P=2.8x10^{-7}), 17p11.2 (*SREBF1*; rs2955382-C, P=2.8x10^{-7}) and 3q22.1 (*TMCC1*; rs73206256-T, P=1.5x10^{-7}) loci suggestively associated with advanced DD (Table 2, Figure 1). Two of the markers suggestively associated with DD in these regions showed significant evidence for replication in the previous NT meta-analysis of the proxy DD traits (*IRX5*: P=2.7x10-5, *SREBF1*: P=0.008), while the third marker (rs73206256 mapping to 3q22.1) or close proxies (R2>0.80) were not present in the replication set (Table 2). Further, replication was also observed in the AFTE meta-analysis, with consistent effect direction of the same alleles being associated with earlier teeth eruption (*IRX5*:P=1.5x10^{-5}, *SREBF1*:P=0.01). The combined meta-analysis of radiographic and NT dental development traits for the top-associated markers in the two loci yielded a P=2.1x10^{-9} in the *IRX5* and P=7.4x10^{-7} in the *SREBF1* regions. After genome-wide meta-analysis we identified variants in three additional novel loci associated with dental development in children: 16q12.2 (*IRX5*; P=3.4x10^{-9}), 7p15.3 (*IGF2BP3*; P=2.7x10^{-8}) and 14q13.3 (*PAX9*; P=6.6x10-^{9}); (Table 3, Figure 2). Further, we replicated at GWS level associations previously reported in seven loci: 2q35 (intergenic), 10q22.2 (*ADK*), 12q14.3 (*HMGA2*), 14q11.2 (C14orf93), 17q21.32 (*IGF2BP1*), 17q22 (*TEX14*) and 17q24.3 (*KCNJ2*). Five of them were nominally associated with DD in the discovery set (2q35, rs12621884, P=0.02; 10q22.2, rs7924176, P=0.001; 12q14.3, rs12422370, P=0.01; 17q21.32, rs1994969, P=0.05; 17q22, rs2257205, P=0.04), while the remaining two were not (14q11.2, rs997154, P= 0.13; 17q24.3, rs8079702, P=0.10). The analysis in LD-hub showed heritability of

Table 4. Significant genetic correlations of *NT15M*; table shows traits for which we obtained significant genetic correlations with *NT15M*; rG-genetic correlation, SE_rG-standard error of genetic correlation, P_rG-p value of genetic correlation

Traits	rG	SE_rG	P_rG
HDL cholesterol	0,16	0,07	0,03
LDL cholesterol	0,17	0,07	0,01
Apolipoprotein A	0,4	0,19	0,05
Isoleucine	-0,3	0,15	0,05
Total cholesterol	0,14	0,06	0,02

Table 5. Observational and causal analysis of growth- and lifestyle determinants with dental development trait using instrumental variable analysis

				Trait:	Dental development			
Exposure	**Instrument**	**n**	**p1**	**Method**	**β**	**95% CI**		**p2**
BMI (kg/m2)[a]	15SNP(34)	2793	<0.001	OBS	0.05	0.04	0.06	<0.001
				TSLS	0.06	-0.09	0.22	0.43
Lean mass fraction (SDS)[b]		2777	0,8	OBS	-0.07	-0.12	-0.01	0.01
				TSLS	-0.97	-14.42	12.48	0.89
Fat % (SDS)[c]		2777	<0.001	OBS	0.13	0.09	0.17	<0.001
				TSLS	0.14	-0.45	0.72	0.65
Serum lipids (mmol\l)								
Cholesterol[a]	97SNP(36)	1837	<0.001	OBS	-0.02	-0.08	0.05	0.58
				TSLS	0.13	-0.32	0.57	0.68
HDL[a]		1839	0,031	OBS	-0.02	-0.16	0.11	0.71
				TSLS	-0.49	-3.11	2.13	0.71
LDL[a]		1838	<0.001	OBS	-0.01	-0.08	0.06	0.83
				TSLS	0.11	-0.41	0.63	0.67
Triglycerides[a]		1833	<0.001	OBS	-0.03	-0.11	0.05	0.49
				TSLS	0.1	-0.35	0.55	0.67

a) Models were adjusted for sex, age(s) of measurement, height, and principal components.
b) Models were adjusted for sex, age(s) of measurement, height, weight, and principal components.
c) Models were adjusted for sex, age(s) of measurement, height, lean mass, and principal components. Abbreviations:p1- First stage regression results p-value; p2 – Observational (OBS) and Two-Stage-Least-Squares (TSLS) regression analysis p-value; β- effect size; 95% CI-95 percent confidence interval; p(AR)- Anderson-Rubin test p-value; BMI- Body Mass Index, HDL- High-density lipoprotein, LDL- Low-density lipoprotein, SDS- Standard deviation score, SNP- Single-nucleotide polymorphism

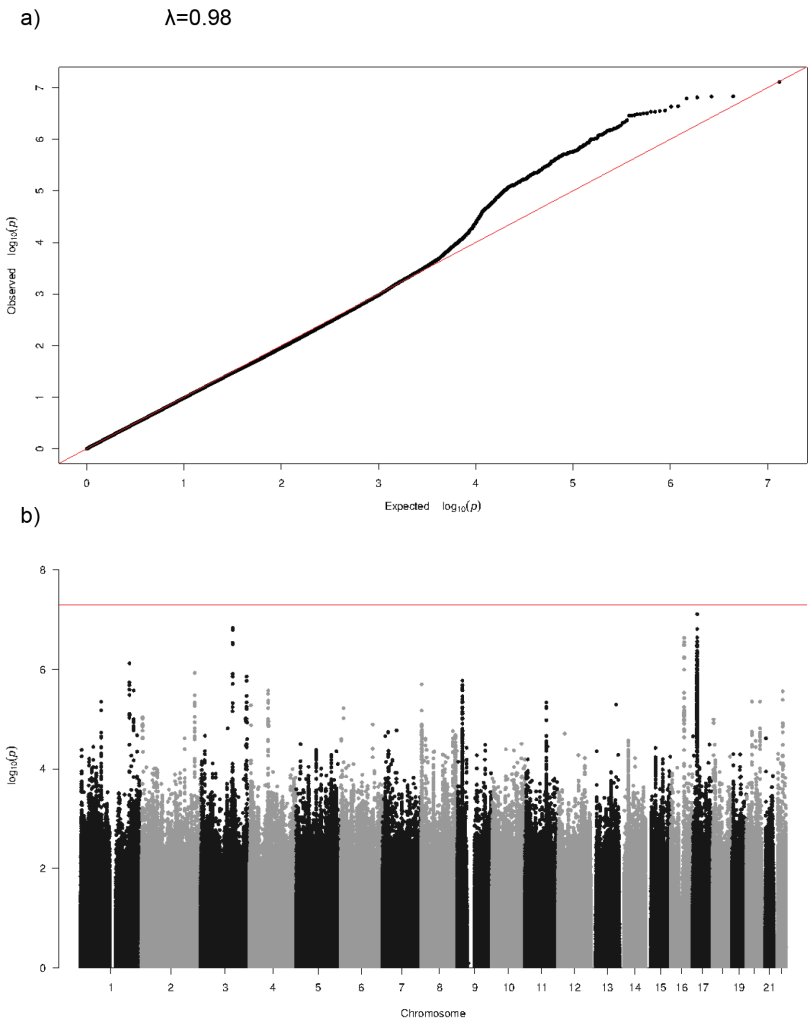

Figure 1. a) Q-Q plot obtained from GWAS of DD in The Generation R Study; plot shows the distribution of expected against observed p-values, diagonal red line represents the null distribution, each dot represents one SNP b) Manhattan plot of association statistics obtained from GWAS of DD in The Generation R Study; each dot represents one SNP, x-axis is its chromosomal position- build 37 NCBI, on the y-axis the –log10(p-value) is reported; horizontal red and blue lines mark the GWS threshold ($P<5x10^{-8}$).

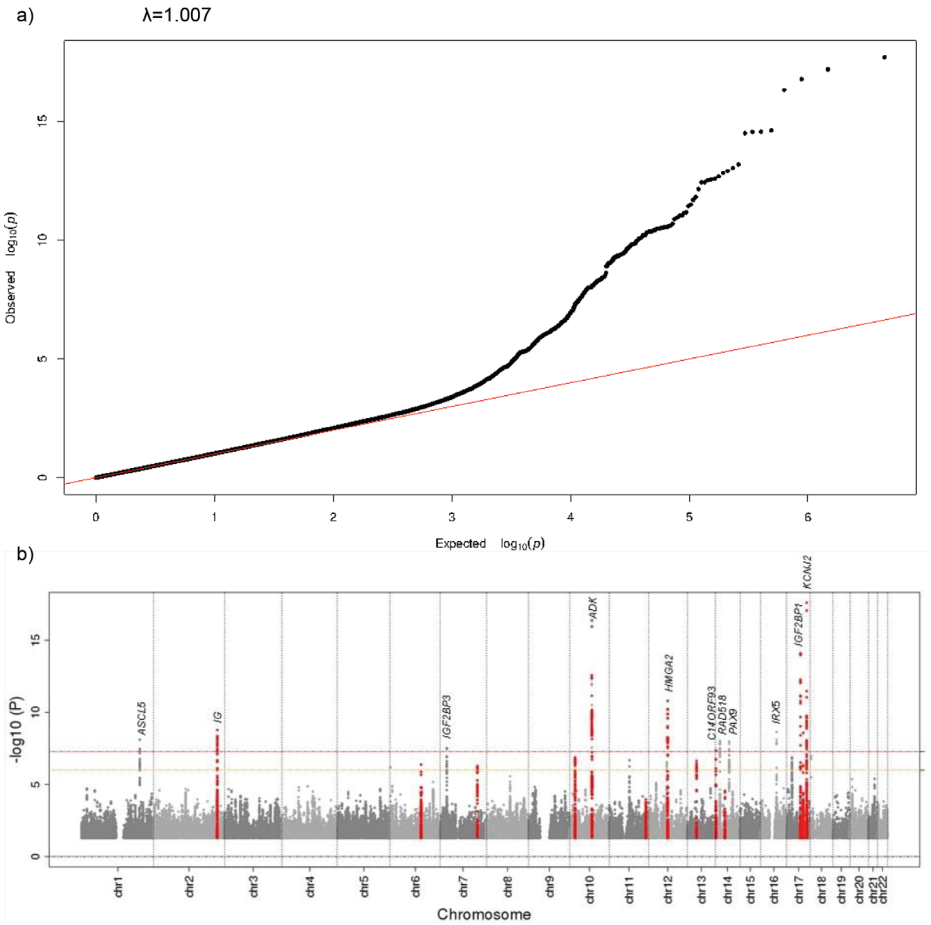

Figure 2. a) Q-Q plot obtained from the combined meta-analysis; plot shows the distribution of expected against observed p-values, diagonal red line represents the null distribution, each dot represents one SNP b)Manhattan plot of association statistics for DD in combined meta-analysis; each dot represents one SNP, x-axis is its chromosomal position- build 37 NCBI, on the y-axis the –log10(p-value) is reported; horizontal red and yellow lines mark the GWS threshold ($P<5x10^{-8}$) and suggestive threshold ($P<1x10^{-6}$),respectively; SNPs which were GWS in previously published meta-analysis investigating NT are colored in red

24.8% and a weak, borderline significant genetic correlation between dental development (using NT as a proxy) and diverse lipid and metabolic traits.

Correlations observed with glycemic and/or anthropometric traits were not significant. Table 4 shows results obtained from LD Hub for the traits which showed significant genetic correlations with NT.

Enrichment and gene prioritization analyses

The most significant 10 gene-sets prioritized with DEPICT for DD are shown in Supplementary Table 1. Among them, three were related to skeletal growth and development including: abnormal craniofacial development, positive regulation of bone mineralization and regulation of developmental growth. As top 10 genes (*MSRB3, HOXB13, RUNX1, HOXB2, HOXB3, HOXB5, HOXB6, RAI1, HMGA2, HOXB13-AS1*), DEPICT prioritized mostly genes from a homeobox gene family known to be involved in embryonic developmental processes. SNPs which showed suggestive associations were connected to genes expressed in different tissues (Supplementary Table 2), none of which included calcified or mineralized tissues (bone or teeth).

Mendelian Randomization

The results of the Mendelian Randomization approach are presented in Table 5. We identified a significant observational association in the Generation R sample between BMI (kg/m2) [β= 0.05, 95% confidence interval (CI)=0.04, 0.06)], LMF (β= -0.07, 95% CI=-0.12, -0.01) and Fat% (β= 0.13, 95% CI=0.09, 0.17). The results of the two-stage least square regression showed no evidence for significant causal effects of BMI, LMF and Fat % on radiographic dental development. Furthermore, we did not identify significant observational associations in the Generation R sample between serum lipids and radiographic dental development, as expected from the significant genetic correlation observed between these variables in the LD score-regression analyses.

DISCUSSION

We report 12 loci associated with DD, including 3 novel ones. The GWAS signals of the novel loci map are within or in the vicinity the following genes: *IRX5, IGF2BP3*, and *PAX9*. Despite the strong epidemiological association between DD and BMI, and the fact that one of the GWS variants for DD maps to *IRX5* (postulated to be a BMI regulatory region), we did not find evidence for a causal association with BMI. Further, despite significant genetic correlation between DD and lipids, we did not find evidence for a causal association after applying a Mendelian randomization approach. These

discoveries provide new insights into the genetic regulation and underlying biology of dental development in children.

IRX5 is a member of the Iroquois homeobox gene family, which is involved the embryonic development of the craniofacial complex and is described as an obesity-associated region through interaction with regulatory elements in the *FTO* locus (37–39). *IRX5* is modulated by direct protein-protein interaction with two GATA zinc-finger proteins, *GATA3* and *TRPS* (37), and it regulates craniofacial development by modulating the migration of progenitor cell populations in the branchial arch (37). In animal models, *IRX5* has been described to regulate retinal cone bipolar cell differentiation, cardiac ventricular repolarization gradient, proximal, anterior limb skeleton formation and lung morphogenesis (40–45). Together with *FOXL2, IRX5* is reported to promote upper jaw development in mice (46). As our findings show that *IRX5* is associated with dental development, it is yet to be determined if this is a pleiotropic effect on both jaw and tooth development, or if it is rather an effect on DD, stimulating jaws to grow as a mechanical reaction to the forces generated by the developing teeth inside (47).

Insulin-like growth factor 2 mRNA-binding protein 3 (*IGF2BP3*) gene encodes a protein that is primarily found in the nucleolus, and it regulates translation of insulin-like growth factor II during late embryonic development in mice and humans (48). Lack of *IGF2* has been associated cleft palate in mouse models (49,50), while the increase is associated with Beckwith–Wiedemann syndrome characterized by abnormal growth of different skull parts, including jaws and tongue (51). Also, an effect of *IGF2BP3* on growth and development is hinted by the fact that overexpression is involved in cell proliferation, migration, and invasion in several kinds of tumors (52–54). Altogether, our findings suggest that *IGF2BP3* plays a role in the dental development of humans.

Pair box 9 (*PAX9*) is a member of paired box family of transcription factors, and it plays an important role in fetal development and cancer growth (55). *PAX9* is a known regulator of odontoblast differentiation (56). Hence, it has been previously implicated in several dental conditions, such as disturbances in the number (including hypodontia (57,58) and oligodontia (59)) and size of teeth (60). Furthermore, *PAX9* is reported to be a risk factor for non-syndromic cleft lip with or without cleft palate (61). Interestingly, although this gene has frequently been associated with dentofacial disturbances, this is the first study which suggests a regulatory role of *PAX9* on the rate of dental development.

Variants in two other loci with suggestive evidence for association in the previous meta-analysis of NT (17), reached GWS in the combined meta-analysis with radiographic dental development. Yet, variants in these loci reached GWS in other GWAS

of primary dentition and mapped to the *ASCL5* (19) and *RAD51B* (paralog *RAD51L1*) (18) regions. Achaete-scute family bHLH transcription factor 5 (*ASCL5*) mapping to 1q32.1 encodes a transcription factor of very limited known function, which has also been associated with permanent teeth eruption (19). In contrast, *RAD51B*, mapping to 14q24.1, encodes a member of the *RAD51* protein family exhibiting central recombinase activity in mammalian cells (62,63), with variants previously shown to be associated with rheumatoid arthritis (64,65). Dental development (DD) is a complex trait with genetic, hormonal and environmental influences likely also including their interactions. The genetic component is confirmed by our study implicating numerous loci associated with DD and an heritability estimate of 24.8%. Several GWAS signals map to genes with high biologic plausibility to be underlying the associations but also to other genes of which little is known about their role in dental or craniofacial development, which may reflect new biology. To better understand potential biological mechanisms underlying DD, we performed DEPICT enrichment and gene prioritization analysis including GWAS variants of the overall DD meta-analysis associated at $P=1x10^{-5}$ significant level. DEPICT identified all gene-sets at 20% FDR, possibly reflecting lack of power. Top gene-sets nominally associated with DD ($9.80x10\text{-}7< P <1.41x10^{-4}$), implicated roles in development, growth, and mineralization. However, the tissue enrichment analyses typified several organs and tissues which do not seem to be relevant to DD, such as the gastrointestinal tract, lung, and hematopoietic system. In addition, we used the number of teeth (NT) dental development meta-analysis to evaluate the genetic correlation with other traits. While several epidemiological studies have implicated BMI in dental development (including our own findings in Generation R), and the fact that *IRX5* has been described as a long-range *FTO*-interacting region (66), we did not find a significant genetic correlation between them. This was corroborated by the MR analysis showing that BMI and the other body composition measurements (lean and fat mass fractions) did not have significant evidence supporting a causal effect on DD. In contrast, we did identify weak evidence for a weak genetic correlation between serum lipids and dental development, but the MR findings indicate that serum lipids are not (casually) associated with DD. Another endocrine mechanism, such as secretion of growth hormone, insulin, thyroid hormones, glucocorticoids, prolactin, and gonadal steroids, are key factors regulating growth and metabolism of the human body (67), including DD and the timing of tooth eruption (8,68). Interestingly, we identified a signal implicating *IGF2BP3* potentially characterizing the role of growth factors in dental development. Nevertheless, as with other complex traits, environmental factors such as general living conditions, nutrition, health status, and stressors have also been shown to

influence growth and development processes (69), which are likely also to mediate human dental development.

One of the limitations of this study is that we employed different methods to assess dental development across the three participating cohorts. Although panoramic radiographs employ a very small amount of radiation (70) and are the most accurate method to determine dental development, to the best of our knowledge, there are no other pediatric cohorts apart from Generation R with these assessments. However, the fact that most of the top hits from both discovery and replication set were at least nominally associated with consistent effect direction indicated they can be used as adequate proxies for each other and to assess DD in general. Similarly, we did not identify evidence for heterogeneity of effects arising from the different phenotype definitions, sex distribution and/or (multi)ethnic background. Still, to fulfill the stringent requirements of the LD score regression as an implement in LD-hub we opted to use the NT trait for estimating genetic correlations. Despite the differences in phenotype definition, the successful use of these proxy definitions suggests that larger and more well-powered GWAS meta-analysis will continue yielding new loci influencing the dental development and which can provide better insight into the genes and pathways currently not prioritized by the DEPICT analysis.

In summary, we have pursued the first meta-analysis on radiographic DD and identified three novel loci affecting dental maturation. Despite epidemiological and genetic correlations potentially implicating BMI and lipid metabolism in the process of dental development, we did not observe any evidence to support (causal) relations between these traits. Altogether our study implicates genes from pathways related to growth and development influencing overall and craniofacial processes in humans, which provide further understanding into the process of dental maturation in children.

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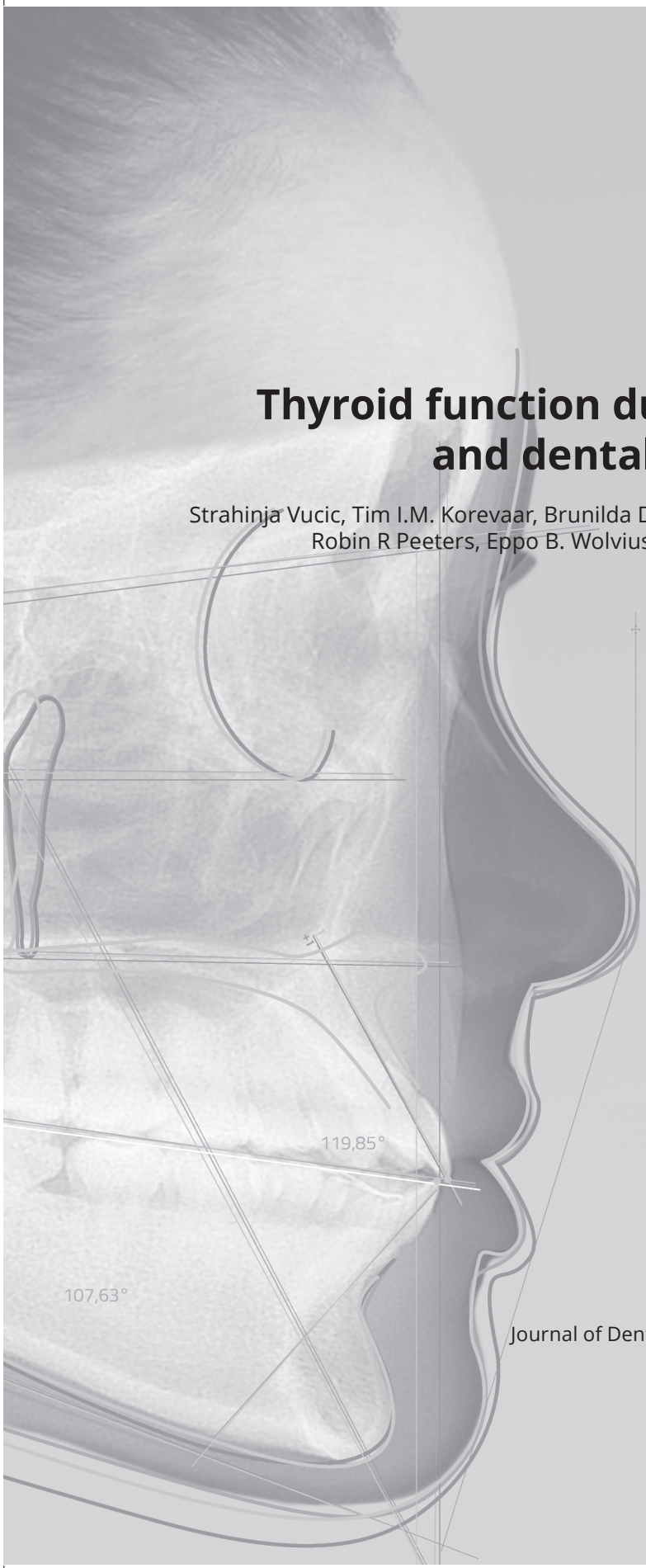
Supplementary Table 1. Gene prioritization

Locus	Nr of Gs in L	Chromosome and position	GWAS P	Ensembl gene ID	Gene	Nominal P	Gene closest to lead SNP	Top cis eQTL SNP	False D
rs12229918	2	chr12:65672423-66036152	8,90E-10	ENSG00000174099	*MSRB3*	9,52E-5	0	rs2270547	<0.20
rs1994969;rs9894411	23	chr17:46210802-47133012	7,62E-15	ENSG00000159184	*HOXB13*	1,71E-4	0	-	<0.20
rs9976876	1	chr21:36160098-37357047	3,98E-6	ENSG00000159216	*RUNX1*	2,55E-4	1	-	<0.20
rs6704363	2	chr1:201008642-201084796	7,52E-9	ENSG00000232237	-	3,45E-4	1	-	<0.20
rs1994969;rs9894411	23	chr17:46210802-47133012	7,62E-15	ENSG00000173917	*HOXB2*	4,38E-4	0	rs1042815	<0.20
rs1994969;rs9894411	23	chr17:46210802-47133012	7,62E-15	ENSG00000120093	*HOXB3*	5,90E-4	0	rs2303487	<0.20
rs1994969;rs9894411	23	chr17:46210802-47133012	7,62E-15	ENSG00000230148	-	7,53E-4	0	-	<0.20
rs1994969;rs9894411	23	chr17:46210802-47133012	7,62E-15	ENSG00000120075	*HOXB5*	1,08E-3	0	rs17634167	<0.20
rs1994969;rs9894411	23	chr17:46210802-47133012	7,62E-15	ENSG00000233101	-	1,11E-3	0	-	<0.20
rs1994969;rs9894411	23	chr17:46210802-47133012	7,62E-15	ENSG00000108511	*HOXB6*	1,18E-3	0	rs1343832	<0.20
rs9891957	8	chr17:17584787-18083116	1,30E-7	ENSG00000108557	*RAI1*	1,41E-3	0	rs11654526; rs12449524	<0.20

Name	MeSH first level term	MeSH second level term	Nominal	FalseD	gene 1	gene 2	gene 3	gene 4
Epithelial Cells	Cells	Epithelial Cells	2.41e-03	>=0.20	LRRC48 (2.2)	RP11-863H1.1 (1.6)	TTLL6 (1.4)	PAX9 (1.1)
Parotid Gland	Digestive System	Gastrointestinal Tract	7.69e-03	>=0.20	PAX9 (4.1)	ASCL5 (2.8)	IRX5 (2.7)	HOXB-AS3 (2.2)
Synovial Membrane	Musculoskeletal System	Skeleton	7.86e-03	>=0.20	MIRLET7I (2.3)	BMP2 (1.9)	C12orf61 (1.8)	STXBP5 (1.7)
Joint Capsule	Musculoskeletal System	Skeleton	7.86e-03	>=0.20	MIRLET7I (2.3)	BMP2 (1.9)	C12orf61 (1.8)	STXBP5 (1.7)
Joints	Musculoskeletal System	Skeleton	7.86e-03	>=0.20	MIRLET7I (2.3)	BMP2 (1.9)	C12orf61 (1.8)	STXBP5 (1.7)
Salivary Glands	Digestive System	Gastrointestinal Tract	9.63e-03	>=0.20	PAX9 (4.2)	IRX5 (2.6)	ASCL5 (2.6)	ITPR2 (2.4)
Lung	Respiratory System	Lung	0.01	>=0.20	FOXF2 (1.4)	STK31 (1.2)	HOXB6 (1.1)	HOXB5 (1.0)
Islets of Langerhans	Digestive System	Pancreas	0.03	>=0.20	HMGA2 (2.0)	JUB (2.0)	HOXB6 (1.9)	ADK (1.9)
Esophagus	Digestive System	Gastrointestinal Tract	0.03	>=0.20	STK31 (3.1)	PAX9 (2.7)	FOXF2 (2.4)	HOXB3 (1.5)
Blood Vessels	Cardiovascular System	Blood Vessels	0.03	>=0.20	BMP2 (2.0)	HOXB8 (1.7)	CALU (1.6)	HMGA2 (1.6)
Serum	Hemic and Immune Systems	Blood	0.03	>=0.20	VCL (2.9)	HMGA2 (2.9)	CALU (1.9)	MSRB3 (1.8)



Chapter 4

Thyroid function during early life and dental development

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ABSTRACT

Children with low levels of thyroid hormones (hypothyroidism) have delayed tooth eruption, enamel hypoplasia, micrognathia, and anterior open bite, whereas children with hyperthyroidism may suffer from accelerated tooth eruption, maxillary, and mandibular osteoporosis. However, it is still unknown whether thyroid function variations within the normal or subclinical range also have an impact on hard dental tissues in healthy children. The objective of this study was, therefore, to investigate the association between thyroid function from the fetal period until early childhood and dental development at school age. This study is embedded in the Generation R Study, a population-based cohort study established in Rotterdam, the Netherlands. Maternal thyroid function (thyroid-stimulating hormone [TSH], free thyroxine [FT4], and thyroid peroxidase antibody [TPOAb] concentrations) was measured during early pregnancy, and thyroid function of the offspring (TSH and FT4) was measured in cord blood at birth and in early childhood (6 y). Dental development was assessed from panoramic radiographs of children of school-going age (9 y). In total, 2,387 to 2,706 subjects were available for the multivariable linear regression analysis, depending on the point in time of thyroid function measurement. There was an inverse association between cord blood and early childhood TSH concentrations with dental development, with a –0.06 lower standard deviation (SD) per 1 mU/L of TSH (95% confidence interval [CI], –0.11 to –0.01) and a –0.06 lower SD per 1 mU/L of TSH (95% CI, –0.11 to 0.00), respectively. There was no association between the maternal thyroid function during pregnancy and the dental development score of the child. However, TPOAb-positive mothers had children with a –0.20 SD (adjusted 95% CI, –0.35 to –0.04) lower dental development score compared with TPOAb-negative mothers. The findings of this study suggest that the thyroid hormone is involved in the maturation of teeth from the early stages of life onward.

INTRODUCTION

Tooth formation is an important process of the human digestive system development. Disturbances in dental development can roughly be divided into 2 types of abnormalities. First, structural, morphological, and positional abnormalities can occur, which often require dental treatment (Crawford and Aldred 2012). Second, a disturbance in the timing of tooth formation may occur, involving delayed tooth eruption and emergence. Such disorders may point to an underlying systemic disorder and influence the timing of orthodontic treatment (Suri et al. 2004).

Tooth formation is a complex process that begins with the development of primary teeth in the eighth week of prenatal development, and it completes postnatally around the age of 18 to 25 y with the formation of third molar roots (Nanci 2014). Teeth go through several growth stages, namely, the initiation, morphogenesis, cytodifferentiation, and matrix secretion (Scheller et al. 2009). Development can be postnatally followed using several methods, of which the most widely used are based on the calcification stages of crown and root as seen on panoramic radiographs (Panchbhai 2011). Tooth formation is regulated by genetic, systemic, and local factors (Nanci 2014). Previous studies showed that the thyroid hormone is an essential element in the regulation of metabolic processes of orofacial mineralized tissues, including teeth (Bochukova et al. 2012; Bassett et al. 2014). Thyroid disorders during childhood were reported to affect the timing of tooth eruption, the mineral content of tooth enamel, maxillary and mandibular bones, the morphology of the tongue, and the susceptibility to periodontal diseases (Ikeda et al. 2008, 2009; Chandna and Bathla 2011; Nanci 2014).

Thyroid hormone availability in serum is regulated through the hypothalamic-pituitary-thyroid axis. Following this pathway, the pituitary gland is stimulated by a thyrotropin-releasing hormone from the hypothalamus to secrete a thyroid-stimulating hormone (TSH) that subsequently stimulates the production of thyroxine (T4) by the thyroid gland (Scanlon and Hall 1989). Subsequently, the thyroid hormone regulates the growth and metabolic functions of various organs and tissue systems, including mineralized tissues, such as the formation of the skeleton and maintenance of bone mass (Pinto and Glick 2002; Bassett and Williams 2003). In addition, thyroid autoimmunity assessed through thyroid peroxidase antibody (TPOAb) is a major risk factor for low thyroid function, especially during pregnancy (Medici et al. 2012; Korevaar et al. 2017). As the fetal thyroid is not functionally mature until 18 to 20 weeks of gestational age, maternal thyroid dysfunction may affect the early

stage of tooth development since the fetus is dependent on the placental transfer of maternal thyroid hormones (Greenberg et al. 1970; Thorpe-Beeston et al. 1991).

Although the effects of thyroid hormones on tooth development are most noteworthy in children with overt thyroid disorders, it is still unknown whether thyroid function variations within the normal or subclinical range have an impact on hard dental tissues in healthy children. To our knowledge, no studies have investigated the association between thyroid function and progress of dental development in the general population.

Therefore, the objective of this study was to investigate the association between thyroid function (TSH, free thyroxine [FT4], and TPOAb concentrations) from the fetal period until early childhood and dental development at school age.

MATERIALS AND METHODS

Design

The Generation R Study is a population-based cohort study ranging from fetal life until adulthood, established in Rotterdam, the Netherlands, at Erasmus University Medical Center. In summary, the Generation R Study was designed to identify early environmental and genetic determinants of growth, development, and health and has been described previously in detail (Kruithof et al. 2014). The study was approved by the Medical Ethics Committee of the Erasmus Medical Centre (MEC-2012-165) in Rotterdam, the Netherlands. At the start of each phase, mothers and their partners were asked for their written, informed consent. This study conformed to Strengthening the Reporting of Observational Studies in Epidemiology guidelines for human observational studies.

Study Population

All mothers who resided in the study area and had a delivery date between April 2002 and January 2006 were eligible (Figure 1). In total, 9,778 mothers were enrolled. After applying general- and thyroid-specific exclusion criteria, 2,867 mothers had a blood sample taken during pregnancy. Furthermore, 2,418 mothers had a blood sample taken from the umbilical cord for thyroid function assessment whose school-aged children (mean age of 9 y) had also undergone a dental development assessment. Also, thyroid function was assessed in children during early childhood (mean age of 6 y; n = 4,212). Of those, children with cancer/chronic disease (n = 12) and thyroid-interfering medication (n = 4) were excluded. The remaining 2,630 children also had dental development assessed at school-going age. The characteristics

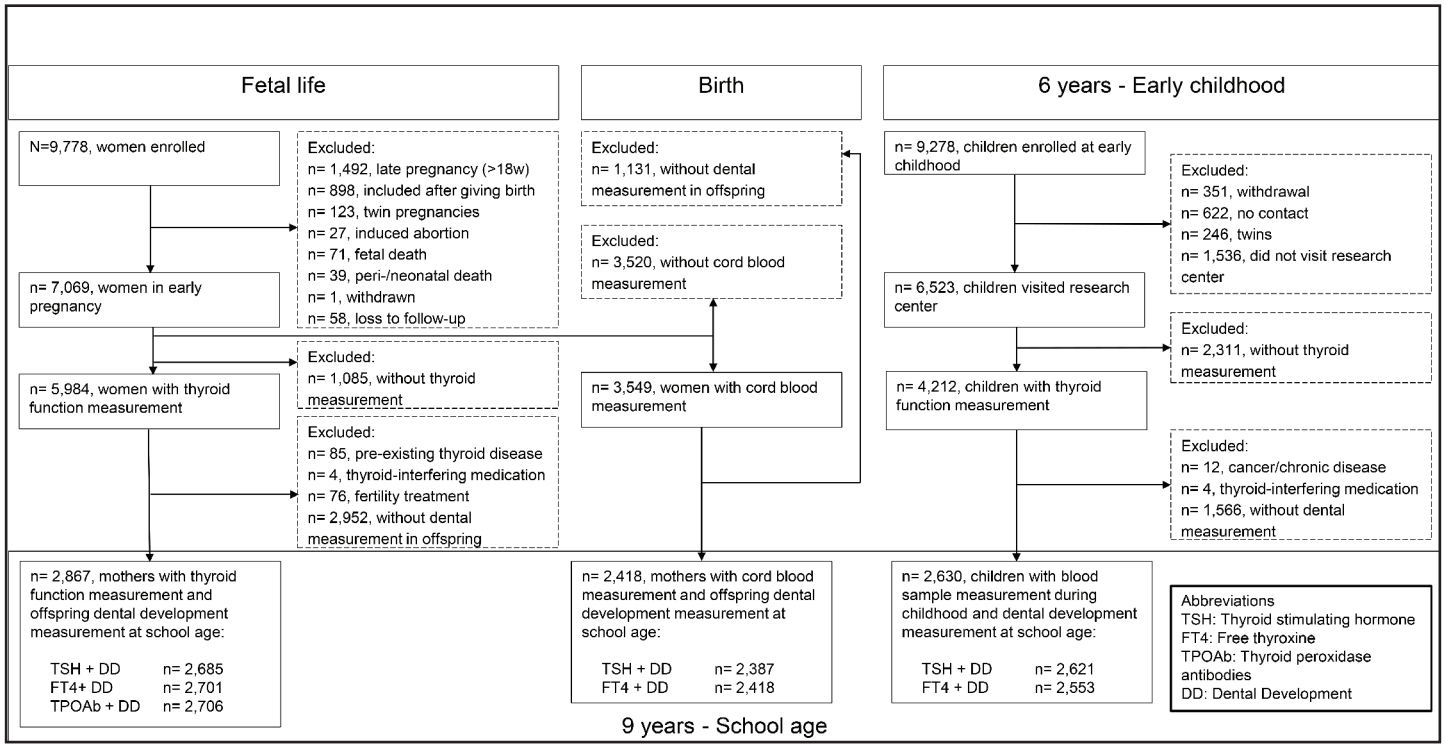

Figure 1. Flow chart of participants.

of the study population and the final number of participants included in the analysis for each thyroid measurement are provided in Tables 1 and 2, respectively.

Thyroid Function Assessment

During the visit to the research center, venipuncture was performed and, subsequently, plain tubes were centrifuged and the serum stored at –80°C. TSH and FT4 concentrations were determined in maternal and cord blood serum samples using chemiluminescence assays (Vitros ECI; Ortho Clinical Diagnostics). The intra- and interassay coefficients of variation were <4.1% for TSH at a range of 3.97 to 22.7 mU/L and <5.4% for FT4 at a range of 14.3 to 25.0 pmol/L. Maternal TPOAbs were measured using the Phadia 250 immunoassay (Phadia AB) and regarded as positive when values were greater than 60 IU/mL (Medici et al. 2012). Childhood TSH and FT4 concentrations were determined using an electrochemiluminescence immunoassay on the Cobas e601immunoanayzer (Roche Diagnostics). The intra- and interassay coefficients of variation were 1.1% to 3.0% for TSH at a range of 0.4 to 0.04 mU/L and 1.6% to 5.0% for FT4 at a range of 1.6 to 24.1 pmol/L. TSH values were logarithmically transformed (log(TSH)) to reduce skewness of distribution and to better reflect biological activity.

Dental Development Assessment

Tooth development of children aged 9.8 ± 0.3 years was quantified on panoramic radiographs using the method described by Demirjian et al. (1973). Following this approach, 7 left mandibular teeth excluding third molars were scored with 1 of the 8 developmental stages (A–H), depending on the crown and root mineralization. The children's overall dental development was established by calculating the mean value of the standard deviation scores (SDS) for the 7 teeth. The overall dental development score was normalized using rank-based transformation due to nonnormal distribution. The interobserver agreement between 2 raters was calculated on a random subsample of 100 subjects for each of the 7 teeth using the intraclass correlation statistic, and coefficients ranged between 0.653 and 0.797, which is considered a substantial agreement according to the conventional criteria (Landis and Koch 1977). Mandibular first incisors were not taken into account for the intraclass correlation statistic due to the lack of variation in the stage of tooth development.

Covariates

Analyses were adjusted for maternal and/or child age, maternal and/or child body mass index (BMI), maternal ethnicity, smoking during pregnancy, and the child's sex. Also, we included thyroid function determinants such as gestational age at the time of blood sampling and gestational age at birth (time of cord blood measurement).

Table 1. Descriptive characteristics of the sample.

Maternal characteristics - Pregnancy	n	Value
Age (years±SD)	3,464	30.8±4.8
Body mass index (kg/m2±SD)	3,443	24.5±4.2
Education (%)		
Primary or lower	277	7
Secondary	1,516	41
Higher	1,908	52
Smoking (%)		
Nonsmokers	2,354	76
Stopped smoking	275	9
Smokers	475	15
Ethnicity (%)		
Dutch	2,247	58
Indonesian	139	4
Cape Verdean	167	4
Moroccan	199	5
Dutch Antilles	75	2
Surinamese	297	8
Turkish	257	7
Other European, North American and Australian	319	8
Other Asian, African and South American	204	5
Thyroid function		
Maternal blood concentrations		
Thyroid stimulating hormone [mU/L; median, (IQR)]	2,685	1.36 (0.87-2.03)
Free thyroxine (pmol/L±SD)	2,701	15.2±3.4
Thyroid peroxidase antibody positive (%)	151	6
Gestational age at sampling (week±SD)	2,809	13.4±1.9
Cord blood concentrations at birth		
Thyroid stimulating hormone [mU/L; median, (IQR)]	2,387	9.30 (6.41-14.30)
Free thyroxine (pmol/L±SD)	2,418	21.0±3.8
Gestational age at birth (week±SD)	3,464	40±1.7
Child characteristics		
Girls (%)	1.974	50
Thyroid function- Early childhood		
Thyroid stimulating hormone [mU/L; median, (IQR)]	2,621	2.31 (1.72- 3.15)
Free thyroxine (pmol/L±SD)	2,553	16.9±1.9
Age at blood sampling (years±SD)	2,630	6.1±0.4
Dental assessments - School age		
Dental development score (SDS±SD)	3,983	0±1
One or more agenetic tooth (%)	147	4
Age at dental assessment (years±SD)	3,983	9.8±0.3

Abbreviations: n- number of subjects in the non-imputed dataset, SD- standard deviation, IQR- interquartile range

We adjusted for hypodontia status as it was associated with delayed dental development (Tunç et al. 2011; Dhamo et al. 2016).

Statistical Analysis

A potential bias related to the loss to follow-up was examined by comparing mother and child characteristics of children with and without data on dental development (children without a dental panoramic radiograph at school age). We investigated the association between the maternal or child thyroid function and the continuous dental development score by performing multivariable linear regression analysis. Covariates were selected according to biological plausibility, the change in beta for the variable of interest, or the residual variability of the model. The Markov chain Monte Carlo imputation method was applied to avoid a possible bias associated with the missing values of covariates. Variables with missing values are provided in the Appendix Table. Mother, child, and cord blood, FT4, and log(TSH) concentrations were considered outliers and excluded if values were outside the range of –3 and +3 standard deviations (SD). We investigated effect modification by adding the following product terms to regression models: SEX × log(TSH), SEX × FT4, and log(TSH) × FT4. The analysis was performed with the statistical software SPSS version 21.0 (SPSS, Inc.) and R statistical package version 3.2.2 (http://www.r-project.org). Results were considered statistically significant if the P value was less than 0.05.

RESULTS

Nonresponse Analysis

Mothers of children without dental data were on average 2.05 y younger (95% confidence interval [CI], 2.26–1.85), were more likely to have a non-Dutch origin (59.4% vs. 40.6%, P < 0.001), were less educated (66.3% vs. 33.7% finished primary or lower education, P < 0.001), and were more likely to continue smoking during pregnancy (62.2% vs. 37.8%, P < 0.001). We also observed a 0.3-pmol/L (95% CI, 0.5–0.1) higher mean FT4 concentration in cord blood of children who had dental data available (P = 0.002), but there was no difference between maternal and child FT4 concentrations. No statistically significant differences were found when groups were compared based on data availability for maternal, cord blood, and child TSH concentrations and maternal TPOAb positivity (data not shown). On the other hand, children of mothers without blood measurements during pregnancy had slightly lower dental development scores (–0.10 SD; 95% CI, –0.17 to –0.3). Dental development of children with and without cord blood samples and early childhood blood samples did not differ significantly.

Table 2. Thyroid function from fetal life until early childhood and dental development of the offspring at school age.

			Dental development SDS at school age			
Time point	**Thyroid function**	**n**	**β**	**95% CI**		**P-value**
A) Fetal life	Maternal blood sample					
	TSH (mU/L)[1]	2,588	-0.03	-0.07	0.01	0.189
	FT4 (pmol/L)[1]	2,588	0.04	-0.01	0.09	0.122
	TPOAb – (reference)		0			
	TPOAb +[1]	151/2,555	-0.17	-0.31	-0.02	0.025
	adjusted TPOAb +[2]	139/2,354	-0.2	-0.35	-0.04	0.012
B) At birth	Cord blood sample					
	TSH (mU/L)[3]	2,334	-0.06	-0.11	-0.01	0.03
	FT4 (pmol/L)[3]	2,334	-0.01	-0.06	0.04	0.698
C) Early childhood	Child's blood sample					
	TSH (mU/L)[4]	2,526	-0.05	-0.1	0	0.039
	FT4 (pmol/L)[4]	2,526	0.04	-0.01	0.08	0.131

1. Model was adjusted for age at thyroid function measurement, age of dental development measurement, child covariates (sex, gestational age at birth and hypodontia), and maternal covariates (age, body mass index, education, smoking and ethnicity).
2. Model was adjusted for maternal TSH and FT4 in addition to variables from 1. Model
3. Model was adjusted for age at thyroid function measurement, age of dental development measurement, child covariates (sex and hypodontia), and maternal covariates (age, body mass index, education, smoking and ethnicity)
4. Model was adjusted for age at thyroid function measurement, age of dental development measurement, child covariates (sex, body mass index, and hypodontia), and maternal covariates (education and ethnicity).
Abbreviations: n - number of subjects included in the model, β - effect size, 95% CI - 95% confidence interval, TSH - thyroid stimulating hormone, FT4 - free thyroxine, TPOAb - Thyroid peroxidase antibody status, SDS - standard deviation score

Study Characteristics

Our study sample predominantly comprised average (41%) and highly educated (52%) mothers of Dutch origin (58%, Table 1). Median maternal TSH was 1.36 mU/L (interquartile range [IQR], 0.87–2.03), and mean maternal FT4 was 15.2 ±3.4 pmol/L during pregnancy. In total, 151 (4%) mothers were TPOAb positive. Median cord blood concentration of TSH measured at birth was 9.30 mU/L (IQR, 6.41–14.30), and mean cord blood FT4 was 21.0 ± 3.8 pmol/L. During early childhood, the median TSH concentration was 2.31 mU/L (IQR, 1.72–3.15), and the mean FT4 concentration was 16.9 ± 2.0 pmol/L. In total, 147 children had agenesis of 1 or more teeth.

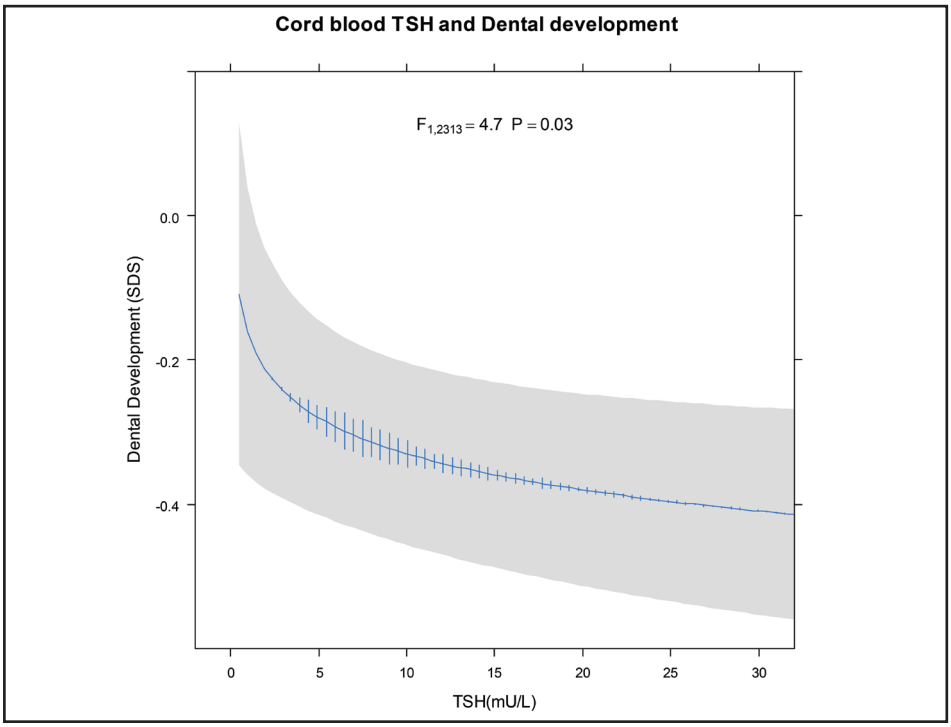

Figure 2. Association between thyroid-stimulating hormone (TSH) concentrations in cord blood at birth and dental development of a child at school age. Shaded area depicts 95% confidence interval, and short vertical lines on the curved regression line depict a data frame containing the original raw data on which the regression model was based. The model was adjusted for age at thyroid function measurement, age of dental development measurement, child covariates (sex and hypodontia), and maternal covariates (age, body mass index, education, smoking, and ethnicity).

Thyroid Function and Dental Development

There was no statistically significant association between maternal TSH or FT4 concentrations during pregnancy and the dental development of the offspring (Table 2A). We also examined the relationship of maternal TPOAb status during pregnancy and dental development score. Children of mothers who were TPOAb positive had a 0.17 SD (95% CI, −0.31 to −0.02) lower dental development score compared with children of TPOAb-negative mothers, even after adjusting for maternal TSH and FT4 concentrations (−0.20; 95% CI, −0.35 to −0.04).

The cord blood TSH concentration was inversely associated with dental development: −0.06 SD change per 1 mU/L (95% CI, −0.11 to −0.01). We plotted a regression curve with 95% CI to examine the relationship between cord blood TSH concentra-

tions and the dental development score (Table 2B). In this model, the increase of TSH from 3 to 30 mU/L (v 2) was associated with a reduction in dental development score from –0.22 SD (95% CI, –0.37 to –0.08) to –0.39 SD (95% CI, –0.53 to –0.25).

The results of the association between thyroid function during early childhood and the dental development score during school age are shown in Table 2C. TSH concentrations were negatively associated with the dental development score, –0.05 SD change per 1 mU/L of TSH (95% CI, –0.10 to 0.00), similar to the TSH concentrations measured in cord blood at birth. On the other hand, FT4 concentrations were positively associated with dental development scores, 0.04 SD change per 1 pmol/L of FT4 (95% CI, –0.01 to 0.08), although they were not statistically significant (P = 0.131).

There was no effect modification by the child's sex in any of the models.

DISCUSSION

The findings of this prospective cohort study suggest that thyroid function is involved in the maturation of teeth from early life into childhood. Children of TPOAb-positive mothers had delayed dental development, and children at school age displayed inverse associations between both TSH concentrations at birth and during early childhood and dental development. There were no overall significant effects of FT4 concentrations on the dental development of the child, which fits with the general notion that TSH is the most reliable/sensitive marker of thyroid function.

During early stages of dental development, the fetus is dependent on the transfer of maternal thyroid hormone via the placenta (Greenberg et al. 1970; Thorpe-Beeston et al. 1991). Interestingly, maternal TPOAb positivity was associated with lower dental development scores, and the effect estimates for maternal TSH and FT4 concentrations point toward an unfavorable effect of high maternal TSH and low FT4 concentrations. In addition, we also found that higher newborn TSH concentrations were associated with lower dental development scores. Both maternal TPOAb positivity and newborn TSH concentrations are a reflection of total thyroid hormone availability during pregnancy. TPOAb-positive women have higher TSH and lower FT4 concentrations (Medici et al. 2012), and recently it was shown that TPOAb-positive women lack the typical increase in thyroid hormone concentrations under the influence of human chorionic gonadotropin stimulation during early pregnancy (Korevaar et al. 2017). The extent of maternal thyroid hormone passage over the placenta is reflected by newborn TSH concentrations, wherein higher TSH concentrations reflect less placental passage (Korevaar et al. 2016). These findings, therefore, suggest that the effects of the thyroid hormone on early dental development occur throughout pregnancy rather than being specific to certain pregnancy timeframes. In addition, our results showed a positive association of both newborn and child-

hood TSH concentrations with development scores. Given the negative feedback system of the hypothalamic-pituitary-thyroid axis, a mild shortage of thyroid hormone availability is reflected by higher TSH concentrations. Therefore, our results indicate that also during postnatal dental development, thyroid hormone-dependent processes may play an essential role.

To our knowledge, this is the first large, population-based study that investigated the association of thyroid function, assessed at multiple time points, with dental development. Data were available on a wide range of potential confounders, lowering the risk that analyses were subdued to residual confounding. Furthermore, we measured dental development by examining calcification stages of certain teeth. As clinical tooth eruption begins when the root has developed around three-fourths of its expected final length, it can be postulated that dental development and eruption represent 2 strongly related aspects (Suri et al. 2004). However, examining calcification stages might be a superior method than tooth eruption, as it has more modalities and can better capture variations among participants. Also, a tooth eruption is subject to non-growth-related influences such as tooth impaction (Suri et al. 2004).

With regards to bone development, the effect of thyroxine has been well described in the process of endochondral ossification, where it promotes chondrocyte proliferation in the cartilaginous center of the bone (Cray et al. 2013). A deficiency of thyroid hormones causes a delay in ossification of cartilaginous centers, named epiphyseal dysgenesis. As opposed to the normal process of cartilage epiphyses ossification, where ossification begins in a single small focus in the center of the cartilage and expands peripherally, in epiphyseal dysgenesis, calcium deposition is delayed and occurs at multiple irregular foci scattered in the cartilage. Although the effect of thyroid hormones on different types of bones has been observed in the literature, the exact mechanism has not been described (Cray et al. 2013). Studies have reported that alveolar bone is less sensitive to alterations in thyroid hormone concentrations, but in the presence of periodontitis, the bone loss might be facilitated by thyroid hormone deficiencies (Feitosa et al. 2009). Shirazi et al. (1999) reported that administration of thyroxine increased orthodontic tooth movement and had a protective effect on root resorption. The protective effect of thyroxine exists due to the alkaline phosphatase activity, which is less damaging to the root during the force-induced remodeling process when thyroxine is administered (Poumpros et al. 1994). Taken together, optimal treatment of thyroid dysfunction and maintenance of a normal range of hormone levels could potentially also have beneficial effects on dental development and preservation, in addition to primary goals of the treatment.

Although the analysis was adjusted for multiple confounders, a residual confounding issue may exist due to numerous genetic, epigenetic, and environmental

factors that regulate the process of human growth and development. For example, environmental factors such as general living conditions, nutrition, health status, and stressors have been strongly associated with growth and development status (Cameron and Bogin 2012; Dasgupta and Hauspie 2013). Due to practical limitations of the study, some confounders were addressed by adding similar variables to the confounders (proxy confounders). For example, we adjusted for the education of mothers, which resembles the socioeconomic class of the family but does not take into account household income and living conditions, although they are highly correlated. BMI is a measurement of food intake, but it does not express eating behaviors qualitatively. Regarding health status and stressors, since our cohort study comprised predominantly healthy subjects, we excluded only those with thyroid-related diseases. Therefore, despite attempts to control for the major confounders in our study, residual confounding issues may remain due to imprecise and unmeasured variables, as in all observational studies.

A potential limitation could be bias related to differences in variables between subjects in our study, including those who were lost to follow-up. For example, if differences in thyroid function measurements between these 2 groups are statistically significant (differential loss of participants), this may introduce bias; if not (nondifferential loss of participants), it may affect the generalizability of our findings. Our assessments indicated that a potential bias due to loss of follow-up and differential for the exposure could have occurred for analyses focusing on cord blood FT4. However, we did not identify any associations of cord blood FT4 with a dental development score. Also, although circadian rhythms in TSH and, to some extent, FT4 levels have been described, these are very minimal, and the TSH peak occurs during nighttime (Pekonen et al. 1988; Russell et al. 2008), making it unlikely to have influenced the current results. Furthermore, as any misclassification of an exposure variable leads to regression to the null, any relevant daily variation that has been missed would increase the current effect estimates. Other hormones, such as parathyroid hormone and cortisol, may play an additional role in tooth maturation and eruption. However, the minimal interference of thyroid hormones with these endocrine axes makes other hormones an unlikely confounder as any other endocrine effect would be mediated through a parallel pathway. Nonetheless, more studies assessing the full clinical endocrine effects on tooth development would be an additive to the field. We quantified dental development as an SDS. The disadvantage of applying SDSs is that the unit of measurement is expressed as SD instead of dental age. We also used a rank-based normalization method to correct for the nonnormal distribution. By applying this procedure, we were able to include, for example, children with extreme values for dental development. As a result, the initial distribution of

dental development scores is narrowed down, implicating that the actual dental development differences might be greater than the observed differences. Still, we avoided the use of dental maturity scores or dental ages. These methods use population-specific standards developed in other samples, which might not apply to our multiethnic study population (Nykanen et al. 1998; Chaillet et al. 2004).

CONCLUSION

This is the first large study to assess the association of thyroid hormone availability throughout early life with dental development. We found that maternal TPOAb positivity during pregnancy, higher newborn TSH concentrations, and higher TSH concentrations during early childhood are associated with lower dental development scores at school age. Therefore, we postulate that lower overall availability of thyroid hormones during pregnancy and early childhood could lead to delayed dental development of the child. Future studies are necessary to provide insight into the underlying mechanism of the observed associations.

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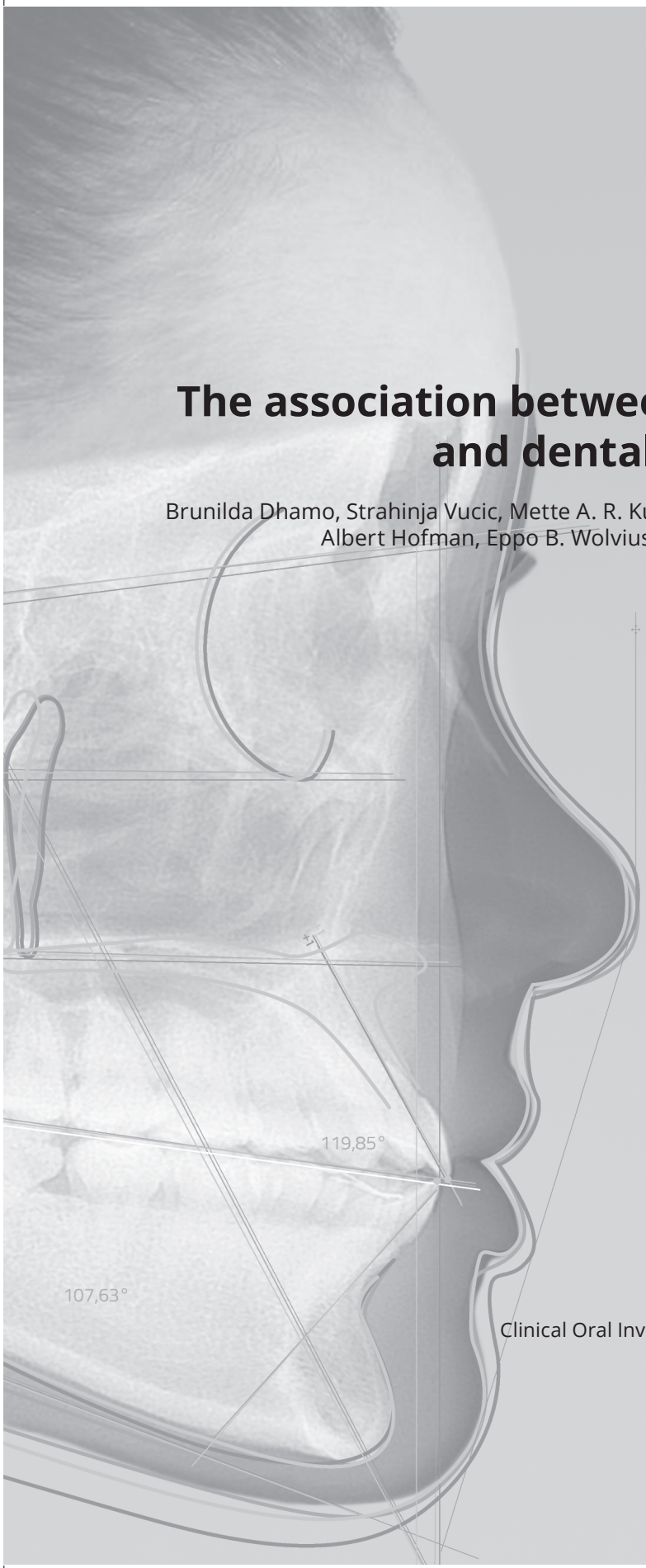

Chapter 5

The association between hypodontia and dental development

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ABSTRACT

Objectives: In this cross-sectional study, we aimed to investigate the pattern of hypodontia in the Dutch population and determine the association between hypodontia and dental development in children with and without hypodontia, applying three different standards, Dutch, French Canadian, and Belgian, to estimate dental age.

Methods: We used dental panoramic radiographs (DPRs) of 1488 children (773 boys and 715 girls), with a mean age of 9.76 years (SD = 0.24) participating in a population-based cohort study in Rotterdam, the Netherlands, born in 2002–2004, and 452 children (219 boys and 233 girls) with a mean age of 9.83 years (SD = 1.09) participating in a mixed-longitudinal, interdisciplinary population-based cohort study in Nijmegen, the Netherlands born in 1960–1968.

Results: The prevalence of hypodontia in the Generation R Study was 5.6 % (N = 84) and 5.1 % (N = 23) in the Nijmegen Growth Study. Linear regression analysis showed that children with hypodontia had a 0.37 [95 % CI (−0.53,-0.21)] to 0.52 [95 % CI (−0.76,-0.38)] years lower dental age than children without hypodontia. The ordinal regression analysis showed a delay in development of mandibular second premolars [1.68 years; 95 %CI (−1.90,-1.46)], mandibular first premolars [0.57 years; 95 % CI (−0.94,-0.20)], and mandibular second molars [0.47 years; 95 % CI (−0.84,-0.11)].

Conclusion: These findings suggest that children with hypodontia have a delayed dental development.

Clinical relevance: The delay of dental development in children with hypodontia should be taken into consideration, and therefore orthodontists should recognize that a later start of treatment in these patients may be necessary.

INTRODUCTION

Hypodontia is defined as the developmental absence of one or more primary or secondary teeth, excluding the third molars (Goodman et al. 1994; Silva Meza 2003). It is classified according to the number of absent teeth: mild if one tooth is absent, moderate if two to five teeth are absent, and severe if more than six teeth are absent (Dhanrajani 2002; Øgaard and Krogstad 1995). It is the most recognized congenital dental anomaly and therefore presents a frequent clinical problem encountered by orthodontists and other dental professionals (Guckes et al. 1998; Kotecha et al. 2013; Worsaae et al. 2007).

Most studies in which the prevalence of hypodontia was investigated were performed in Caucasians. These studies showed a prevalence of hypodontia of 5.5 % in European, 3.9 % in North American, and 6.4 % in the Australian population (Polder et al. 2004). The highest prevalence of hypodontia, 6.9 %, was found in an Asian population (Shimizu and Maeda 2009). Investigations in other populations are scarce. In the Dutch population, the prevalence of hypodontia is similar to the prevalence observed in European studies and is estimated to be 5 % (van den Boogaard et al. 2012). The prevalence of hypodontia is substantially higher in some disorders such as ectodermal dysplasia (Daniel et al. 2002; Johnson et al. 2002), Down syndrome (Acerbi et al. 2001; Mestrovic et al. 1998), Witkop syndrome (Nieminen et al. 2003; Zabawski and Cohen 1999), and cleft lip or palate (Bartzela et al. 2010). The most frequently affected tooth is the mandibular second premolar, followed by the maxillary second incisor and the maxillary second premolar (Polder et al. 2004). Although statistically significant differences were inconsistent throughout the literature, most reported a higher occurrence of hypodontia in females (Aasheim and Ögaard 1993; Davis 1987; Medina 2012).

Few studies have investigated whether an association exists between non-syndromic hypodontia and dental development (Ben-Bassat et al. 2014; Ruiz-Mealin et al. 2012; Tunç et al. 2011; Uslenghi et al. 2006). In a previous study, a significantly delayed dental development in subjects with hypodontia was reported (Uslenghi et al. 2006). Furthermore, the same authors reported that isolated hypodontia could impact the development of adjacent teeth by decreasing the crown size, changing crown and root morphology, delaying development, or inducing taurodontism. Another report identified a similar result of delayed dental development in children with hypodontia (Tunç et al. 2011). On the other hand, researchers reported a non-significant difference of dental development between children with hypodon-

Table 1. Characteristics of children included in the study (N=1940)

	Generation R sample (N=1488)			Nijmegen sample (N=452)		
	Controls (N=1404)	**Hypodontia (N=84)**	**P-value***	**Controls (N=429)**	**Hypodontia (N=23)**	**P-value***
Gender (N, %)			0.94			0.62
Boys	729 (52)	44 (52)		209 (52)	10 (52)	
Girls	675 (48)	40 (48)		220 (48)	13 (48)	
Age (years; mean, SD)	9.76 (0.24)	9.73(0.20)	0.3	9.85(1.05)	9.47(1.56)	0.1
Ethnicity (N, %)			0.24			
Dutch	934 (67)	52 (62)		429 (100)	23 (100)	
Non-Dutch	438 (31)	32 (38)		0	0	
Maternal age (years; mean, SD)	30.82(4.89)	31.34(5.14)	0.35	29.86(5.79)	30.92(5.56)	0.46
Dental age (years; mean, SD)						
French-Canadian standards						
Method 1[a]	11.31(1.15)	9.84(1.46)	<0.05	11.59(1.63)	9.90(2.03)	<0.05
Method 2[b]	11.31(1.15)	10.76(1.07)	<0.05	11.57(1.61)	10.86(1.94)	<0.05
Method 3[c]	11.32(1.12)	10.62(1.18)	<0.05	11.61(1.63)	10.77(1.86)	<0.05
Belgian standards						
Method 1[a]	13.56(2.95)	12.48(2.57)	<0.05	14.19(3.40)	13.17(3.55)	0.16
Method 2[b]	13.56(2.95)	13.11(2.80)	0.17	14.22(3.41)	13.73(3.71)	0.5
Method 3[c]	13.57(2.95)	13.01(2.77)	0.09	14.22(3.41)	13.63(3.62)	0.42

Abbreviations: N- number of children, SD- standard deviation *Differences were tested using independent t-test for continuous variables and chi-squared test for categorical variables. Dental age was calculated if both matching mandibular teeth were missing by scoring them: a) as non-developed teeth (developmental stage=0); b) as a stage calculated from regression equations developed by Nyström et al. (2000); c) as a developmental stage of the (left) matching maxillary tooth.

tia and their matched controls (Ben-Bassat et al. 2014). These inconsistent findings prompted us to conduct a study with a large-sized sample in the general population.

In this cross-sectional study, we aimed to determine the association between hypodontia and dental development in children with and without hypodontia using three different standards, Dutch, French Canadian, and Belgian, to obtain the best estimation of dental age in relation to chronological age.

MATERIALS AND METHODS

Study population

Our cross-sectional study aims to represent Dutch population over time, so we used 1940 dental panoramic radiographs (DPR) of 1940 children, obtained from two cohorts in different cities in the Netherlands, the Generation R Study in Rotterdam and the Nijmegen Growth Study (Table 1).

The Generation R Study is a population-based prospective cohort study from fetal life until young adulthood established in the city of Rotterdam in the Netherlands (Hofman et al. 2004; Jaddoe et al. 2012; Kruithof et al. 2015). From the still ongoing fourth examination phase, we used 1488 DPRs taken of 773 girls and 715 boys, with a mean age of 9.76 ± 0.24 years and born between 2002 and 2003. At the start of each phase, mothers and their partners received written and oral information about the study and they were asked for their written informed consent. The study was approved by the Medical Ethics Committee of the Erasmus Medical Centre in Rotterdam, the Netherlands (MEC-2012-165).

The second sample was derived from the Nijmegen Growth Study, a mixed-longitudinal, interdisciplinary population-based cohort study in healthy Dutch children conducted from 1971 to 1976 at the Radboud University Medical Centre in Nijmegen, the Netherlands. The design of this cohort was described in the past (Prahl-Andersen et al. 1979). Children were enrolled at 4, 7, and 9 years of age and followed until 9, 12, and 14 years. From this cohort, we used 452 DPRs of 219 boys and 233 girls, with a mean age of 9.83 ± 1.09 years and born between 1960 and 1968. Prior to the collection of general, physiological, dental, and anthropometric measurements of children, informed consents were obtained from their parents. Children who were not born in the Netherlands and nonwhite children were excluded from the

study. The participants in this study had no recognizable syndrome associated with hypodontia.

The assessment of hypodontia

One experienced examiner ascertained hypodontia from the DPRs. Children were included in the hypodontic group if they missed at least one tooth (no sign of formation or calcification showed in DPR).

Dental development assessment

Dental development was defined using the Demirjian method (Demirjian et al. 1973). One experienced examiner determined one of the eight developmental stages (A, B, C, D, E, F, G, and H) for each of the seven teeth located in the lower left quadrant. In order to estimate the developmental stage of the hypodontic teeth, we applied two methods. In Method 1, we applied regression equations (Nyström et al. 2000), which take into account the development of the remaining teeth in the lower left quadrant and age of a child to calculate dental age. In Method 2, we assessed the stage of development for a hypodontic tooth in the left mandible from the corresponding right mandibular tooth if it was present or from a corresponding maxillary tooth if that tooth was missing in both sides of the mandible. In the case when no corresponding tooth was present, stage 0 was assigned to that tooth. Obtained stages of dental development were used to calculate the dental maturity score by summing up the weighted scores from Dutch, French-Canadian, and Belgian dental age standards (Chaillet and Willems 2004; Demirjian et al. 1973; Leurs et al. 2005). Lastly, we used standard tables to convert the dental maturity score to dental age (Chaillet and Willems 2004; Demirjian et al. 1973; Leurs et al. 2005).

Statistical analysis

We calculated the intra-class correlation coefficient to determine agreement between two independent examiners who assessed the presence of hypodontia and stages of development (A to H) for each of the seven left mandibular teeth in a subsample of 20 DPRs from the study population.

The association between hypodontia and dental development in children was analyzed with linear regression models and by adjusting for confounders in three consecutive steps. In the first model, we analyzed the crude dependence of dental age on the hypodontia status of children. In the second model, we additionally adjusted for gender, age, and study population. The study population was taken into account to avoid any possible cohort effect. Lastly, in the third model, variables ethnicity and maternal age at the birth of a child were added. Maternal age at birth was added because previous studies showed that certain maternal factors might have an in-

fluence on the condition of hypodontia and dental development of children (Keene 1966).

To study the association between hypodontia and the developmental stage for each of the observed teeth separately from the lower left quadrant, we performed an ordinal regression analysis. Dental development stages (A to H) were converted into numbers (1 to 8) and used as a dependent variable while the independent variables were added in three consecutive steps, as previously described for the linear regression analysis. In order to avoid possible errors of the two methods for assigning the stage of development 0 to hypodontic teeth, we excluded stage 0 from being a dependent variable in the ordinal regression model.

We tested for interaction terms between gender, ethnicity, and hypodontia in relation to dental development. Since no significant interaction terms were found, we did not stratify our analyses for these interaction terms. The Markov Chain Monte Carlo imputation method was used to reduce potential bias associated with missing data on maternal age at birth in 99 children (5 %) (Sterne et al. 2009). As a result, five imputed datasets were generated from which a pooled effect estimate was calculated. The result was considered statistically significant for a P value ≤0.05. All statistical analyses in this study were performed using statistical software SPSS version 21.0 (SPSS Inc. Chicago, IL, USA).

RESULTS

Inter-examiner agreement for the study population

The inter-examiner reliability of the study population was performed by two independent researchers in a subsample of 20 DPRs. We found an excellent agreement between the examiners for the scoring of the central incisors, with an intra-class correlation coefficient (ICC) equal to 1.00. The intra-class correlation coefficient was the lowest for the first molars (ICC = 0.49), while the range of ICC values for the rest of the scored teeth ranged from good to excellent (ICC = 0.79–0.94).

Prevalence of hypodontia

The distribution of tooth agenesis is presented in Supplementary Table S1. The prevalence of hypodontia in the Generation R Study was 5.6 % (N = 84) and 5.1 % (N = 23) in the Nijmegen Growth Study. The most common hypodontic teeth in the Generation R Study and the Nijmegen Growth Study were the mandibular second premolars, 51.8 % (N = 72); 50.0 % (N = 20), respectively; P = 0.84, and the maxillary lateral incisor, 15.8 % (N = 22); 27.5 % (N = 11), respectively; P = 0.09. None of the children had more than five hypodontic teeth. The prevalence of hypodontia was similar in

Table 2. Linear regression models: Association between hypodontia and dental age using Dutch standards

	Model 1			Model 2			Model 3		
	β	**95% CI**	**P-value**	**β**	**95% CI**	**P-value**	**β**	**95% CI**	**P-value**
Method 1[a]									
Hypodontia									
No (ref.)	0	–	–	0	–	–	0	–	–
Yes	-0.46	(-0.65,-0.27)	<0.05	-0.36	(-0.52,-0.20)	<0.05	-0.37	(-0.53,-0.21)	<0.05
Method 2[b]									
Hypodontia									
No (ref.)	0	–	–	0	–	–	0	–	–
Yes	-0.57	(-0.76,-0.38)	<0.05	-0.52	(-0.68,-0.35)	<0.05	-0.52	(-0.69,-0.36)	<0.05

Abbreviations: β –regression coefficients, CI – confidence interval, ref.-reference. Dental age was calculated if both matching mandibular teeth were missing by scoring them: a) as a developmental stage calculated from regression equations developed by Nyström et al. (2000); b) as a developmental stage of the (left) matching maxillary tooth.

both sexes in the Generation R Study sample (P = 0.94) and the Nijmegen Growth study sample (P = 0.62) (Table 1).

Crude analysis

The calculated dental age using Dutch (10.35 ± 0.91), French-Canadian (11.29 ± 1.35), and Belgian (13.65 ± 3.07) standards was statistically significantly higher than the chronological age (9.78 ± 0.57) of children (P ≤ 0.05) (Table 1). We observed a statistically significant lower dental age in children with hypodontia, compared to controls by applying the two methods to score hypodontic teeth using Dutch standards, French-Canadian standards, and Belgian standards (P ≤ 0.05). The mean difference between chronological and dental age was the least when using Dutch standards. For this reason, a dental age defined by Dutch standards was used in the linear regression analysis (Figure 1).

Linear regression analysis: association between hypodontia and dental age

The association between dental age and hypodontia was investigated by three linear regression models separately for each of the two methods and is presented in Table 2. Univariate linear regression analysis showed that a child with hypodontia had a 0.46 [95 % CI (−0.65,-0.27)] to 0.57 [95 % CI (−0.76,-0.38)] years lower dental age compared to a child without hypodontia. After additionally adjusting Model 2 for age, sex, and study population, the effect estimate of the hypodontia status variable changed, resulting in a 0.36 [95 % CI (−0.52,-0.20)] to 0.52 [95 % CI (−0.68,-0.35)] years lower dental age in children with hypodontia. The effect estimates and

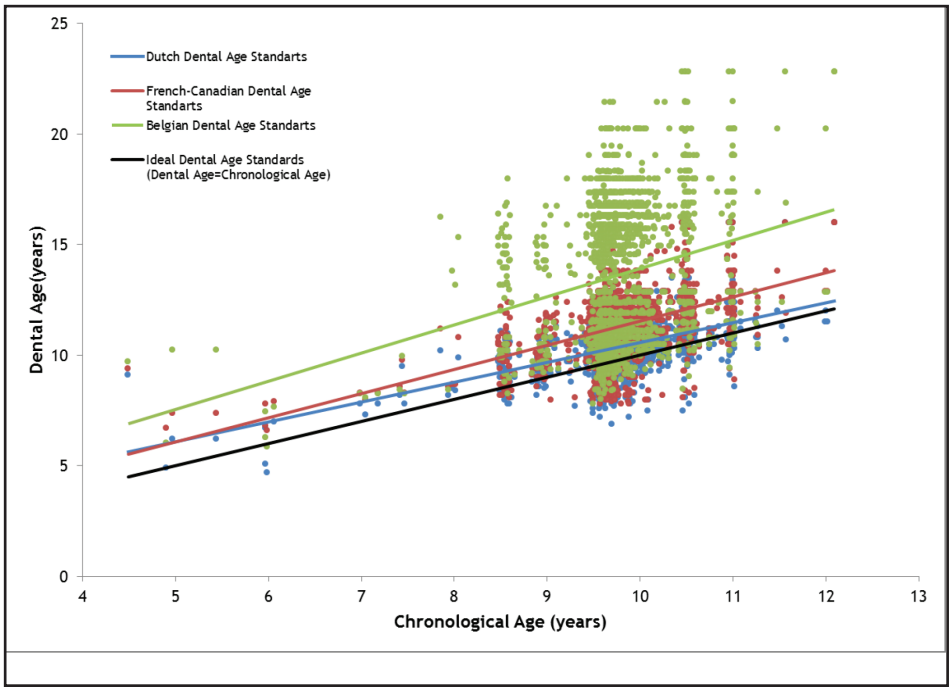

Figure 1. Dental age of study population assessed from Dutch, French-Canadian, and Belgian standards are presented as a function of chronological age of children.

statistical significance barely changed by taking into account the ethnicity of a child and maternal age at birth, in the fully adjusted model.

Ordinal regression analysis: association between hypodontia and stages of dental development

Results for the left mandibular second molar, first molar, second premolar, first premolar, canine, and lateral and central incisors are shown in Figure 2. The following regression coefficients and P values are reported from the third model (fully adjusted model) of ordinal regression. The greatest difference in obtained developmental stages was observed for the left mandibular second premolar, where the results of the ordinal regression analysis showed that children with hypodontia tend to have lower dental developmental stages than the controls [−1.68 years; 95 % CI (−1.90,-1.46)]. In addition, similar negative and significant associations were observed for the left mandibular first premolar [−0.57 years; 95 % CI (−0.94,-0.20)] and for the left

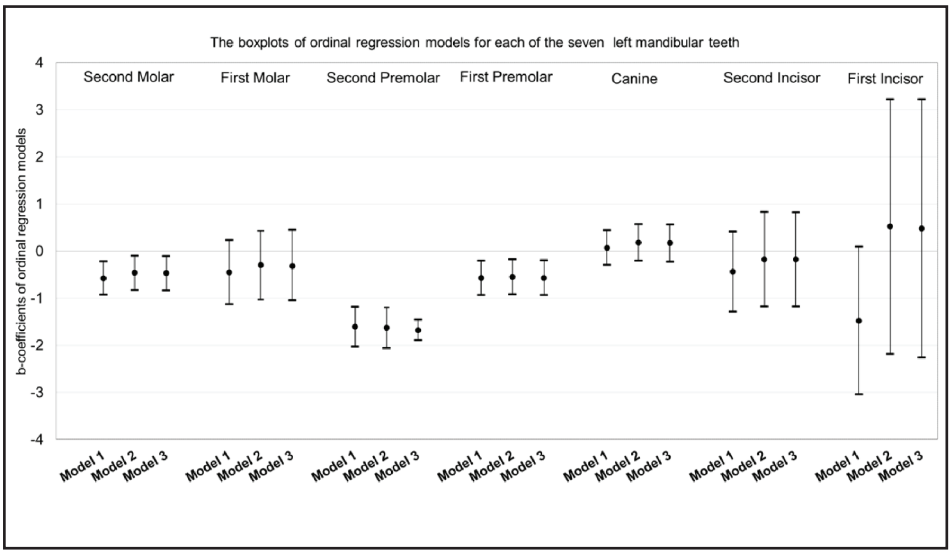

Figure 2. Association of hypodontia with stages of dental development for each of seven left mandibular teeth, expressed by estimates of b-coefficients and 95% confidence intervals; assessed from ordinal regression model using developmental stage (A/1, B/2, C/3, D/4, E/5, F/6, G/7, H/8) as a dependent variable and hypodontia status (No-ref., Yes) as a determinant in Model 1. Model 2 was additionally adjusted for age, gender and study population. Model 3 was adjusted for variables used in the previous model and additionally for ethnicity and maternal age at birth of a child.

mandibular second molar [−0.47 years; 95 % CI (−0.84,-0.11)]. Developmental stages between children with hypodontia and controls did not significantly differ for the central incisor [0.48 years; 95 % CI (−2.26, 3.22)], lateral incisor [−0.18 years; 95 % CI (−1.18, 0.82)], canine [0.17 years; 95 % CI (−0.23, 0.56)], and first molar [−0.32 years; 95 % CI (−1.05, 0.42)].

DISCUSSION

The findings of our study suggest a significant delay of 0.37–0.52 years in the dental development of children with hypodontia, supporting the overall mean of earlier studies of 1.04 years delay in dental development presented in Figure 3. Different results on the association between hypodontia and dental development have been observed possibly because of different methods used to define the developmental stage of hypodontic teeth (Table S2). Accordingly, previous investigators have proposed different techniques to tackle this problem. Uslenghi (2006) used a meth-

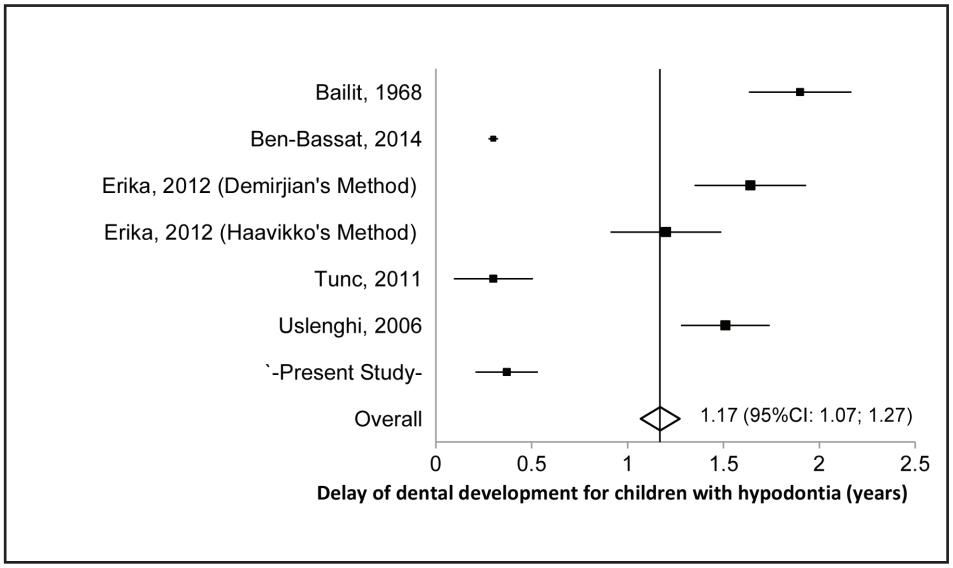

Figure 3. The forest plot of studies on the association between hypodontia and dental development.

od and data from Haavikko's scoring system to overcome the problem of scoring a hypodontic tooth (Haavikko 1970). On the other hand, Tunc (Tunc and Koyuturk 2008) used an adapted Demirjian method which relies on the development stages of three teeth only: left mandibular canine, first premolar, and second molar. We used two methods to estimate the developmental stage of the hypodontic teeth. The advantage of using Method 1 in patients with hypodontia is that the developmental stage is obtained from mathematical formulas for each missing tooth separately (Nyström et al. 2000). By using Method 2, we tested the suitability of regression equations from Method 1 as they were derived from a Finnish population. Method 2 may be more suitable when assessing dental age in children with mild hypodontia because in using Method 1, the underlying population stays an important factor in establishing the imputations formulas. However, in cases of severe hypodontia in which the same tooth is missing in all four quadrants, Method 1 may be more advantageous for the calculation of dental age than Method 2. The limitation of the two methods used in this study might be the dependence of calculated dental age on the estimated stage of development for the hypodontic tooth. We tried to overcome the problem related to assessing dental development in children with hypodontia by using ordinal regression models in which stage 0 of development of every left mandibular tooth (hypodontic teeth) was not used in the analysis, and the effect

of hypodontia is assessed directly from the eight stages of dental development for every single tooth.

A combination of several methods for determining dental development is generally recommended for a better estimation of dental age (Ben-Bassat et al. 2014). We used three different dental age standards (Dutch, French-Canadian, and Belgian) in order to approach dental age to chronological age of the children the best. The French-Canadian standard is the most used in the literature although studies were not performed in Canada. Our assumptions were that dental age assessed by Dutch standards would resemble chronological age of our sample better than Belgian Standards and that dental age assessed by Belgian standards would resemble chronological age better than French-Canadian, because of the geographical proximity of the Dutch and Belgian population. Belgian standards were indeed better than French-Canadian's in defining a dental age for boys, but the estimated dental age for girls was at least 6 years higher than their real age. The calculations we did showed that the inaccuracy of Belgian standards was not in the scores they presented, but in the polynomial equations that they used to define a dental age for girls. Although chronological age was closer to dental age estimated from Dutch standards than to dental age estimated from French-Canadian or Belgian standards, still a statistically significant difference existed between Dutch dental age and chronological age. A better approach to Dutch standards needs to be performed in a larger sample of Dutch population in the future.

The frequency of hypodontia in the cohorts of the Nijmegen Growth Study and the Generation R Study coincided with an earlier prediction of 5 % in the Dutch population (van den Boogaard et al. 2012). It has been hypothesized that prevalence of hypodontia in permanent teeth increases over the years (Mattheeuws et al. 2004). We compared the prevalence of hypodontia in 1970 and 2010 between the cohorts of the Nijmegen Growth Study (5.1 %) and the Generation R Study (5.6 %) and found no statistically significant difference. A higher prevalence has been reported in females than in males, with a ratio of 3:2 (Polder et al. 2004) but in our study, the frequency of hypodontia did not differ by gender or by ethnicity.

The results from ordinal regression models showed that the delay in dental development was caused mainly by the second premolar [1.68 years; 95 % CI (−1.90,-1.46)], the last in the row of premolars which is also the most prevalent hypodontic tooth in our study, consistently with previously published literature (Polder et al. 2004; Shimizu and Maeda 2009). As a consequence of evolution, what is less needed is going to disappear naturally (Smith 1978). This may explain the major absence of the third molar, which is the latest developing tooth and molar, and may be explained in the same way for the last premolar, the second premolar and lateral incisor (Parkin et

al. 2009). At the age of ten, we observed little variation for central, lateral incisors, and first molars because they were in the final stage of development, common for 9–10-year-old children. However, to test whether there is delayed dental development of incisors and first molars, DPRs of children of younger ages need to be taken when these teeth have not yet reached the final stage of development. The effect of hypodontia in the development of the canine, important in our dentition, was not statistically significant. Cases of hypodontic canines are rarely reported (Polder et al. 2004; Shimizu and Maeda 2009). Following this line of thought, the trend of tooth loss throughout the evolution of mankind could explain the association between hypodontia and delayed dental development. Although an association between delayed dental development and hypodontia was found in our cross-sectional study, it currently remains uncertain whether hypodontia causes a delay of dental development or vice versa (Kerekes-Máthé et al. 2015). The nature of this association would be better determined by genetic investigations in humans, taking into consideration the different pathways of PAX9, MSX1, and AXIN2 acting on both hypodontia and delayed dental development (Cobourne 2007; Dhanrajani 2002; Matalova et al. 2008).

CONCLUSIONS

The findings of our study indicate a lower dental age in children with hypodontia. The delay varied from 0.37 to 0.52 years of dental age between the groups of hypodontia and non-hypodontia and the difference in development was most pronounced for the second lower premolars, first lower premolar, and second lower molars.

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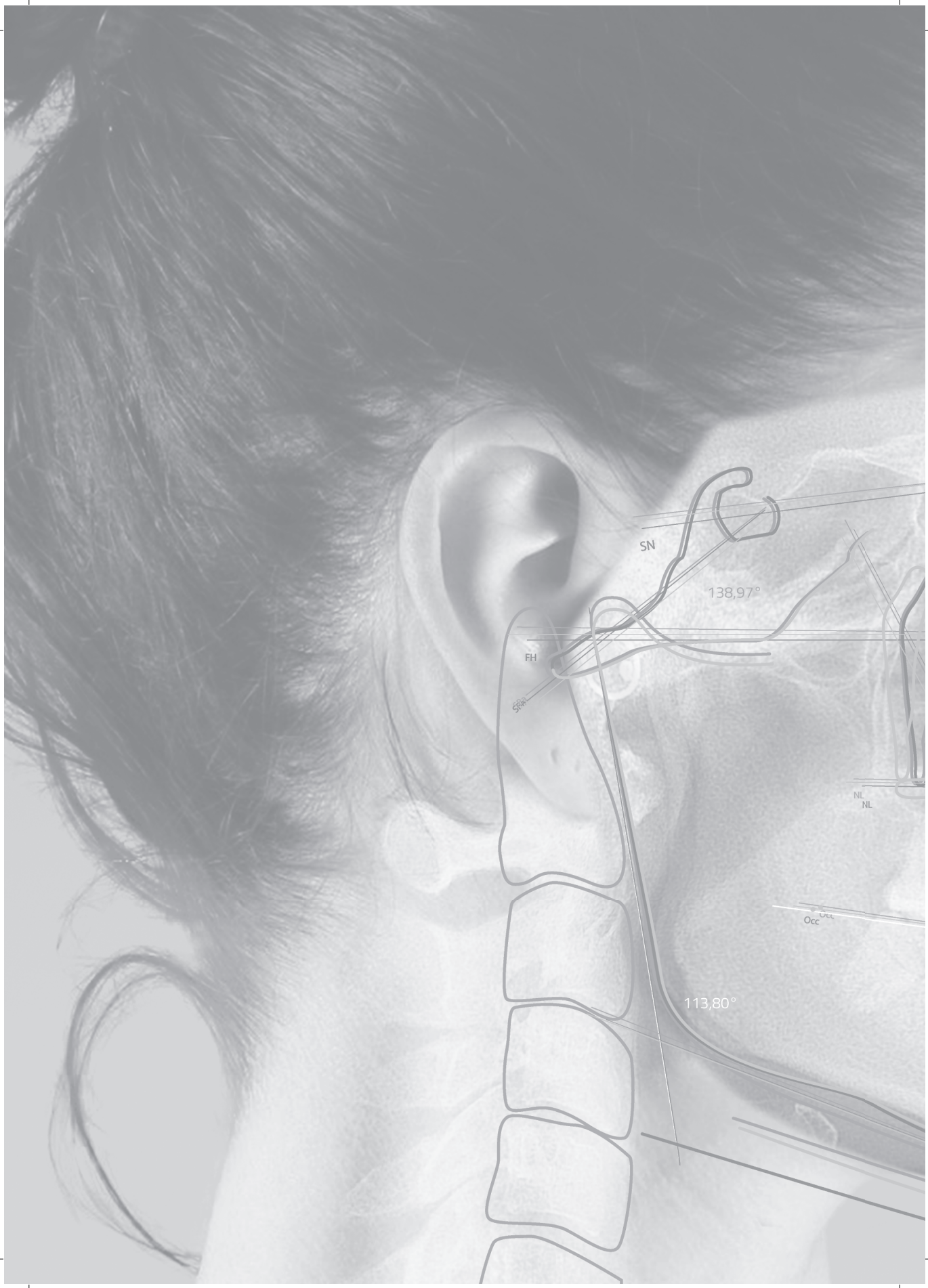
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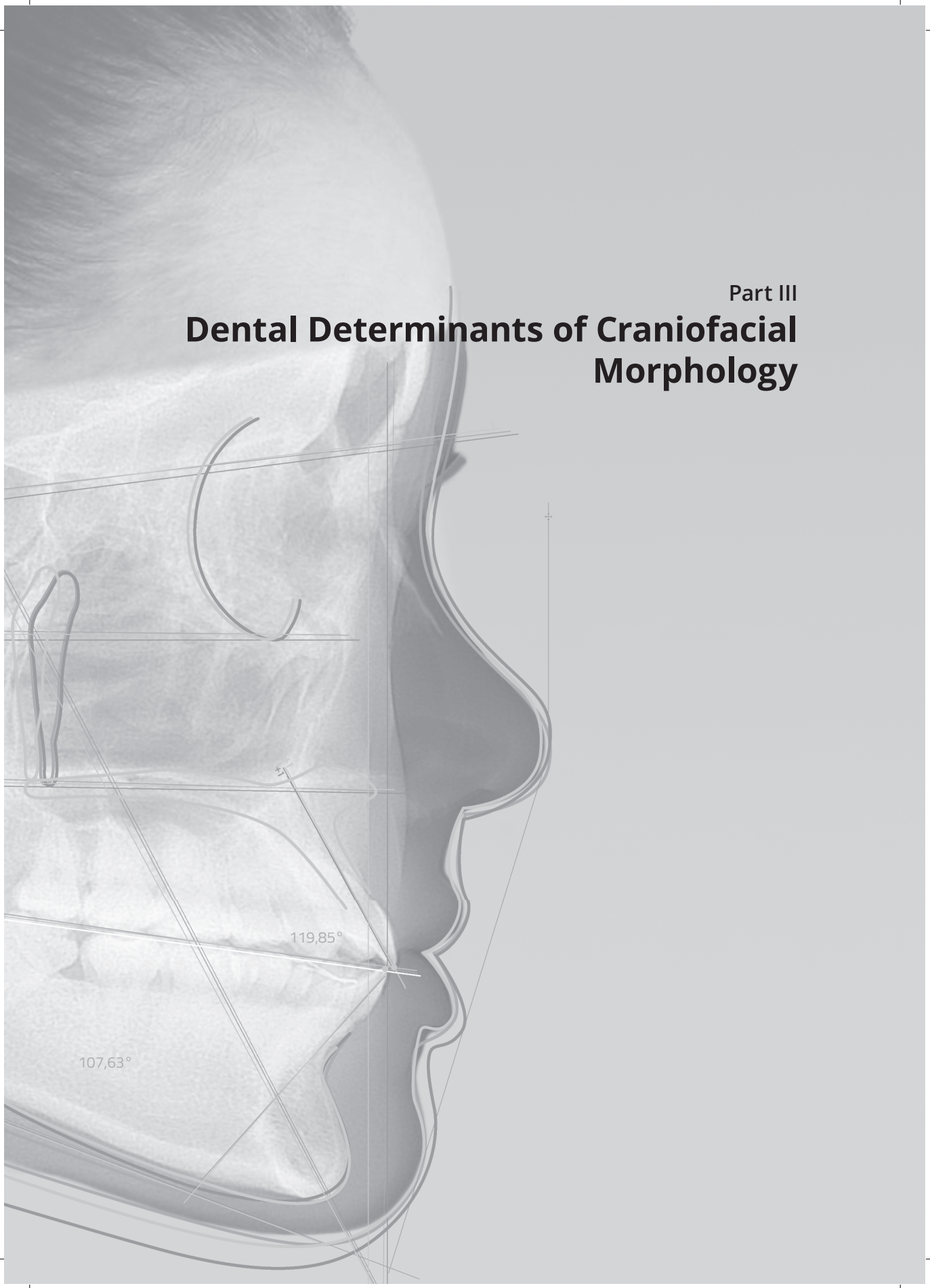
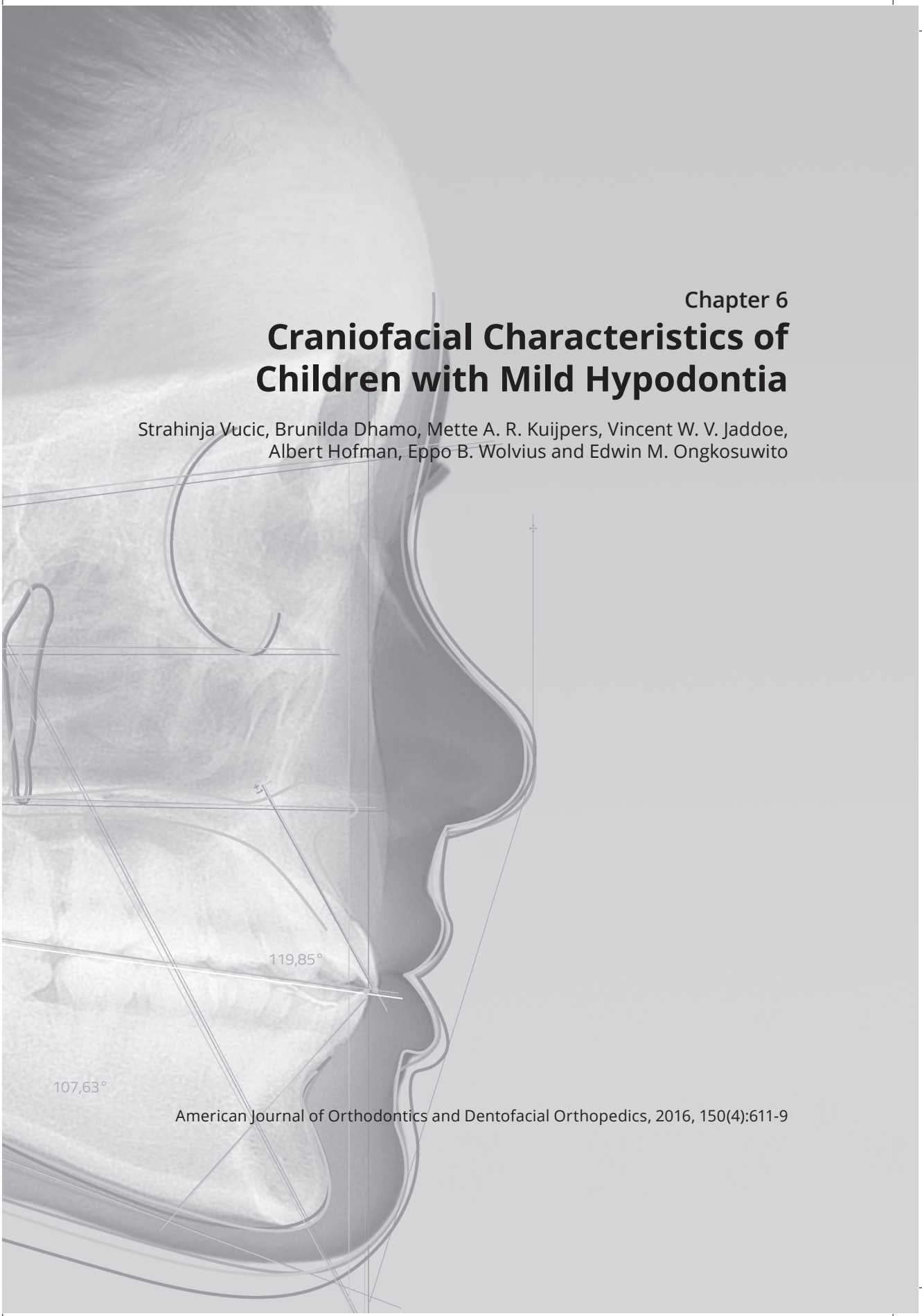


Part III

Dental Determinants of Craniofacial Morphology



Chapter 6

Craniofacial Characteristics of Children with Mild Hypodontia

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ABSTRACT

Introduction: The aim of our study was to evaluate the craniofacial characteristics of children with mild hypodontia using conventional and principal component (PC) analysis.

Methods: We used radiographic images of 124 children (8-12 years old) with up to 4 missing teeth (55 boys, 69 girls) and of 676 reference children (365 boys, 311 girls) from the Rotterdam Generation R Study and the Nijmegen Growth Study in The Netherlands. Fifteen cephalometric measurements of children with hypodontia were compared with those of the reference children. Moreover, cephalometric parameters were combined into standardized PC scores using PC analysis, and the components were compared between the 2 groups.

Results: PC analysis showed common dental characteristics for all types of hypodontia: a significant increase of the interincisal angle, and decreases of the maxillary and mandibular incisor angles. Other findings were consistent when both methods were applied: (1) anterior hypodontia was significantly associated with the high-angle (hyperdivergent) craniofacial pattern, (2) the tendency toward a Class III malocclusion was identified in maxillary hypodontia, and (3) we observed a significant reduction of lower posterior facial height in children with posterior and mandibular hypodontia.

Conclusions: Our findings suggest that children with mild hypodontia have distinctive skeletal and dental features.

INTRODUCTION

Hypodontia is the most prevalent developmental tooth disorder in which a person has at least 1 missing tooth, excluding the third molars (Larmour et al. 2005). The population prevalence of hypodontia varies between 3.9% and 6.9%, and a slightly higher prevalence was reported in females than in males (Medina 2012; Polder et al. 2004). Hypodontia mostly occurs in its mildest form, with the highest percentage of just 1 tooth (49%) and lower percentages for 2 (35%), 3 (7%), or 4 missing teeth (6%) (Polder et al. 2004). Previous studies have reported that isolated hypodontia could affect the dental morphology of adjacent teeth and the relationship between jaws (McKeown et al. 2002; Uslu et al. 2009). More severe cases of hypodontia (6 or more missing teeth) are rare and often combined with specific syndromic disorders (Bartzela et al. 2010; Johnson et al. 2002). In this study, we examined children with mild hypodontia, with up to 4 missing teeth; they are referred to as children with hypodontia (Dhanrajani 2002; Ogaard and Krogstad 1995).

Cephalometric studies performed on people with hypodontia showed that they have a distinctive facial morphology, including the following characteristics: maxillary retrognathism (Acharya et al. 2010; Ben-Bassat and Brin 2003; 2009; Kreczi et al. 2011; Nodal et al. 1994; Ogaard and Krogstad 1995; Roald et al. 1982; Sarnas and Rune 1983), mandibular retrognathism, (Acharya et al. 2010; Ben-Bassat and Brin 2003; 2009; Kreczi et al. 2011; Nodal et al. 1994) or prognathism, (Nodal et al. 1994) increased overjet, (Kreczi et al. 2011; Nodal et al. 1994; Sarnas and Rune 1983) increased overbite, (Kreczi et al. 2011) reduction in vertical jaw relationship, (Acharya et al. 2010; Bondarets and McDonald 2000; Nodal et al. 1994; Ogaard and Krogstad 1995) higher interincisal angle, (Endo et al. 2004; Ogaard and Krogstad 1995) and a tendency toward a Class III malocclusion (Acharya et al. 2010; Chung et al. 2000). Contradictory results of previous studies could be attributed to varying sizes and genetic backgrounds of the samples, and different methods for quantifying hypodontia and measuring the morphology of the dentofacial complex.

Most of the previous similar studies divided patients into groups, depending on the number of missing teeth (Kreczi et al. 2011; Nodal et al. 1994; Ogaard and Krogstad 1995). Other studies compared craniofacial measurements between hypodontia and reference subjects by dividing them into groups depending on the location of the missing teeth (Ben-Bassat and Brin 2003; Endo et al. 2006) or by combining both criteria (Acharya et al. 2010; Ben-Bassat and Brin 2009).

On the other hand, the most common method for measuring linear and angular parameters of the facial profile is conventional 2-dimensional cephalometric analysis (Ludlow et al. 2009). Although there are numerous parameters available from different types of analyses, investigators often select linear, angular, and other de-

rived cephalometric parameters that reflect the best morphology of the facial profile region that they are investigating. These led to inclusion of 9 up to 65 parameters in previous similar studies that examined the craniofacial characteristics in subjects with hypodontia (Acharya et al. 2010; Ben-Bassat and Brin 2003; 2009; Endo et al. 2006; Endo et al. 2004; Kreczi et al. 2011; Nodal et al. 1994; Ogaard and Krogstad 1995; Roald et al. 1982; Sarnas and Rune 1983). Consequently, including more parameters in the analysis increases the number of statistical comparisons, which, if not considered, may present a potential issue of discovering false significant results, also known as a multiple comparisons problem (Shaffer 1995). Therefore, increasing the significance threshold or reducing the number of comparisons by unifying correlated parameters may be a solution to overcome this issue (Aickin and Gensler 1996; Cupples et al. 1984).

Several cephalometric studies have suggested a principal component (PC) analysis to reduce the number of parameters in their analyses (Halazonetis 2004; 2007; Moreno Uribe et al. 2013). This statistical technique uses the correlation between a set of variables to create a set of new variables, named PCs (Abdi and Williams 2010; Wold et al. 1987). Application of a PC analysis might be a suitable adjunct method next to conventional analysis for craniofacial studies with many cephalometric parameters and for revealing hidden underlying structures and making stronger conclusions than by using each parameter independently (Cleall et al. 1979).

The aim of this study was to determine the cephalometric characteristics of children with mild hypodontia using conventional cephalometric analysis and PC analysis.

MATERIAL AND METHODS

We used radiographic images of 124 children (8-12 years old) with up to 4 missing teeth (55 boys, 69 girls) that we compared with the images of 676 reference children (365 boys, 311 girls) from the Nijmegen Growth Study and the Generation R Study in Rotterdam in The Netherlands (Table I).

The Nijmegen Growth Study is a mixed longitudinal population-based cohort study conducted from 1971 to 1976 at the Radboud University Medical Center in Nijmegen, The Netherlands (Prahl-Andersen and Kowalski 1973). This study included 3 cohorts: children were enrolled at 4, 7, and 9 years of age and followed until they were 9, 12, and 14 years of age. We used only 1 radiographic image per child taken between 8 and 12 years. If a child had more than 1 radiographic image available, we selected the one taken at the age that was closer to the mean age of the Generation R Study sample. In total, 7 children with hypodontia and 203 reference children were

Table 1. Baseline characteristics of the study population (N= 800)

	Hypodontia	
	No (N= 676)	**Yes (N= 124)**
General characteristics		
Gender (N, %)		
Males	365 (56)	55 (46)
Females	311 (44)	69 (54)
Age-years (years; mean, SD)	9.67 (0.35)	9.77 (0.24)
Study Population (N, %)		
Nijmegen	203 (30)	7 (6)
Generation R	473 (70)	117 (94)
Tooth agenesis (N, %)		
Region		
Incisive and canine region	-	39 (31)
Premolar and molar region	-	85 (69)
Jaw		
Maxilla	-	29 (23)
Mandible	-	86 (69)
Both jaws	-	9 (7)
Frequency (N, %)		
1 agenetic tooth	-	68 (55)
2 agenetic teeth	-	46 (37)
3 or more agenetic teeth	-	10 (8)

included from the Nijmegen Growth Study, with an average age of 9.39 ± 0.32 years. Before the inclusion of the participating children in the Nijmegen Growth Study, signed consents were obtained from the parents.

The Generation R Study is a population-based cohort study from fetal life to adulthood, established in Rotterdam, The Netherlands, at the Erasmus University Medical Centre (Jaddoe et al. 2012). From the fourth data collection phase, we used data from 117 children with hypodontia and 473 healthy children with a mean age of 9.67 ± 0.40 years. The study was approved by the medical ethics committee of the Erasmus Medical Centre (MEC-2012-165) in Rotterdam. At the start of each phase, mothers and their partners received written and oral information about the study and were asked for their written informed consent.

Hypodontia in children was assessed from their dental panoramic radiographs. One dentist (B.D.) determined the number and position of the missing teeth for each

subject. In total, 124 children had hypodontia (Table I). Of those, 39 children had anterior hypodontia (tooth agenesis of incisors and canines), and 85 children had posterior hypodontia (tooth agenesis of premolars and molars). Also, we classified hypodontia based on the jaw in which the tooth was missing. Our sample consisted of 29 subjects with maxillary hypodontia, 86 subjects with mandibular hypodontia, and 9 subjects who had missing teeth in both jaws. No child had more than 4 missing teeth or a combination of anterior and posterior hypodontia.

We selected 14 landmarks on cephalograms (Table II); from these cephalometric points, we measured 12 angular parameters, 1 distance, and 2 derived proportions. The mean values of the cephalometric parameters for boys and girls are given in Table III and Table IV, respectively. Lines Mx (palatal plane) and Mn (mandibular plane) were obtained by connecting points ANS and PNS, and Go and Me, respectively. Line Ui passes through the axis of the maxillary central incisors by connecting points Is and Rs, and line Li goes through the axis of the mandibular central incisors by connecting points Ii and Ri. Before each measurement, the image was recalibrated depending on the magnification of the cephalometric radiograph. We included the most frequently used cephalometric parameters from previous studies that investigated cephalometric differences between hypodontia and reference groups (Acharya et al. 2010; Ben-Bassat and Brin 2003; 2009; Chung et al. 2000; Endo et al. 2006; Endo et al. 2004; Kreczi et al. 2011). In both the Nijmegen Growth Study and the Generation R Study, cephalometric landmarks were digitized from which linear and angular cephalometric parameters were calculated. Cephalometric points in the Generation R Study were digitized by a dentist (S.V.) using Viewbox software (version 4.0; dHAL Software, Kifissia, Greece).

We applied a PC analysis to combine correlated cephalometric parameters into a new set of uncorrelated PCs, each representing a distinct craniofacial pattern. A detailed description of the PC procedure used in this study is provided in the Supplemental material. Briefly, we explored first the intercorrelation among cephalometric parameters using Pearson correlation (Table S1). Secondly, a standardized score was created for each PC with a mean value of 0 and a standard deviation of 1. If a cephalometric parameter had a loading greater than 0.5 for a component, we interpreted the increase or decrease of that component as an increase or a decrease of that cephalometric parameter. If a cephalometric parameter had a loading lower than −0.5, an inverse principle was applied. Cephalometric parameters that are included in 1 PC are bolded in Table S2.

Interobserver agreement for the cephalometric measurements in the Generation R sample was tested using intraclass correlation on a subsample of 20 randomly selected cephalograms scored by 2 independent observers (S.V. and B.D.). Values

Table 2. Cephalometric landmarks used to derive 15 cephalometric parameters

No.	Abbreviation	Name	Definition
1	S	Sella	Centre of sella turcica
2	N	Nasion	The most anterior limit of the frontonasal suture
3	Ar	Articulare	A point where the posterior outline of the condyle passes over the posterior and lower margin of the cranial base
4	Go	Gonion	The midpoint of the angle of the mandible
5	Me	Menton	The most inferior point on the symphysis of the mandible
6	Pg	Pogonion	The most anterior point on the symphysis of the mandible
7	B	B-point	The deepest point on the contour of the mandible
8	A	A-point	The deepest point on the contour of the premaxilla
9	ANS	Anterior nasal spine	Tip of the anterior nasal spine
10	PNS	Posterior nasal spine	The most posterior point in the sagittal plane on the bony hard palate
11	Is	Incision superius	The incisal tip of the most anterior maxillary central incisor
12	Rs	Upper incisor apex	The root apex of the most prominent upper incisor
13	Ii	Incision inferius	The incisal tip of the most anterior medial mandibular central incisor
14	Ri	Lower incisor apex	The root apex of the most prominent lower incisor

Table 3. Cephalometric characteristics of hypodontic and nonhypodontic boys (N= 420)

	Hypodontia							
	No			Yes				
Cephalometric measurements	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**Difference**	**P-value**
S-Ar-Go (°)	364	139.99	5.93	55	139.28	5.4	0.71	0.41
N-S-Ar (°)	365	123.82	5.26	55	125.14	4.72	-1.32	0.08
Ar-Go-Me (°)	363	129.07	5.06	55	128.68	5.15	0.38	0.6
S-N-A (°)	361	81.08	4.04	55	80.74	3.87	0.34	0.56
S-N-B (°)	363	77.26	3.92	55	77.07	3.91	0.19	0.74
A-N-B (°)	359	3.81	2.34	55	3.67	1.92	0.13	0.69
S-N-Pg (°)	363	77.63	3.83	55	77.27	3.87	0.36	0.52
Mx-SN (°)	364	6.83	3.31	55	6.7	3,18	0.13	0.79
Mn-SN (°)	364	32.21	5.44	55	33.03	5.03	-0.82	0.29
IIA (°)	342	126.28	9.38	55	125.12	9.91	1.15	0.4
Li-Mn (°)	344	98.31	6.35	55	98.14	6.64	0.17	0.86
Ui-Mx (°)	354	109.85	5.75	55	110.4	7.22	-0.55	0.53
LAFH (%)	363	58.17	2.52	55	57.77	2.52	0.4	0.27
LPFH (%)	364	60.38	3.63	55	60.52	3.6	-0.14	0.79
Overjet (mm)	362	3.96	1.94	55	4.07	1.58	-0.11	0.69

IIA- Interincisal Angle, Mn- Mandibular Plane, Mx- Palatal plane, LAFH- Lower anterior facial height, LPFH- Lower posterior facial height. Mean differences were compared by using independent samples t-test (*Adjusted P ≤ 0.004).

Table 4. Cephalometric characteristics of hypodontic and nonhypodontic girls (N= 380)

	Hypodontia							
	No			Yes				
Cephalometric measurements	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**Difference**	**P-value**
S-Ar-Go (°)	310	140.24	6.19	69	141.13	6.05	-0.89	0.28
N-S-Ar (°)	310	124.3	5.18	69	123.86	5.51	0.45	0.52
Ar-Go-Me (°)	310	129.53	5.05	69	127.97	5.33	1.56	0.02
S-N-A (°)	310	81.25	3.6	69	80.1	3.74	1.15	0.02
S-N-B (°)	311	77.1	3.51	69	76.5	3.59	0,6	0.2
A-N-B (°)	310	4.14	2.06	69	3.6	2.58	0.54	0.11
S-N-Pg (°)	311	77.35	3.57	69	77	3.74	0.35	0.46
Mx-SN (°)	310	7.22	3.34	69	6,37	3.15	0,85	0.06
Mn-SN (°)	311	33.35	5.37	69	32.79	4.62	0.55	0.43
IIA (°)	300	126.04	9.6	67	128.08	8.86	-2.04	0.11
Li-Mn (°)	301	97.59	6.59	68	96.92	6.44	0.67	0.45
Ui-Mx (°)	303	110.11	6.77	67	108.61	5.77	1.5	0.09
LAFH (%)	311	58.06	2.47	69	57.79	2.21	0.27	0.41
LPFH (%)	310	60.65	3.45	69	60	3.3	0.64	0.16
Overjet (mm)	307	4.26	2.02	67	4.26	2.23	-0.05	0.85

IIA- Interincisal Angle, Mn- Mandibular Plane, Mx- Palatal plane, LAFH- Lower anterior facial height, LPFH- Lower posterior facial height. Mean differences were compared by using independent samples t-test (*Adjusted P ≤ 0.004).

Table 5. Association of hypodontia with cephalometric parameters of children

		Hypodontia Subtypes			
	Hypodontia N= (124)	**Anterior (N=39)**	**Posterior (N=85)**	**Maxilla (N=38)**	**Mandible (N=95)**
	β (95% CI) *P*-Value	**β (95% CI) *P*-Value**	**β (95% CI) *P*-Value**	**β (95% CI) *P*-Value**	**β (95% CI) *P*-Value**
S-N-Pg (°)	–0.82 (–1.53, –0.10) P = 0.025*	–1.00 (–2.19, 0.20) P = 0.103	–0.73 (–1.56, 0.10) P = 0.086	–0.45 (–1.65, 0.75) P = 0.465	–0.67 (–1.47, 0.12) P = 0.099
S-N-B (°)	–1.00 (–1.71, –0.30) P = 0.005**	–1.36 (–2.54, –0.18) P = 0.024*	–0.84 (–1.66, –0.01) P = 0.046*	–0.69 (–1.88, 0.50) P = 0.256	–0.81 (–1.60, –0.03) P = 0.043*
A-N-B (°)	–0.22 (–0.65, 0.21) P = 0.313	–0.66 (–1.38, 0.06) P = 0.071	–0.02 (–0.52, 0.48) P = 0.931	–1.15 (–1.86, –0.43) P = 0.002***	0.14 (–0.33, 0.62) P = 0.558
S-N-A (°)	–1.22 (–1.95, –0.48) P = 0.001***	–2.02 (–3.25, –0.79) P = 0.001***	–0.86 (–1.72, 0.00) P = 0.050*	–1.84 (–3.07, –0.60) P = 0.004***	–0.66 (–1.48, 0.15) P = 0.111
Mn-SN (°)	0.64 (–0.38, 1.66) P = 0.221	0.44 (–1.26, 2.15) P = 0.611	0.72 (–0.47, 1.92) P = 0.234	0.49 (–1.22, 2.20) P = 0.575	0.59 (–0.55, 1.72) P = 0.311
S-Ar-Go (°)	0.70 (–0.41, 1.81) P = 0.237	0.17 (–1.78, 2.12) P = 0.864	0.94 (–0.42, 2.30) P = 0.175	–0.66 (–2.61, 1.29) P = 0.507	1.20 (–0.10, 2.49) P = 0.069
N-S-Ar (°)	0.57 (–0.44, 1.58) P = 0.275	1.79 (0.07, 3.49) P = 0.040*	0.02 (–1.17, 1.22) P = 0.974	1.39 (–0.30, 3.08) P = 0.112	–0.04 (–1.17, 1.09) P = 0.943
Mx-SN (°)	–0.04 (–0.65, 0.56) P = 0.891	–0.06 (–1.10, 0.99) P = 0.918	–0.04 (–0.77, 0.69) P = 0.918	0.21 (–0.82, 1.24) P = 0.693	–0.23 (–0.92, 0.46) P = 0.510
IIA (°)	1.87 (0.10, 3.64) P = 0.039*	2.34 (–0.62, 5.30) P = 0.121	1.65 (–0.42, 3.72) P = 0.118	3.07 (0.10, 6.05) P = 0.043*	1.48 (–0.49, 3.45) P = 0.141
Ui-Mx (°)	–1.04 (–2.26, 0.17) P = 0.091	–0.83 (–2.85, 1.20) P = 0.423	–1.14 (–2.56, 0.27) P = 0.114	–0.24 (–2.28, 1.79) P = 0.813	–1.37 (–2.71, –0.02) P = 0.047*
Ar-Go-Me (°)	–0.64 (–1.64, 0.35) P = 0.203	–1.54 (–3.18, 0.10) P = 0.068	–0.24 (–1.38, 0.90) P = 0.685	–0.27 (–1.94, 1.40) P = 0.753	–0.58 (–1.68, 0.52) P = 0.303

Table 5. (Continued)

		Hypodontia Subtypes			
	Hypodontia N= (124)	**Anterior (N=39)**	**Posterior (N=85)**	**Maxilla (N=38)**	**Mandible (N=95)**
	β (95% CI) *P*-Value	**β (95% CI) *P*-Value**	**β (95% CI) *P*-Value**	**β (95% CI) *P*-Value**	**β (95% CI) *P*-Value**
Li-Mn (°)	–1.41 (–2.62, –0.20) P = 0.022*	–1.86 (3.89, 0.16) P = 0.071	–1.21 (–2.63, 0.20) P = 0.094	–2.97 (–4.99, –0.94) P = 0.004***	–0.87 (–2.21, 0.47) P = 0.202
Overjet (mm)	0.22 (–0.16, 0.61) P = 0.258	0.20 (–0.45, 0.84) P = 0.553	0.23 (–0.22, 0.69) P = 0.308	–0.26 (–0.91, 0.39) P = 0.426	0.35 (–0.08, 0.78) P = 0.112
LAFH (%)	–0.39 (–0.88, 0.09) P = 0.109	–0.40 (–1.20, 0.41) P = 0.336	–0.39 (–0.96, 0.17) P = 0.172	–0.50 (–1.31, 0.31) P = 0.227	–0.37 (–0.90, 0.17) P = 0.180
LPFH (%)	–0.83 (–1.48, –0.18) P = 0.014*	–0.72 (–1.82, 0.39) P = 0.206	–0.88 (–1.59, –0.18) P = 0.025*	–0.30 (–1.37, 0.77) P = 0.597	–0.83 (–1.55, –0.12) P = 0.027*

IIA- Interincisal Angle, Mn- Mandibular Plane, Mx- Palatal plane, LAFH- Lower anterior facial height, LPFH- Lower posterior facial height.
Values are regression coefficients (β) with 95% CI that indicate the differences in cephalometric parameters between the reference sample (N=676) and specific types of hypodontia. Models are adjusted for age, sex, study sample and ethnicity of children.
*P ≤ 0.05; **P ≤ 0.01; ***Adjusted P ≤ 0.003.

Table 6. Association of hypodontia with principal components (PC) derived from cephalometric parameters of children

		Hypodontia Subtypes			
	Hypodontia N= (124)	**Anterior (N=39)**	**Posterior (N=85)**	**Maxilla (N=38)**	**Mandible (N=95)**
	β (95% CI)	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
	***P*-Value**	***P*-Value**	***P*-Value**	***P*-Value**	***P*-Value**
Facial Divergence (PC1)	–0.23 (–0.42, –0.04) P = 0.019*	–0.33 (–0.65, –0.01) P = 0.043*	–0.18 (–0.41, 0.04) P = 0.106	–0.20 (–0.52, 0.12) P = 0.227	–0.16 (–0.38, 0.05) P = 0.132
Posterior Facial Height (PC2)	–0.15 (–0.34, 0.04) P = 0.134	–0.02 (–0.34, 0.30) P = 0.889	–0.20 (–0.43, 0.02) P = 0.077	0.09 (–0.23, 0.41) P = 0.582	–0.22 (–0.44, –0.01) P = 0.041*
Incisor Angulation (PC3)	–0.26 (–0.45, –0.07) P = 0.006**	–0.34 (–0.65, –0.02) P = 0.038*	–0.23 (–0.45, –0.01) P = 0.041*	–0.37 (–0.69, –0.05) P = 0.022*	–0.22 (–0.43, –0.01) P = 0.038*
Mandibular Base (PC4)	–0.02 (–0.22, 0.17) P = 0.812	–0.11 (–0.43, 0.22) P = 0.512	0.01 (–0.21, 0.24) P = 0.898	0.13 (–0.19, 0.46) P = 0.431	–0.06 (–0.27, 0.16) P = 0.603
Anterior Facial Height (PC5)	0.11 (–0.08, 0.31) P = 0.253	0.19 (–0.13, 0.52) P = 0.243	0.08 (–0.15, 0.31) P = 0.506	0.32 (–0.01, 0.65) P = 0.054	0.02 (–0.19, 0.24) P = 0.830
Dental Relation-ship (PC6)	0.02 (–0.18, 0.21) P = 0.868	–0.09 (–0.42, 0.23) P = 0.581	0.07 (–0.16, 0.29) P = 0.574	–0.33 (–0.65, 0.00) P = 0.047*	0.13 (–0.09, 0.34) P = 0.236

Values are regression coefficients (β) with 95% CI that indicate the differences in standardized PC scores between the reference sample (N=676) and specific types of hypodontia. Models are adjusted for age, gender, study sample and ethnicity of children.
*P ≤ 0.05; ** P ≤ 0.01.

of the correlation coefficient ranged from 0.699 to 0.972. The reliability of the measurements done in the Nijmegen Growth Study was published earlier, and it was considered highly reliable (Ligthelm-Bakker et al. 1995). We added study sample as a covariate in the linear regression analysis to adjust for the potential measurement differences between the Nijmegen Growth Study and the Generation R Study.

Statistical analysis

The differences of individual cephalometric parameters and craniofacial patterns (PCs) between the hypodontia and reference groups were compared using multiple linear regression models. All linear regression models were adjusted for age, sex,

study sample, and ethnicity of the children. We tested for statistical interaction by adding the interaction term in the linear regression model.

The Markov Chain Monte Carlo imputation method was used to reduce potential bias associated with missing data (Sterne et al. 2009).The numbers of missing values for each variable are provided in Table S3. As a result, 5 imputed data sets were generated, and a pooled effect estimate was calculated. The analysis was performed with the statistical software SPSS for Windows (version 21.0; IBM, Armonk, NY). We used the following thresholds of the P values: 0.05, 0.01, and the Bonferroni-adjusted threshold that takes into account the number of statistical comparisons and the average cross-correlation coefficient of outcome variables given the alpha level of 0.05 (Wright 1992).

RESULTS

We did not observe any significant interactions between hypodontia and any of the adjusting covariates (age, sex, ethnicity, study population) in the regression model. Hence, the results were grouped and assessed per classification.

For the association of hypodontia and individual cephalometric parameters, the results of the linear regression analysis are presented in Table V. Children with hypodontia had retrognathism of the maxilla and the mandible, with significantly reduced angles: S-N-Pg, −0.82° (95% confidence interval [CI], −1.53, −0.10); SNB, −1.00° (95% CI −1.71, −0.30); and SNA, −1.22° (95% CI, −1.95, −0.48). Subcategorizing hypodontia into maxillary and mandibular or anterior and posterior hypodontia showed that the SNA value was significantly smaller in children with anterior hypodontia, −2.02° (95% CI, −3.25, −0.79), and maxillary hypodontia, −1.84° (95% CI, −3.07, −0.60), compared with the reference children. Children with maxillary hypodontia had more negative ANB angles (P ≤0.003) than both reference children and children with mandibular hypodontia, −1.15° (99.7% CI, −1.86, −0.43), and −1.27° (99.7% CI, −2.11, −0.44) (not presented in Table V), respectively. Also, angle N-S-Ar was significantly increased in children with anterior hypodontia, 1.79° (95% CI, 0.07, 3.49), compared with the reference children.

Furthermore, children with hypodontia had an increased interincisal angle and a reduced mandibular incisor angle, 1.87° (95% CI 0.10, 3.64), and −1.41° (95% CI, −2.62, −0.20), respectively; these were especially prominent in children with maxillary hypodontia: 3.07° (95% CI, 0.10, 6.05) and −2.97° (95% CI, −4.99, −0.94), respectively.

The maxillary incisor angle was significantly reduced in children with mandibular hypodontia, −1.37 (95% CI, −2.71, −0.02).

For the association of hypodontia and specific craniofacial patterns (PCs), the results of the linear regression analysis with standardized PC scores are presented in Table VI. Our findings indicate that children with hypodontia have increased interincisal angles with retrusion of both maxillary and mandibular incisors (incisor angulation [PC3] = −0.23; 95% CI, −0.42, −0.04). Also, we observed a tendency toward high-angle (hyperdivergent) facial patterns in the case of hypodontia (PC3 = −0.26; 95% CI, −0.45, −0.07), especially prominent in anterior hypodontia (PC3 = −0.33; 95% CI, −0.65, −0.01). A subgroup analysis showed that children with mandibular hypodontia had reduced posterior facial height and N-S-Ar (posterior facial height [PC2] = −0.22; 95% CI, −0.44, −0.01). Children with maxillary hypodontia have a tendency toward Class III malocclusion (decreased overjet and ANB; dental jaw relationship [PC6] = −0.33; 95% CI, −0.65, 0.00).

DISCUSSION

The findings of our study suggest that children with mild hypodontia have distinctive skeletal and dental characteristics. The main difference in the results of the 2 methods, conventional and PC analyses, is that PC analysis showed that children with all types of hypodontia have a decreased PC3, indicating an increased interincisal angle and retroclination of the maxillary and mandibular incisors. Furthermore, children with hypodontia, especially those with anterior hypodontia, had a hyperdivergent (high angle) facial pattern. Changes in individual cephalometric parameters observed in the PC analysis mostly agree with the conventional analysis. However, this and previous studies have suggested PC analysis as a preferred method because the statistical relationship between individual cephalometric parameters cannot be investigated with a conventional analysis (Halazonetis 2004; Wellens et al. 2013).

Many previous studies were consistent in reporting retroclination of the maxillary incisors (Chung et al. 2000; Endo et al. 2006; Endo et al. 2004; Sarnas and Rune 1983). Ogaard and Krogstad (1995) suggested that retroclination of the maxillary incisors occurred because of reduced lingual support. Most studies reported retroclination (Chung et al. 2000; Endo et al. 2006; Ogaard and Krogstad 1995) or a neutral position of the mandibular incisors (Kreczi et al. 2011). In our study, retroclination of the mandibular incisors was particularly prominent in children with maxillary hypodontia (P = 0.004). One explanation for this finding is that children with maxillary hypodontia have an underdeveloped maxilla (reduced SNA angle; P = 0.004). Previous studies in

children with 10 or more missing teeth reported a significant decrease of the SNA angle (Ben-Bassat and Brin 2003; 2009; Ogaard and Krogstad 1995). Although Kreczi et al. (2011) observed a reduced SNA in children with hypodontia, the subgroup analysis showed that SNA was significantly reduced only in children with missing teeth in both jaws. The severity of hypodontia also had an influence on the extent of SNA angle reduction: it was reported to decrease by 0.3° with each missing tooth (Acharya et al. 2010).

The exact cause of maxillary retrognathism in subjects with hypodontia is still being disputed. One explanation is that the maxilla either could be underdeveloped, resulting in a shorter sagittal length, or could be more posteriorly positioned in relation to the cranial base (Roald et al. 1982; Sarnas and Rune 1983). Factors contributing to the shortening of the maxilla were a bone reduction of the maxillary tuberosity in posterior hypodontia and bone reduction of the anterior alveolar process in anterior hypodontia (Endo et al. 2004). Our findings partly confirm these arguments, since we demonstrated a substantial reduction of the SNA angle in children with anterior hypodontia. A second mechanism was suggested by Ogaard and Krogstad (1995) who proposed that maxillary retrusion occurs as a consequence of anterior mandibular rotation because of lack of support of the posterior teeth. A third mechanism could be an increased cranial base angle in children with hypodontia (Sarnas and Rune 1983). We did not measure the cranial base angle. However, we identified a slight increase of the N-S-Ar angle, which was significant only in children with anterior hypodontia.

The facial divergence component (PC1), characterized mainly by parameters S-N-Pg, SNA, SNB, Mx-SN, and Mn-SN, was lower in children with hypodontia. All of these angles describe the vertical relationship or the high or low angle craniofacial pattern (Halazonetis 2004; Wellens et al. 2013). Therefore, a child with hypodontia will have the angles Mx-SN and Mn-SN increased but will also have both angles SNA and SNB decreased (corresponds to a lower PC1 value); this indicates a high-angle (hyperdivergent) facial pattern because the cranial base is inclined at a greater angle (i.e., sella is low) (Creton et al. 2010). Bondarets and McDonald (2000) showed, however, that patients with hypodontia had low-angle (hypodivergent) face types because of a reduction in anterior face height. In our study, the opposite findings may be due to a decrease of lower posterior facial height in children with hypodontia. Studies in other populations reported no significant association between high or low angle craniofacial pattern and hypodontia (Celikoglu et al. 2010; Chung et al. 2008).

The position of the mandible in relation to the cranial base, which was expressed by angles SNB and S-N-Pg, indicated a significant mandibular retrognathism of around 1°, compared with the reference children. Acharya et al. (2010) also found

a significant reduction of the SNB angle but no change in the S-N-Pg angle. Quite opposite, some authors reported increased SNB angles in children with hypodontia (Endo et al. 2004; Roald et al. 1982) or observed no differences in hypodontia subjects (Sarnas and Rune 1983). Subgroup analysis in this study showed that reduced SNB angles were evident in both anterior and posterior hypodontia. Regarding the jaw where the tooth was missing, we only observed a significant reduction of the angle SNB in children with mandibular hypodontia. Somewhat similar findings were reported by Kreczi et al. (2011), who identified a significantly reduced SNB angle in subjects with mandibular or both-jaw hypodontia. Although hypodontia is associated with an anterior alveolar process and chin bone reduction, our findings demonstrate greater bone reduction at the alveolar process, particularly in children with mandibular hypodontia.

In our study, we observed a significant reduction of the angle ANB and decreased overjet (corresponds to a lower PC6 value) in children with maxillary hypodontia compared with children with mandibular hypodontia or compared with the reference children. This might indicate that in mandibular hypodontia both jaws are equally retruded. However, in maxillary hypodontia, retrusion of the maxilla is much greater than in the mandible; this might contribute to the Class III relationship between the jaws. Other studies have identified a smaller ANB angle (Sarnas and Rune 1983) or a normal ANB angle in subjects with hypodontia (Ben-Bassat and Brin 2009). Thus, we can conclude that a decreased ANB angle is to be expected mostly in patients with maxillary hypodontia caused by maxillary retrognathism.

Our findings also suggest a reduction of LPFH in children with hypodontia. Also, a subgroup analysis showed that a posterior and mandibular hypodontia was significantly associated with the reduction of PC2. Other studies, however, showed a decrease of LAFH in subjects with hypodontia (Acharya et al. 2010; Bondarets and McDonald 2000; Ogaard and Krogstad 1995). In our study, children with maxillary hypodontia had a reduced but nonsignificant value of the LAFH parameter (PC5; $P = 0.054$). The reduction of vertical parameters could be attributed to lack of the tooth support and the compensating increase of the angle S-Ar-Go as indicated by the increase of PC2-posterior facial height in children with mandibular hypodontia.

Previous case-report studies have pointed out that sometimes it may be problematic to identify hypodontia from panoramic radiographs because of late calcification of the second premolars at the expected age, giving the false impression of agenesis (Bicakci et al. 2012; Memmott et al. 1985). However, given the sporadic character of these cases and the age of the children with hypodontia in our sample (all older

than 9 years), it may be unlikely that these cases occur and even less likely that they influence our results.

The PC analysis is a useful adjunct to the standard cephalometric analysis, but it carries potential limitations. Intercorrelation and, therefore, a combination of cephalometric parameters within a particular PC is based on the statistical and not the biological aspects (Halazonetis 2004). As a result, it may be difficult to find the linkage and, therefore, interpret the output of the standardized PC scores. Moreover, with standardized PC scores, it is hard to express the effect size because of the absence of measuring units. We compensated for this by also analyzing conventional cephalometric parameters. Also, although eigenvalues, scree plots, statistical tests, cross-correlations, and interoperability may serve as guides, the selection of PCs is always a subjective question for the investigator (Moreno Uribe et al. 2013).

CONCLUSIONS

The findings of our study suggest that children with mild hypodontia have distinctive skeletal and dental characteristics: (1) anterior hypodontia was significantly associated with the high-angle (hyperdivergent) craniofacial pattern, (2) the tendency toward a Class III malocclusion was identified in subjects with maxillary hypodontia, (3) we observed a significant reduction of lower posterior facial height in children with posterior and mandibular hypodontia, and (4) PC analysis showed a common dental characteristic for all types of hypodontia: a significant increase of the interincisal angle, and decreases of the maxillary and mandibular incisor angles. By reducing the number of parameters in the cephalometric analysis, PC analysis proved to be a useful tool for simplifying the analysis and identifying patterns of changes in craniofacial structures.

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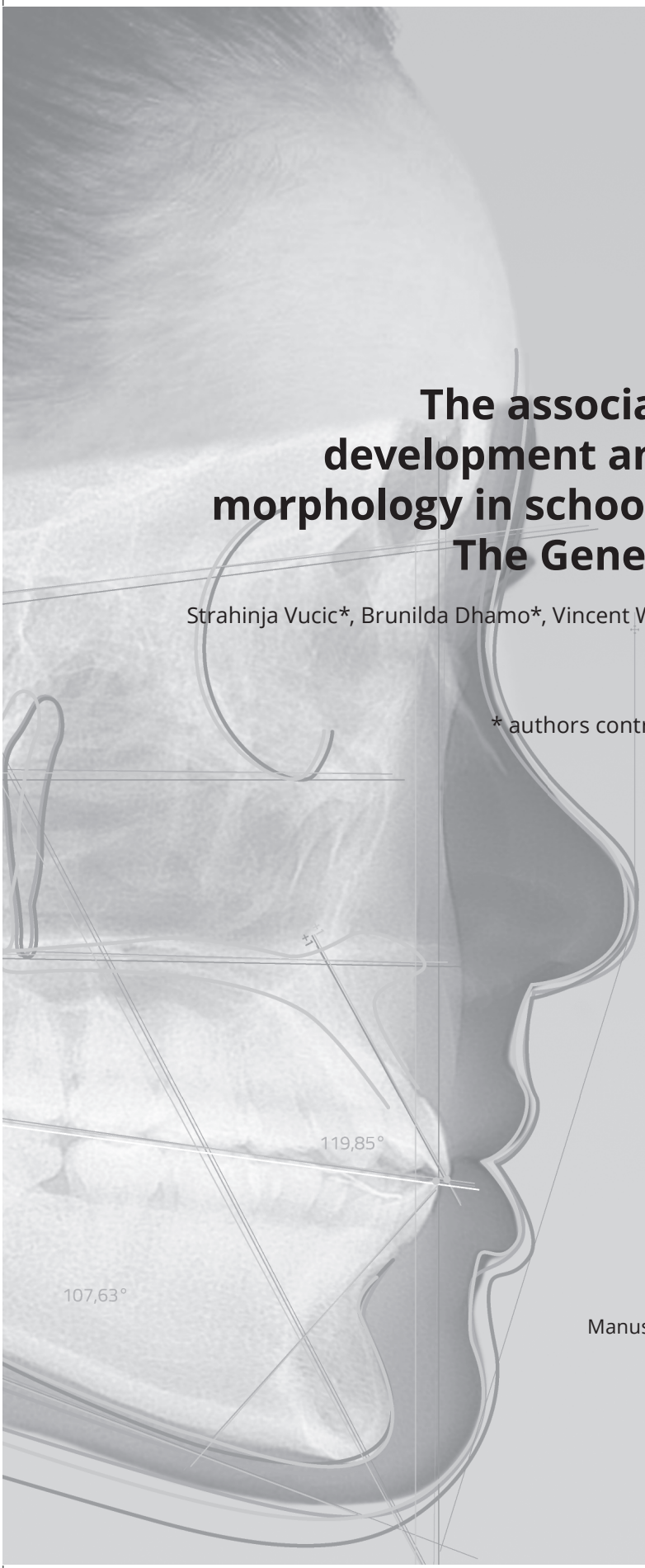

Chapter 7

The association of dental development and craniofacial morphology in school-age children: The Generation R study

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ABSTRACT

Introduction: The growth of the craniofacial complex is important for establishing a balanced relationship between the teeth, jaws and other facial structures. However, there is still a lack of information about craniofacial parameters that are affected by the rate of dental development. The aim of this study is to investigate the association between dental development and craniofacial morphology in school-age children.

Methods: This study was embedded in the Generation R Study, Rotterdam, the Netherlands. In 3,896 children between 8 and 11 years, dental development was assessed from panoramic radiographs and craniofacial morphology was assessed by combining cephalometric parameters into nine uncorrelated principal components, each representing a distinct skeletal or dental craniofacial pattern. The statistical analysis was performed using (non)linear regression models.

Results: Dental development was positively associated with the bimaxillary growth (β=0.04, 95%CI: 0.01, 0.08). Children with above-average dental development had a tendency towards Class II jaw relationship (β=-0.08, 95%CI: -0.13, -0.04). As regard to dental parameters, the proclination increased for incisors and lips with advanced dental development (β=0.15, 95%CI: 0.10, 0.19 and β=0.13, 95%CI: 0.09, 0.17, respectively), still the incisor proclination was more pronounced in children that had above-average dental development.

Conclusions: The findings of this large population-based study show that dental development is associated with specific dental and skeletal cephalometric characteristics in school-age children. Further longitudinal studies are necessary to confirm observed effects over time.

INTRODUCTION

The growth of the craniofacial complex is important for establishing a balanced relationship between teeth, jaws and other facial structures that participate in the formation of occlusion. Disturbances in the development of craniofacial structures may lead to malocclusions, which require orthodontic treatment or sometimes even orthognathic surgery. Therefore, understanding genetic, epigenetic and environmental factors which affect the occurrence of these disturbances have significant clinical value (Dixon et al. 1997).

Genes which regulate the migration of ectomesenchymal cells and the cells of neural crest are responsible for the beginning of facial development around 28 days of gestation (Nanci 2007). Strong genetic influence is also evident in studies that investigated the variability of craniofacial parameters in populations with different ethnic background (Wen et al. 2015), and in studies that examined craniofacial characteristics in patients with specific congenital syndromes (Buchanan et al. 2014). On the other hand, the epigenetic and environmental component of craniofacial development is still largely unknown. Biological indicators of craniofacial morphology that have been previously investigated are nutrition (Sadeghianrizi et al. 2005), growth and other hormones (Pirinen 1995), height (Pelin et al. 2010; Shrestha et al. 2015) and skeletal maturation (Flores-Mir et al. 2004; Helm et al. 1971; Mellion et al. 2013; Verma et al. 2009).

Dentition and the rate of its development play a role in the development of surrounding tissues of the face. For instance, children with a vertical growth pattern or a long face have advanced dental development compared to children with horizontal growth pattern or short face subjects (Neves et al. 2005). Also, the change of vertical dimensions of occlusion and the occurrence of malocclusions occur most often during the eruption of deciduous and permanent teeth (Crawford and Aldred 2012). During the process of eruption and simultaneously to the development of teeth, important changes in the growth patterns may occur in the adjacent hard and soft tissues. Ultimately, this process facilitates the movement of the teeth until reaching the plane of occlusion. Furthermore, local or general disturbances in the dental development are associated with structural, morphological and positional abnormalities of the teeth (Crawford and Aldred 2012; Dhamo et al. 2016; Suri et al. 2004; Vucic et al. 2017), which also impact the facial morphology represented by cephalometric parameters.

Although previous studies acknowledge the importance of considering dental eruption and type of dentition when examining dental, skeletal and soft tissue relationships in the facial region (Baccetti et al. 2007; Thilander et al. 2001), studies, which investigated the impact of the rate of dental development on craniofacial

morphology, are scarce (Esenlik et al. 2014; Neves et al. 2005). Furthermore, studies on this topic in a large population-based cohort are lacking.

Therefore, the aim of this population-based prospective cohort study was to investigate whether the rate of dental development is associated with specific dental and skeletal characteristics in the craniofacial region of school-age children.

METHODS

Design

This study was embedded in the Generation R Study, a multi-ethnic population-based prospective cohort study from fetal life onwards, which was initiated to identify early environmental and genetic determinants of growth, development, and health (Kruithof et al. 2014). The study was approved by the Medical Ethics Committee of the Erasmus Medical Centre (MEC-2012-165) in Rotterdam, the Netherlands. At the start of each phase, mothers, and their partners were asked for their written informed consent. This study conformed to Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for human observational studies.

Study Population

Out of 8548 children invited to participate in the Generation R Study at the age of nine, 2501 were not available for the study and 185 twins were excluded. The remaining 5862 children visited the research center (Figure 1). Out of those children, 1301 had no dental panoramic radiograph (DPR), 114 had a DPR of bad quality, and in 75 dental development could not be assessed due to symmetric hypodontia in the lower jaw. Finally, 476 children without proper cephalometric assessment were excluded. The remaining 3896 singleton children (1950 boys and 1946 girls) aged 9.8±0.3 years were eligible for the study.

Dental Development Assessment

DPRs and cephalograms of children were exposed in a standardized manner by trained personnel using a digital dental imaging unit (OP/OC 200D, Tuusula, Finland). Tooth development was quantified on DPRs using the method described by Demirjian (Demirjian et al. 1973). Following this approach, seven teeth excluding third molars located on the left side of the mandible were scored with one of the eight developmental stages (A-H), depending on the calcification of the crown and root. Each child's overall dental development was established by calculating the mean value of the standard deviation (SD) scores for the seven teeth. The overall dental development score was normalized using rank-based transformation due

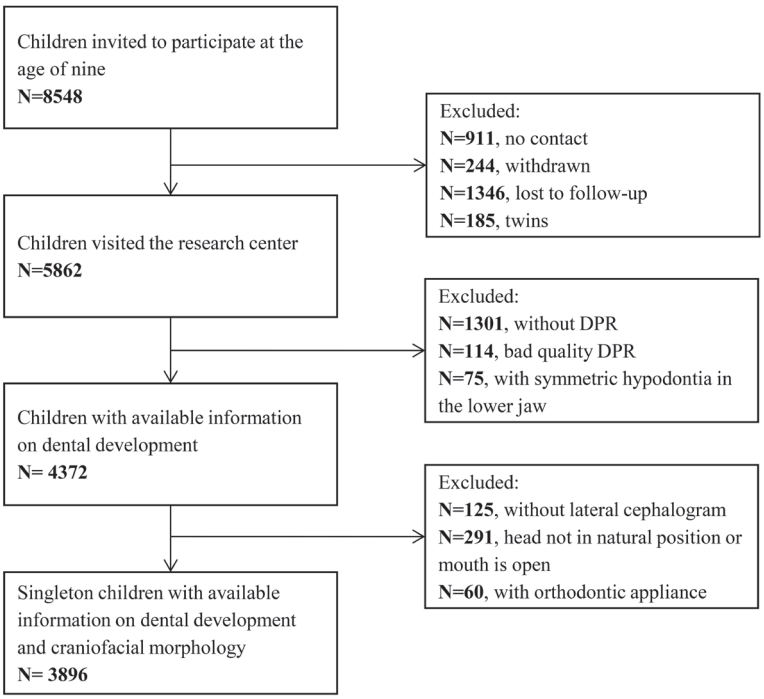

Figure 1. Flowchart of the participants included in the study. Abbreviation: DPR- dental panoramic radiograph; N- the number of subjects.

to non-normal distribution. So, for example, children whose dental development was more advanced were referred to as having an above average SD score and children whose dental development was more delayed were referred to as having a below average SD score. The inter-observer agreement between two raters was performed on a random subsample of 100 subjects for each of the seven teeth using the intraclass correlation statistic, and coefficients ranged between 0.653-0.797 which is considered to be a substantial agreement according to the conventional criteria (Landis and Koch 1977). Central incisors were not taken into account due to the absence of variation in the stage of tooth development.

Cephalometric Parameters Assessment

In total, 22 cephalometric landmarks were used in this study and from these points, 35 cephalometric parameters were derived; 16 angular, 15 linear, and 4 indices (Table S1 and S2). A cephalometric analysis including measures adopted from the analyses of Down, Steiner, Ricketts, and Pancherz was performed on each tracing.

(Downs 1956; Pancherz 1982; Ricketts 1960; Steiner 1959). Cephalometric points were digitized by a trained investigator using Viewbox software, version 4.0 (dHAL Software, Kifissia, Greece). Interobserver agreement was calculated based on the subsample of 93 subjects, and intraclass correlation statistic ranged between 0.710-0.931 which is considered to be a substantial agreement according to the conventional criteria (Landis and Koch 1977). To efficiently reduce the number of cephalometric parameters we combined highly correlated parameters using the principal component (PC) analysis (Al-Moraissi and Ellis 2014; Halazonetis 2004). The use of this method in our study sample has been described in a previous study (Vucic et al. 2016). Briefly, 47 cephalometric parameters were combined into nine PCs, each representing a distinct skeletal or dental craniofacial pattern (Figure 2 and Table S3). In total, we identified six skeletal craniofacial patterns: Facial divergence, Bimaxillary growth, Sagittal jaw relationship, Ramus height, Lower Anterior facial height and Cranial base angle. Lip position, Incisor angulation, and Overjet were identified as dental craniofacial patterns.

Covariates

Information on child's sex and date of birth were available from medical records and hospital registries. The age of a child was calculated as the interval between the date when the DPR was taken and the date of birth. Height and weight of the children were measured by trained personnel at the research center following a previously described protocol and, subsequently, BMI was calculated. We obtained information on ethnicity and maternal educational level using questionnaires. Ethnicity and educational attainment were defined according to the classification of Statistics Netherlands. One experienced examiner ascertained hypodontia from the DPRs. Children with hypodontia had one or more congenitally missing teeth (no sign of formation or calcification showed in DPR). Covariates were included in the regression models based on previous literature or a change of >10% in effect estimates.

Statistical Analysis

The association between dental development with each of the nine PC of craniofacial morphology was analyzed using linear regression models. Regression models were adjusted for age, sex, BMI, height, ethnicity, maternal education and hypodontia. The non-linearity of exposure variables was tested by utilizing restricted cubic splines with 3 to 5 knots. Values of variables dental development score and craniofacial PCs were considered outliers and excluded if values were outside the range, -3 and +3 SD. Statistical interaction between dental development and child's sex was investigated by adding a product term of this two variables in the linear regression analysis. Missing data were handled by generating five imputed datasets using the

Markov Chain Monte Carlo method, from which the pooled effect estimates are presented in this study [effect size (β); 95% confidence intervals (CI) and p-value]. Results were considered statistically significant for a p-value ≤0.05. Statistical analyses in this study were performed using statistical software SPSS version 21.0 (IBM, New York, USA) and R statistical package version.3.3.2 (R, Vienna, Austria).

RESULTS

Subject characteristics

The characteristics of the study population are provided in Table 1. Among all children included in this study, 2305 (59.2%) were Dutch, and 1502 (38.6%) were of other ethnicity. One-sided hypodontia was found in 144 (3.7%) cases. The largest group of mothers attained higher education (N=1845; 47.4%) followed by those with secondary education (N=1450, 37.2%), and 273 (7.0%) mothers attained primary education. The median stage of development for mandibular canines, first premolars, second premolars, and second molars was six (out of eight); while mandibular central incisors, second incisors, and first molars almost reached the full development, presenting a median stage of 8. The mean value of child's BMI was 17.62±2.79 kg/m2 and children were on average 141.70±6.72 cm tall. Craniofacial PC and dental development were converted to standard deviation scores with mean value = 0 and SD= 1 (not shown in the table).

The rate of dental development and craniofacial morphology

The results of the association between dental development and nine cephalometric patterns are presented in Table 2 and illustrated in Figure 2. Advancement in dental development was associated with a decrease in the Sagittal jaw relationship PC (β=-0.08, 95%CI: -0.13, -0.04), indicating a tendency towards skeletal Class II relationship. However, by applying a non-linear transformation to the dental development, we observed that tendency toward Class II relationship was mainly present in children with above-average dental development (dental development score >0 SD; Figure 3A). Also, dental development was positively associated with the Bimaxillary growth PC (β=0.04, 95%CI: 0.01, 0.08). The results of the linear regression analysis showed that with increasing dental development score, the Ramus height PC (β=0.04, 95%CI: 0.00, 0.09), and the Lower anterior facial height PC increased (β=0.04, 95%CI: 0.00, 0.09) with borderline significance (P=0.05). No significant association was observed for dental development with the Facial divergence PC and Cranial Base Angle PC.

As regards to dental parameters, the Lip position PC and Incisor angulation PC were increasing along with dental development (β=0.15, 95%CI: 0.10, 0.19 and β=0.13,

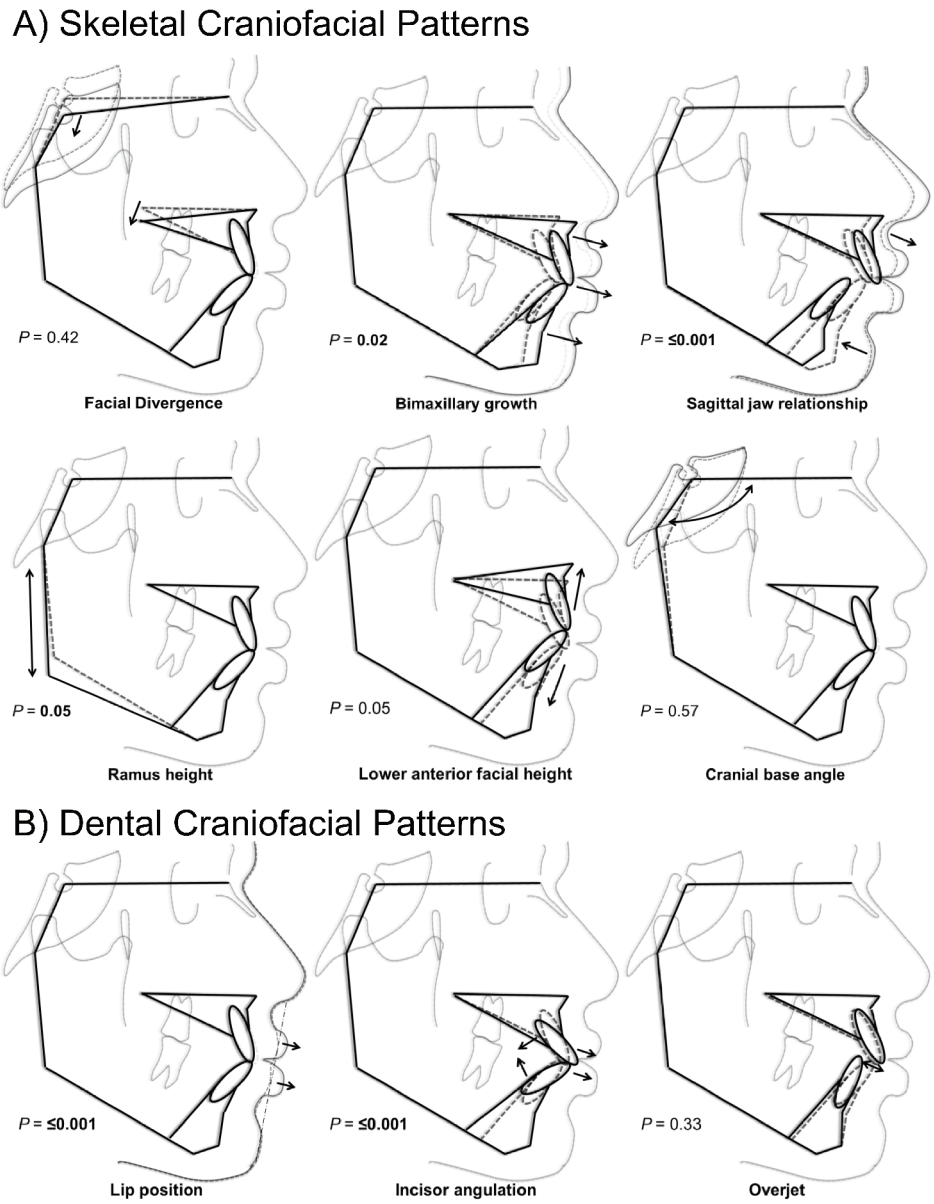

Figure 2. Changes in cephalometric parameters with increasing dental development score. Craniofacial parameters are represented by principal components (PC) each representing a specific skeletal (A) or dental (B) craniofacial pattern. P value denotes a significance level of the association between dental development and craniofacial PCs.

Table 1. Characteristics of subjects included in the study (N=3896).

General characteristics	Value
Girls (N, %)	1946 (49.9)
Chronological age [mean (SD); years]	9.81 (0.33)
Ethnicity (N, %)	
Dutch	2305 (59.2)
Non-Dutch	1502 (38.6)
Missing	89 (2.3)
Maternal education (N, %)	
Primary	273 (7.0)
Secondary	1450 (37.2)
Higher	1845 (47.4)
Missing	328 (8.4)
Body mass index [median, (IQR); kg/m2]	16.96 (14.4, 23.1)
Height [mean, (SD); cm]	141.73 (6.6)
Dental characteristics	
Stage of tooth development [median, (IQR)])	
Central incisor	8 (8-8)
Lateral incisor	8 (8-8)
Canine	6 (5-7)
First premolar	6 (5-7)
Second premolar	6 (5-7)
First molar	8 (7-8)
Second molar	6 (4-7)
Hypodontia cases (N, %)	144 (3.7)

Values for categorical variables and continuous variables with a skewed distribution are represented as frequency (N) with a percentage (%) or median value with interquartile range (IQR). Values for continuous variables with a normal distribution are represented as mean values with standard deviation (SD).

Table 2. The association between dental development and craniofacial morphology.

Dental development (SDS)	Craniofacial patterns								
	Skeletal						Dental		
	Facial divergence	**Bimaxillary growth**	**Sagittal jaw relationship**	**Ramus height**	**Lower anterior facial height**	**Cranial base angle**	**Lip position**	**Incisor angulation**	**Overjet**
β	-0.02	0.04	-0.08	0.04	0.04	0.01	0.13	0.15	0.02
95% CI	-0.06; 0.03	0.01; 0.08	-0.12; -0.03	0.00; 0.09	0.00; 0.09	-0.03; 0.06	0.09; 0.17	0.10; 0.19	-0.02; 0.06
P-value	0.42	0.02	≤0.001*	0.05	0.05	0.57	≤0.001	≤0.001*	0.33
N	3.878	3.875	3.876	3.883	3.872	3.878	3.871	3.875	3.851

*significant non-linear effect (P ≤0.05)
Models are adjusted for age, sex, BMI, height, ethnicity, maternal education and hypodontia.
Abbreviations: BMI– body mass index, β –regression coefficients, CI – confidence interval; SDS- standard deviation score; N- the number of subjects in the imputed dataset and after exclusion of outliers (±3SD).
Significant p-values are presented in bold.

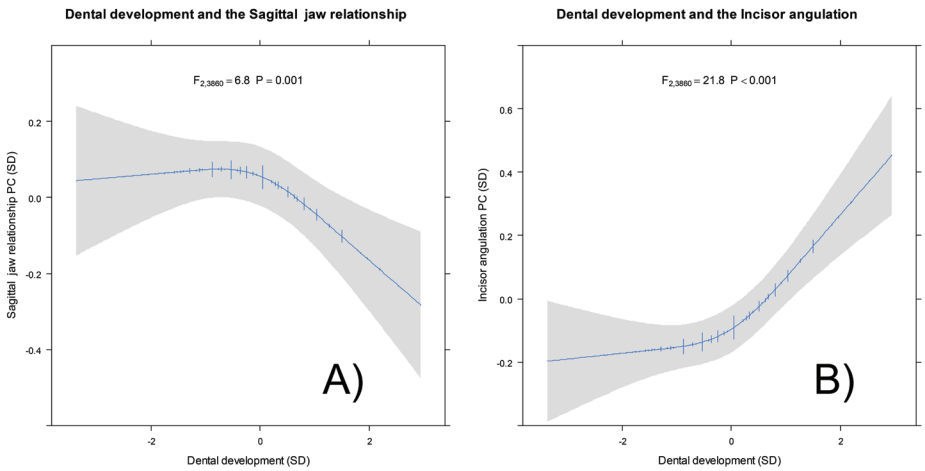

Figure 3. Nonlinear association between Dental development and Craniofacial Principal Components.
The x-axis indicates a change in standard deviation score of dental development. Lower values indicate delayed dental development and higher values indicate advanced dental development.
The y-axis indicates a change in standard deviation score of:
A) Sagittal jaw relationship principal component. Lower values indicate a tendency toward skeletal Class II relationship and higher values indicate a tendency toward skeletal Class III relationship.
B) Incisor angulation principal component. Lower values indicate an incisor retroclination and increased interincisal angle, and higher values indicate incisor proclination and decreased interincisal angle.
The model was adjusted for age, gender, body mass index, height, ethnicity, maternal education and hypodontia.

95%CI: 0.09, 0.17, respectively), indicating an increased proclination for both incisors and lips. Still, the incisor proclination was more pronounced in children that had above-average dental development (dental development >0 SD, Figure 3B), based on the non-linear transformation of dental development. We did not observe a significant association between dental development and Overjet PC.

Statistical Interaction

We did not observe a significant statistical interaction between dental development and child's sex.

DISCUSSION

The results of this study indicate that the rate of dental development is associated with the bimaxillary growth of the craniofacial complex and to some extent changes in vertical facial parameters, as represented by the increased ramus height and the lower anterior facial height. Furthermore, children with advanced dental development showed a tendency toward Class II jaw relationship. This tendency was more pronounced in children with above-average dental development compared to children with normal and below-average dental development. The strongest effect of advanced dental development was shown for dental parameters pronounced in the incisors proclination and lip protrusion.

Our findings indicate that dental development is positively associated with vertical and sagittal jaw growth. A common genetic background is the most probable explanation why the development of these two traits is closely related. *BARX1, PITX2, MSX, DLX* are active genes which are involved in the development of first pharyngeal arch from which facial bones, maxilla and mandible are derived. *BARX1* regulates jaw, muscle, and tongue development (Tissier-Seta et al. 1995), *PITX2*, regulates the development of oral ectoderm (Mitsiadis et al. 1998), *MSX* is involved in the migration of neural crest and mesenchymal cells (Blin-Wakkach et al. 2001), and *DLX* is involved in the development of the maxillary and mandibular arch (Wu et al. 2015). The same genes are involved in the process of odontogenesis. *BARX1* takes part in the early stage of odontogenesis (Nanci 2007; Thesleff and Sharpe 1997), *PITX2* is expressed in all cells of the tooth bud (Mitsiadis et al. 1998), *MSX* shows regulatory capabilities specific to root formation (Yamashiro et al. 2003) and *DLX* regulates amelogenesis (Zhang et al. 2015). Therefore, we postulate that *BARX1, PITX2, MSX, DLX* genes have a biologically pleiotropic effect on both craniofacial and dental development. This could also explain why similar craniofacial changes are also present in subjects with

tooth agenesis (Vucic et al. 2016). Further genetic studies are necessary to investigate the genetic background of the complex dentofacial growth.

In addition to the known conventional genetic components that control craniofacial skeletal growth, the literature raises the importance of the growth of craniofacial bones as a mechanical response to the development of functional matrices, such as teeth, muscles, salivary glands, sinuses and other tissues (Moss 1997). Acting as a functional matrix, dental development contributes to the sagittal and vertical growth of the maxilla and mandible, which undergo a rapid remodeling process in the age period from 3 to 18 years of age (Bjork and Skieller 1977; Moss 1997). In our study, development of the teeth contributes to the increase of vertical facial parameters, as a result of the increased ramus height and the lower anterior facial height. A possible mechanism which explains our findings is that the eruption and development of the maxillary and mandibular teeth, as well as the growth of the maxillary bone in the vertical direction, triggers the compensatory vertical growth of the mandible. Thus, the plane of occlusion is maintained in a straight line (Nanci 2007). However, if the dental development and eruption are accelerated, the lowered maxillary arch comes in earlier contact with the mandibular teeth in the posterior region which leads to downward and backward rotation of the mandible (Bjork 1969). This is why we also observed a tendency toward Class II occlusion in children with advanced dental development, which was also reported in previous studies (Celikoglu et al. 2011; Esenlik et al. 2014). Another explanation for the tendency toward class II might be a difference in the response between the maxilla and the mandible to the teeth which grow inside them. As the maxilla is a fixed bone with a spongy structure, the effect of the forces generated by the developing teeth would also be increased growth of the upper jaw. In contrast, the mechanical forces created by the growing teeth inside the mandible are reduced by the compact structure of the bone. Therefore, it seems that the growth of the teeth favors the growth of the upper jaw. However, by looking into the nonlinear relationship between dental development and sagittal jaw relationships (Figure 3A), we demonstrated that the tendency toward Class II was only present in the children with above-average dental development. In contrast, normal and delayed dental development did not have an effect on the sagittal jaw relationship.

We also demonstrated the incisor proclination and the lip protrusion in relation to the nose chin line in children with advanced dental development. Further, the lip protrusion is independent of the effect of incisor and vice versa, due to the weak correlation between PCs. We can postulate that with earlier development of the upper teeth, incisors erupt in a more labial direction to increase the length of the arch.

Again, by looking at the non-linear relationship between dental development and Incisor angulation PC (Figure 3B), we observed that incisor proclination is only present in children with higher than the average dental development. In contrast, we did not observe any association between dental development and incisor angulation in children with normal or delayed dental development probably due to the compensatory growth of maxilla, which comes into balance with developing maxillary teeth. Other studies reported an increased incisor proclination during the mixed dentition, which later stabilizes in the permanent dentition (Baccetti et al. 2007; Thilander et al. 2001). Therefore, careful interpretation of our findings is necessary as a consequence of catch-up growth of maxilla late mixed and permanent dentition period. With respect to the soft tissue parameters, we assume that the nose and chin are located more backward in children with advanced dental development, from which lips seem more protruded.

The differences in facial growth patterns between boys and girls have been studied in the past. Studies have reported sex- specificity changes in hard and soft tissue parameters (Kau and Richmond 2008). Some studies showed that malocclusions of II or III are more prevalent in males. On the other hand, it is reported in the literature that facial growth patterns do not differ until about 12 years of age (Bittner and Pancherz 1990; Nanci 2007). In line with previous studies, we adjusted for child's sex in the regression analysis to take into account gender differences. However, we did not analyze boys and girls separately as some previous studies did, due to non-significant interaction term between child's sex and dental development score.

The findings of this study could be used as a clinical guide to the early diagnosis, treatment planning and prognosis of the orthodontic treatment. Early assessment of the rate of dental development in a child could help orthodontist estimate the facial type or jaw relationship in the later stage of life. For instance, identifying an advanced dental development or eruption might indicate a tendency towards Class II jaw relationship, bimaxillary and lip protrusion or incisor proclination. Further, assessing the rate of dental development could serve as a favoring or impeding prognostic factor for the orthodontic treatment. Therefore, the next step would be to develop a predictive model that could estimate craniofacial development at a later stage of life or even predict the occurrence of craniofacial related anomalies, based on the dental maturity score calculated and filled in by the clinicians. Further studies are necessary that will take into account clinical parameters to develop and validate a predictive model of craniofacial development. Ultimately, the final deci-

sion for the adequate treatment would remain primarily based on individual patient characteristics, clinical parameters and the expertise of a clinician.

Strengths and limitations

A prior strength of our study is the inclusion of a large number of subjects from a multi-ethnic population-based prospective cohort design, with exclusive measurements of dental development and craniofacial characteristics.

In studies which analyze the human face, large inter- and intrapopulation variation occurs due to numerous genetic, epigenetic and environmental factors which regulate the process of human craniofacial growth and development. (Wen et al. 2015) In spite of adjusting analysis for multiple confounders, residual confounding may still be an issue in our study. For example, environmental factors such as general living conditions, nutrition, health status and stressors have been strongly associated with growth and development status (Cameron and Bogin 2012; Dasgupta and Hauspie 2013; Vucic et al. 2017). Due to practical limitations of the study, some confounders were addressed by adding similar variables to the confounders (proxy confounders). For example, we adjusted for the education of mothers which resembles a socio-economic class of the family, but it does not take into account household income and living conditions, although they are highly correlated. BMI is a measurement of food intake, but it does not express eating behaviors qualitatively. As previous studies showed that skeletal maturation correlates significantly with craniofacial growth (Bjork and Helm 1967; Helm et al. 1971), we attempted to minimize the influence of skeletal maturation on the association between dental maturation and craniofacial characteristics by adding the height of a child in the analysis which served as a proxy for general growth and skeletal maturation (Beunen et al. 1997; Moore et al. 1990; Ranjitkar et al. 2006) . Further, we quantified dental development as standard deviation score. The disadvantage of applying standard deviation scores is that unit of measurement is expressed as standard deviation instead of dental age. We also used rank-based normalization method to correct for the non-normal distribution. By applying this procedure, we were able to include, for example, children with extreme values for dental development. As a result, the initial distribution of dental development scores is narrowed down, implicating that the actual dental development differences might be greater than the observed differences. Also, PC analysis is a good method to group correlated cephalometric parameters into a single trait. However, determining the number craniofacial patterns, choosing the method of factor rotation, and interpretation is a subjective question

for the investigator. Therefore, we opted to minimize subjectivity by using analysis protocols reported in previous studies (Halazonetis 2004; Vucic et al. 2016).

CONCLUSIONS

The findings of this large population-based study show dental development is associated with specific dental and skeletal cephalometric characteristics in school-age children. We observed an increased sagittal and vertical growth of the dentofacial structures in children with advanced dental development. Furthermore, children with above-average dental development showed a tendency toward Class II occlusion and increased incisor and lip protrusion. Further longitudinal studies are necessary to explore the stability of observed effects over time.

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Table S1. Description of the cephalometric landmarks.

Abbreviation	Name	Definition
S	Sella	Centre of sella turcica
N	Nasion	The most anterior limit of the frontonasal suture
Ar	Articulare	A point where the posterior outline of the condyle passes over the posterior and lower margin of the cranial base
Go	Gonion	The midpoint of the angle of the mandible
Me	Menton	The most inferior point on the symphysis of the mandible
Pg	Pogonion	The most anterior point on the symphysis of the mandible
B	B-point	Deepest point on the contour of the mandible
A	A-point	Deepest point on the contour of the premaxilla
ANS	Anterior nasal spine	Tip of the anterior nasal spine
PNS	Posterior nasal spine	Most posterior point in the sagittal plane on the bony hard palate
Is	Incision superius	Incisal tip of the most anterior maxillary central incisor
Rs	Upper incisor apex	Root apex of the most prominent upper incisor
Msc	Molar superius cusp	The mesiobuccal cusp tip of the maxillary first molar; when double projection gives rise to two points, the midpoint is used
Ii	Incision inferius	Incisal tip of the most anterior medial mandibular central incisor
Ri	Lower incisor apex	root apex of the most prominent lower incisor
G’	Glabella	The most prominent anterior point in the midsagittal plane of the forehead
NT’ (P)	Pronasale	The most prominent point of the nose
MP’	Steiner S-point	Steiner S-point
RN’	Retro-nasale	The point at which the columella (nasal septum) merges with the upper lip in the midsagittal plane
Ls’	Labrale superius	The most prominent point of the vermilion border of the upper lip
Li’	Labrale inferius	The most prominent point of the vermilion border of the lower lip
PG’	Soft tissue Pogonion	The most anterior point on the chin

Table S2. Definition of cephalometric parameters by facial region and descriptive statistics.

Facial region	Abbreviation (Unit of measurement)	Definition	N	Mean	SD
Cranial base	SArGo (º)	Articular angle formed by points S, Ar and Go	3.896	141,8	6,1
	NSAr (º)	Saddle angle formed by points N, S and Ar	3.896	124,1	5,2
	ArGoMe (º)	Gonial angle formed by points Ar, Go and Me	3.896	127,9	5,3
Jaw relationship	SNA(º)	Angle formed by points S, N and A according to Steiner analysis	3.896	81,1	3,8
	SNB (º)	Angle formed by points S, N and B according to Steiner analysis	3.896	77,3	3,5
	ANB (º)	Angle formed by points A, N and B according to Steiner analysis	3.896	3,8	2,2
	SNPg (º)	Angle indicating chin prominence formed by points S, N and Pg	3.896	77,6	3,5
	MxSN (ANSPNS-SN) (º)	Angle formed by plane connecting points S and N, and palatal plane (plane formed by points ANS and PNS)	3.896	6,7	3,2
	MnSN (GoMe- SN) (º)	Angle formed by plane connecting points S and N, and mandibular plane (plane formed by points Go and Me)	3.896	33,9	5,1
	MxMn (AN-SPNS-GoMe) (º)	Angle formed by palatal (plane formed by points ANS and PNS) and mandibular planes (plane formed by points Go and Me)	3.896	27,3	5,1
	N-A-Pg (º)	Angle of convexity formed by points N, A and Pg according to Downs' analysis	3.896	172,8	5,4
	A⊥ NPg (mm)	Convexity of Point A is the distance between point A and facial plane (plane formed by points N and Pg) according to Ricketts analysis	3.896	3,1	2,3
Dental parameters	Interincisal angle (º)	Angle formed by the lines going through axes of upper and lower incisors according to Steiner analysis	3.896	126,2	10
	LI-Mn (º)	Incisor-Mandibular Plane Angle formed by the intersection of the mandibular plane (plane formed by points Go and Mn) with a line passing through the incisal edge and the apex of the root of the mandibular central incisor according to the Downs' analysis	3.896	97,2	6,7

Table S2. (Continued)

Facial region	Abbreviation (Unit of measurement)	Definition	N	Mean	SD
	UI-Mx (º)	Incisor-Palatal Plane Angle formed by the intersection of the mandibular plane (plane formed by points ANS and PNS) with a line passing through the incisal edge and the apex of the root of the maxillary central incisor	3.896	109,4	6,7
Vertical indices	LAFH = (ANSMe/NMe)	Percentage of lower anterior facial height (distance between points ANS and Me) as a fraction of total anterior face height (distance between points N and Me)	3.896	57,4	2,3
	LPFH = (ArGo/SGo)	Percentage of lower posterior facial height (distance between points Ar and Go) as a fraction of total posterior face height (distance between points S and Go)	3.896	58,9	3,5
	Jarabak Ratio (SGo/NMe)	Percentage of total posterior face height (distance between points S and Go) as a fraction of total anterior face height (distance between points N and Me)	3.896	64,2	4,3
	NPNS/PNSMe	Ratio between distance connecting points N and PNS and distance connecting points PNS and Me	3.896	1	0,1
Soft tissue	G'-RN'-PG'(º)	Angle of facial convexity for soft tissue is formed by points G', RN' and Pg'	3.896	165,9	5,3
	Ls'-Sl (Steiner line) (mm)	Distance between point Ls' and Steiner line (line connecting points Pg' and MP')	3.896	0,6	2,4
	Ls'-El (Esthetic Rickets line) (mm)	Distance between point Ls' and Esthetic line (line connecting points Pg' and NT')	3.896	1,3	2,7
	Li'-Sl (mm)	Distance between point Li' and Steiner line (line connecting points Pg' and MP')	3.896	-0,3	2,6
	Li'-El (mm)	Distance between point Li' and Esthetic line (line connecting points Pg' and NT')	3.896	0,7	2,8
Sagittal analysis of Pancerz	A ⊥ OLp (mm)	Distance between point A and line going through point S perpendicularly on occlusal plane	3.896	70,4	4,5
	Pg ⊥ OLp (mm)	Distance between point Pg and line going through point S perpendicularly on occlusal plane	3.896	74,2	5,9
	Is ⊥ OLp (mm)	Distance between point Is and line going through point S perpendicularly on occlusal plane	3.896	78,1	5,4

Table S2. (Continued)

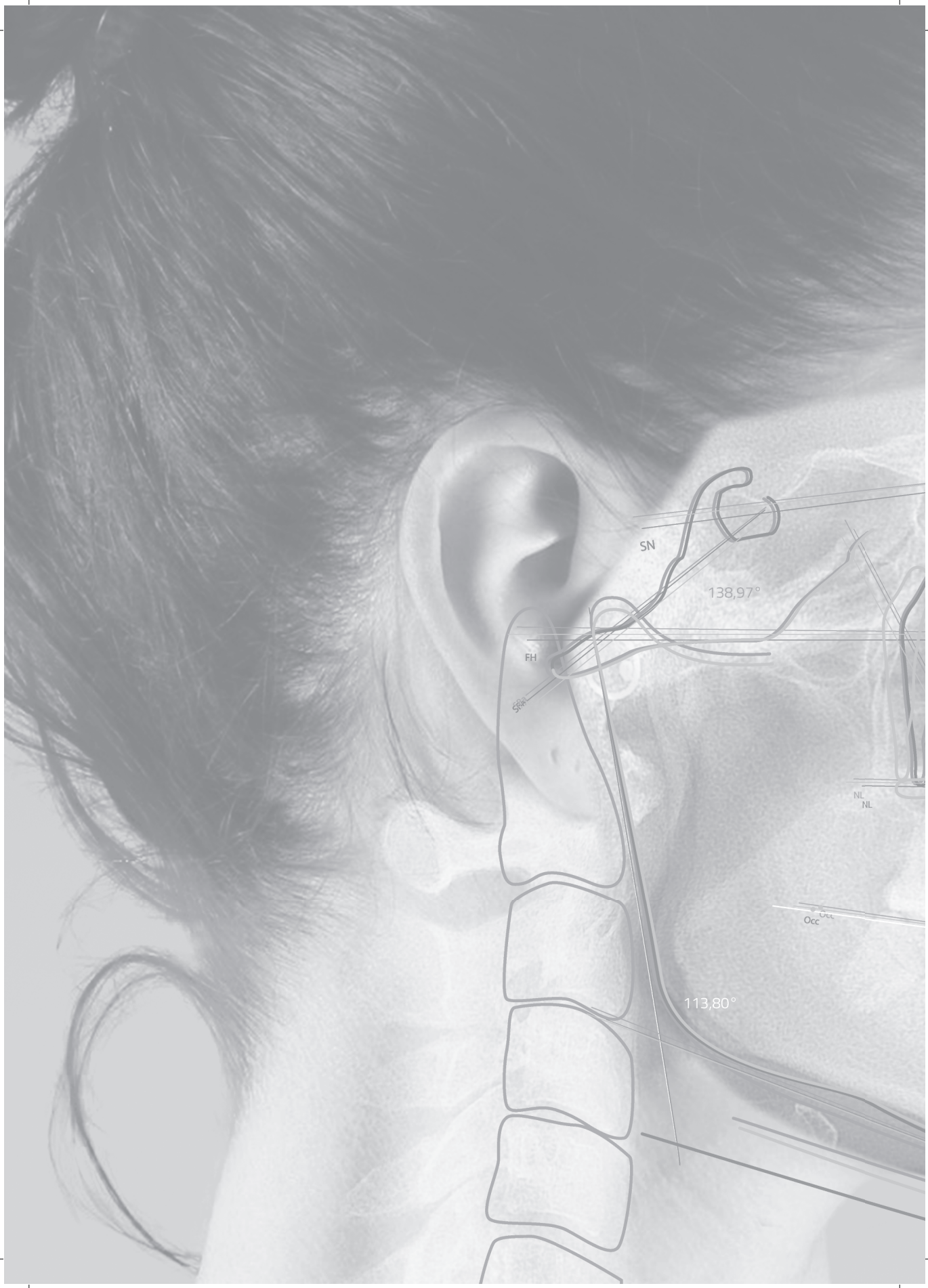
Facial region	Abbreviation (Unit of measurement)	Definition	N	Mean	SD
	Ii ⊥ OLp (mm)	Distance between point Ii and line going through point S perpendicularly on occlusal plane	3.896	73,9	5,4
	IsOLp–IiOLp (Overjet) (mm)	Distance Is ⊥ OLp minus distance Ii ⊥ OLp	3.896	4,3	1,9
	AOLp–PgOLp (mm)	Distance A ⊥ OLp minus distance Pg ⊥ OLp	3.896	-3,8	3,5
	IsOLp–AOLp (mm)	Distance Is ⊥ OLp minus distance A ⊥ OLp	3.896	78,1	5,4
	IiOLp–PgOLp (mm)	Distance Ii ⊥ OLp minus distance Pg ⊥ OLp	3.896	73,9	5,4
Vertical analysis of Pancerz	ANS-Me (mm)	Distance between points ANS and Me	3.896	58,7	4,7
	Is ⊥ Mx (mm)	Distance between point Is and palatal plane (plane formed by points ANS and PNS)	3.896	25,5	2,5
	Ii ⊥ Mn (mm)	Distance between point Ii and mandibular plane (plane formed by points Go and Mn)	3.896	35,5	2,9

*Occlusal plane is a plane that goes through points Is and Msc; Abbreviations: SD- standard deviation, N- number of subjects

Table S3. Description of craniofacial patterns explained through principal component analysis.

Facial region		Interpretation			Principal Component Analysis1	
		Craniofacial pattern	Positive value of PC	Negative value of PC	Cephalometric parameters 2	Explained variability (%)
Skeletal	PC2	Facial divergence	Hypodivergent/ Low-angle face	Hyperdivergent/High-angle face	+SNB; +SNPg; +SNA; –NPNS/PNSMe	16
	PC3	Bimaxillary growth	Towards bimaxillary protrusion	Towards bimaxillary retrusion	+Pg ⊥ OLp; +A ⊥ OLp; +Is ⊥ OLp; + Ii ⊥ OLp	12
	PC4	Sagittal jaw relationship	Towards Class III relationship	Towards Class II relationship	–ANB; +N-A-Pg; +A⊥ NPg; +G'-RN'-PG'; –AOLp–PgOLp	9
	PC6	Ramus height	Increased ramus height	Decreased ramus height	–ArGoMe; + LI-Mn; –GoMe- SN; +Jarabak Ratio	5
	PC7	Lower anterior facial height	Increased Lower anterior facial height	Decreased Lower anterior facial height	+LAFH; +ANS-Me; –MxSN; +Is ⊥ Mx; +Ii ⊥ Mn; +AN-SPNS-GoMn	5
	PC8	Cranial base relationship	Increased cranial base angle	Decreased cranial base angle	–SArGo; +NSAr;+LPFH;	4
Dental	PC1	Lip position	Protruded lips	Retruded lips	+Ls'-SL; +Li'-Sl; +Ls'-El; +Li'-El	25
	PC5	Incisor angulation	Incisor proclination and decreased interincisal angle	Incisor retroclination and increased interincisal angle	+UI-Mx; –Interincisal angle; +IiOLp–PgOLp; +IsOLp–AOLp	8
	PC9	Overjet	Increased Overjet	Decreased Overjet	+Overjet	4

1. Cephalometric parameters described a principal component based on the primary (strongest) loading of a cephalometric parameter from the pattern matrix of the principal component analysis using direct oblimin rotation procedure. Secondary and subsequent factor loadings of cephalometric parameters were not higher than 0.56 and, therefore, not taken into account.
2. + denotes positive and – negative correlation of a cephalometric parameter with a PC; Description of cephalometric parameters is provided in the supplemental file (Tables S1 and S2)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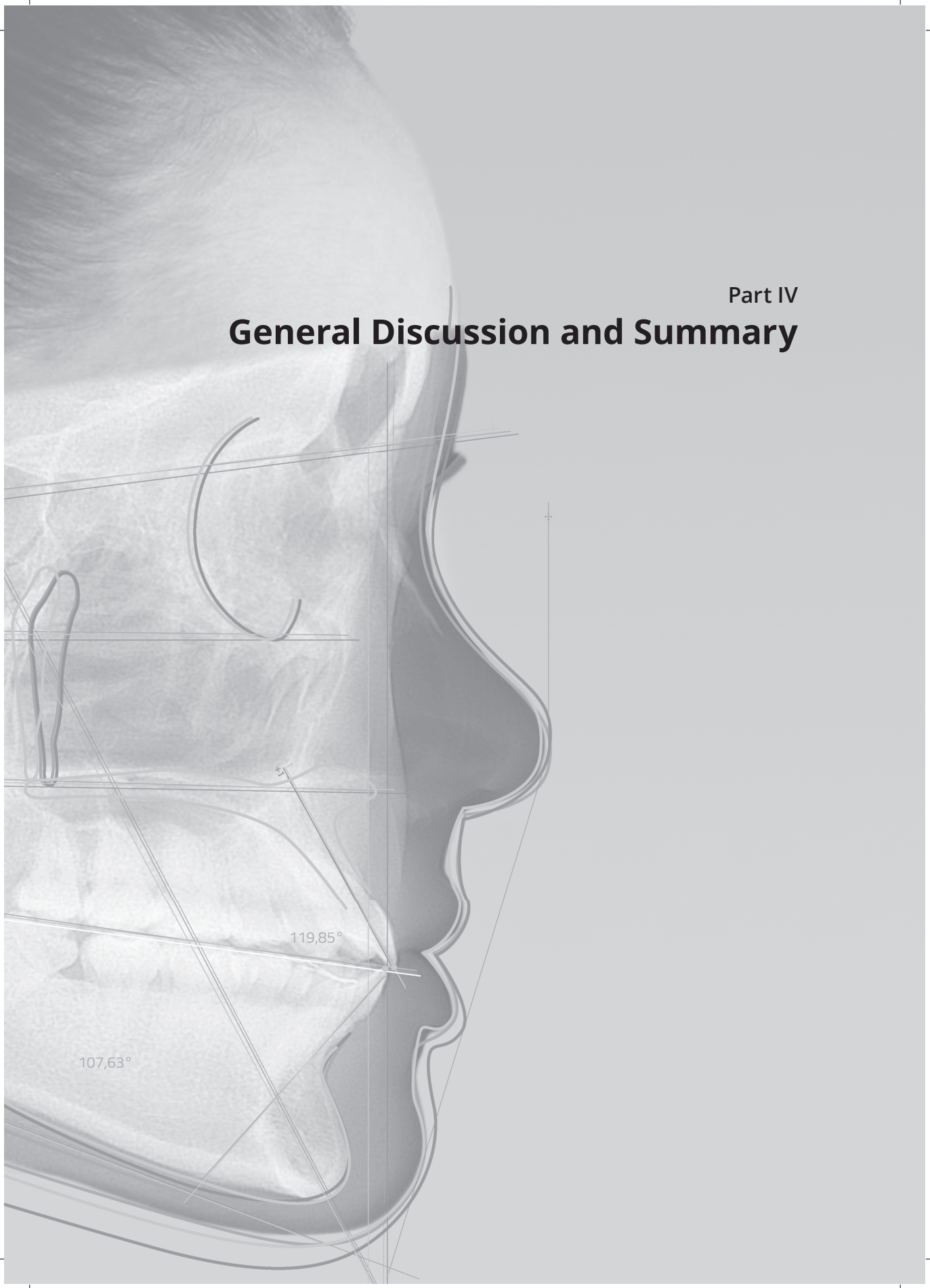
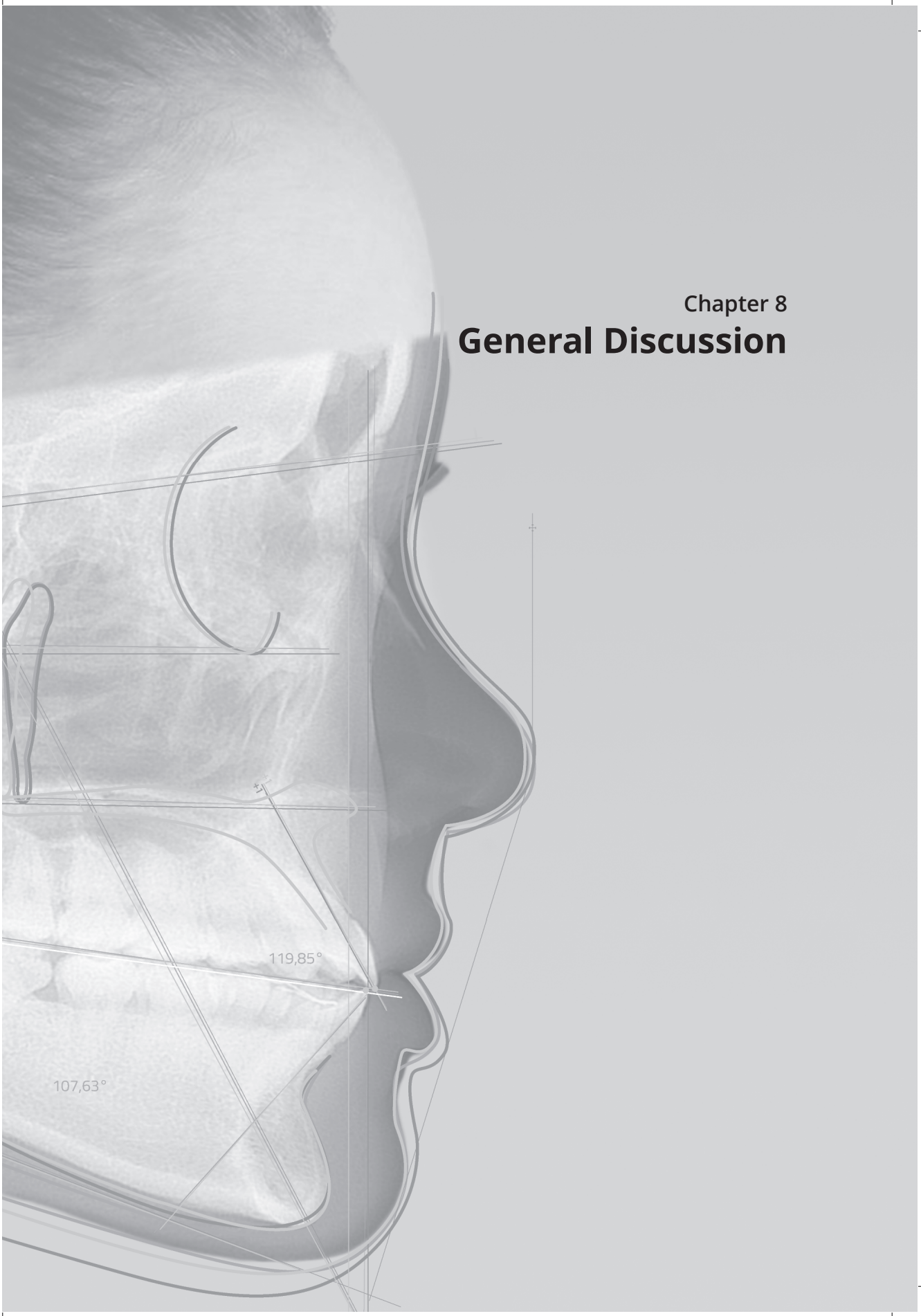
Part IV

General Discussion and Summary





Chapter 8

General Discussion

DISCUSSION

In this thesis, the patterns of dental and craniofacial development in children were investigated. We found several genetic, endocrine and dental factors, which were involved in the regulation of the rate of dental development, offering additional insight into how the morphology of craniofacial region is shaped. Dental development was assessed using a protocol described by Demirjian (Demirjian et al. 1973), and craniofacial morphology was assessed by combining multiple cephalometric measurements into principal components, each representing a certain craniofacial pattern (Halazonetis 2004; 2007). This chapter provides an overview of the main findings of the studies presented in this thesis, and also presents important methodological considerations, clinical implications, and future directions.

MAIN FINDINGS

The concept of dental development

The concept of assessing dental development has been a point of interest for many disciplines, including forensic medicine, archeology, pediatrics, dentistry and orthodontics (Manjunatha and Soni 2014). Various methods were developed to be used to identify markers of dental development, including those based on clinical tooth eruption as well as the calcification stages of tooth structures evaluated from panoramic radiographs (Garn et al. 1959; Haavikko 1974; Logan and Kronfeld 1933; Moorrees et al. 1963; Schour and Massler 1940). One of the most widely used methods is the Demirjian dental age standard that was established in the French-Canadian population (Demirjian et al. 1973). Based on this method, developmental stages of teeth, which correspond to one of the eight stages in the Demirjian atlas, are weighted by normative data from the general French-Canadian population. Further, the scores of the left mandibular seven teeth are summarized and converted into dental age using available tables (Demirjian et al. 1973). However, studies which used French-Canadian standards in other geographical regions and ethnic groups found that these standards were not always applicable to other populations (Al-Tuwirqi et al. 2011; Chaillet et al. 2005). Shortly after these publications, other investigators determined their own population-specific standards. However, it is also disputable whether population-specific standards are applicable to other samples of the same ethnicity, given that several reports have suggested that dental development is subject to secular variation (Cardoso et al. 2010; Heuze and Cardoso 2008; Jayaraman et al. 2013; Sasso et al. 2013).

Chapter 2 of this thesis investigates the secular changes of dental development in the Dutch population. Using the same reference standards in three samples of

Dutch children born between 1961 and 2004, we observed an almost 1.5 years advanced dental development in children born later compared to the children of the same age born 40 years earlier. The contributing factors to secular changes in dental development are still being debated. Environmental and lifestyle factors, better healthcare, and other factors, such as skeletal and general somatic development, height, BMI, and the onset of puberty, were reported to be important in these time trends (Fredriks et al. 2000a; Fredriks et al. 2000b; Mul et al. 2001). Results from Chapter 2 also contradict previous studies which underline that dental development is a biologically stable process and independent of certain environmental factors (Bagherian and Sadeghi 2011; Elamin and Liversidge 2013). On the contrary, we demonstrated that dental development standards derived from a specific population are not always generalizable to other populations, most likely due to the influence of environmental factors. As many studies have published their own dental age standards, care should be exercised not only when applying these norms to other populations, but also when applying them to the same population after a certain period of time. Therefore, we recommend that the year of birth should also be taken into account when investigating a population across a wider time span.

Endocrine regulation of dental development

As in growth processes of other organs, endocrine regulation plays an important role in dental development (Garn et al. 1965). Previous studies investigated the relationship between dental development and calcitonin (Mallek et al. 1979), growth hormone (Kjellberg et al. 2000), insulin-like growth factor-I (Young 1995), parathyroid hormone (Sakakura 1987) and the adrenal gland (Carlos Fabuel et al. 2010; Hagg and Taranger 1984).

Thyroid function and dental development

Thyroid function is known to regulate the metabolic processes and development of the human body. Yet, little is known about the effects of thyroid function on dentofacial structures. Previous studies have shown that the concentration of thyroid hormones has an effect on the timing of tooth eruption, the mineral content of maxillary, mandibular bones and tooth enamel, the morphology of the tongue, and the susceptibility to periodontal diseases (Chandna and Bathla 2011; Ikeda et al. 2008; 2009; Nanci 2007). In our study, we observed that TBOAb positivity in mothers during pregnancy and TSH at birth and during early childhood were inversely associated with dental development (Chapter 4). We did not observe an overall significant effect of FT4 concentrations on the dental development, which fits the general notion that the TSH is the most reliable/sensitive marker of thyroid function. Taken

together, our findings suggest that the thyroid hormone is involved in the maturation of teeth from the early stages of life onward.

The underlying biological mechanisms for these associations between thyroid function and dental development remain largely unknown. With regard to the development of mineralized tissues, the effects of thyroid hormones have been well-described in the process of endochondral ossification (Cray et al. 2013). Thyroxine enhances cartilage matrix mineralization by stimulating expression of genes that control chondrocyte maturation, and it also regulates the function of bone-forming osteoblasts and bone-resorbing osteoclasts (Williams 2013). From the perspective of teeth and supporting tissues, a previous study reported that alveolar bone is less susceptible to thyroid hormone changes, except in the presence of periodontitis when bone loss is facilitated by a deficiency of thyroid hormones (Feitosa et al. 2009). Furthermore, administered thyroxine was reported to reduce root resorption during the force-induced remodeling process (Poumpros et al. 1994). These findings suggest that thyroid hormones might also regulate the function of tooth-forming cells, ameloblasts, odontoblasts and cementoblasts, however further studies are necessary to investigate the underlying biological mechanisms.

Genetic basis of dental development

Understanding the genetic basis of dental development enables us to pinpoint genes associated with disturbances in dental development. So far, the genetic background has been studied mostly on animal models, in human syndromes associated with disturbances in dental development, family-based studies, and twin studies. Genome-wide association (GWA) analysis is a relatively new approach for investigating genetic variants, known as Single Nucleotide Polymorphism (SNP) associated with a certain disease (Bush and Moore 2012). Previous GWA studies identified the *ADK, AJUBA, BMP4, CACNB2, CACNA1S, CALU/OPN1SW, CDON, DLEU7, EDA, HMGA2, HOXB2, IGF2BP1, KCNJ2/KCNJ16, MSRB3, RAD51C, TEX14/RAD51B* and *TNP1* loci associated with the eruption of teeth (Fatemifar et al. 2013; Geller et al. 2011; Pillas et al. 2010). The study described in Chapter 3 is the first GWAS on radiographic dental development. Furthermore, we investigated the genetic correlation of dental development with other traits using LD- score regression, and potential causal pathways between them using a Mendelian randomization approach.

In our GWAS, we confirmed 9 loci which were previously associated with the eruption of teeth and identified 3 new loci, *IGF2BP3, IRX5*, and *PAX9*. The novel loci, *IGF2BP3* regulates translation of insulin-like growth factor II. Polymorphisms in this gene have been associated with cleft palate (Baker et al. 1993; Eggenschwiler et al. 1997), while the increased expression this gene was associated with abnormal growth of different skull parts, including jaws and tongue (Sun et al. 1997). Taken together, our

findings suggest that *IGF2BP3* plays a role in human dental development. A member of the Iroquois homeobox gene family, *IRX5* has been previously associated with upper jaw development in mice (Jeong et al. 2008). The link between jaw and dental development in the context of *IRX5* could point to a potentially pleiotropic effect of *IRX5* on dental and jaw development. Finally, along with the well-described role of *PAX9* in tooth agenesis, cleft lip, and cleft palate, we also report the involvement of this gene in the later stages of dental development (Lee et al. 2012; Seki et al. 2015). By applying additional analyses, we attempted to reduce the possibility that the genes identified in dental development are regulated through parallel pathways of other traits that correlate with dental development (i.e. pleiotropy). Further larger GWA studies and functional follow up studies are necessary to gain broader insight into the genetic basis of dental development.

Disturbances of dental development

Tooth agenesis is a condition characterized by teeth failing to develop, and, with a population prevalence of 4-6%, is the most common congenital dental development disturbance (Polder et al. 2004). In a healthy population, 97.3 % tooth agenesis involves up to five missing teeth, and is referred to as non-syndromic hypodontia (Polder et al. 2004). Non-syndromic hypodontia presents a significant burden to the society as it is associated with functional, psychological and esthetic problems which often require multidisciplinary treatment (Tunc et al. 2011). Subjects with non-syndromic hypodontia may experience various types of malocclusion, periodontal damage, lack of alveolar bone growth, reduced chewing ability, and pronunciation problems (Rakhshan 2015). With regard to dental changes, teeth adjacent to missing teeth were reported to have decreased crown size, differential root morphology, or even taurodontism (Uslenghi et al. 2006). On the other hand, studies were inconsistent when reporting dental development. Some studies reported a delayed dental development in children with hypodontia (Medina et al. 2016; Tunc et al. 2011) whereas others observed no significant differences (Ben-Bassat et al. 2014).

Tooth agenesis and dental development

In Chapter 5 we explored the association between hypodontia and dental development, demonstrating that children with hypodontia had delayed dental development. Interestingly, the observed difference of dental development between children with hypodontia and controls was the largest for mandibular second premolars, which are also the most prevalent agenetic teeth (Khalaf et al. 2014; Polder et al. 2004). According to Brook et al., subjects with hypodontia have smaller teeth than subjects with supernumerary teeth (Brook 1984). Similarly, studies observed that a delay in development results in smaller teeth, whereas a precocious or earlier

development results in larger teeth (Pinho et al. 2009; Schalk-van der Weide and Bosman 1996). Considering that tooth development is a polygenic trait regulated by multiple genes, where an increasing number of genes are invooved as the tooth enters later stages of development (Bei 2009), a potential underlying mechanism linking tooth agenesis and delayed dental development may lie in the number of mutations present; if relatively few mutations exist, a delayed dental development may be observed whereas if many genes contain mutations, dental development arrests completely leading to tooth agenesis. Another theory suggests that certain genes are important at certain stages of dental development, such that mutations in genes which are more specific for early stages of development cause tooth agenesis while mutation in genes which regulate later stages cause delayed dental development. An explanation may also be that innervation plays a role in the development and eruption. Further studies are necessary to investigate whether tooth agenesis is a severe form of delayed dental development.

Tooth agenesis and craniofacial morphology

The impact of tooth agenesis goes beyond the oral cavity, as previous studies reported that it might also determine facial-skeletal relationships. Studies were consistent in reporting the reduction of vertical jaw relation (Nodal et al. 1994; Ogaard and Krogstad 1995). However, many studies reported maxillary retrognathism (Acharya et al. 2010; Ogaard and Krogstad 1995), while others reported mandibular retrognathism (Nodal et al. 1994) or bimaxillary prognathism (Ben-Bassat and Brin 2009; Chung et al. 2000; Kreczi et al. 2011). Furthermore, subjects with hypodontia were reported to have increased overjet, overbite and interincisal angle (Endo et al. 2004; Kreczi et al. 2011). Chapter 6 of this thesis explores the cephalometric characteristics of children with hypodontia. Using conventional and principal component cephalometric analysis, we observed that hypodontia generally has a biggest impact on the retroclination of incisors, resulting in a more posteriorly tipped upper and lower incisors, unless teeth were missing in the anterior region were children showed a more hyperdivergent skeletal profile. Interestingly, there was a discrepancy between cephalometric parameters depending on whether tooth agenesis was localized in the upper or the lower jaw. Hypodontia in the upper jaw was associated with the restriction of the sagittal growth of the upper jaw. This restricted sagittal growth is characterized by the tendency toward (pseudo) Class III jaw relationship. On the other hand, lower jaw hypodontia was associated with reduced vertical facial parameters in the posterior region. In contrast to changes in incisor position and vertical dimensions, which are most likely to be explained by the functional adaptation mechanisms of the neighboring structures (Ogaard and Krogstad 1995; Roald et al. 1982), the maxillary retrognathism and hyperdivergent facial pattern could be

partly explained by the altered growth pattern in children with hypodontia (Sarnas and Rune 1983). Therefore, future genetic studies on craniofacial parameters could be crucial for identifying pleiotropic genes with a known regulatory role in dental development.

Dental development and craniofacial morphology

To date, limited work has investigated the relationship between dental and facial growth. Specific craniofacial characteristics were identified in children with delayed or advanced dental development. For example, subjects with advanced dental development had a higher chance of a vertical facial growth pattern. In Chapter 5 of this thesis, we reported that impaired dental development in the form of tooth agenesis was already associated with changes in craniofacial growth. However, most of these changes could be attributed to the functional compensation of facial structures in the region of missing teeth. Therefore, to exclude the effect of compensational mechanisms on facial structures, in Chapter 7 we investigated whether there is also an association between dental development and craniofacial morphology. We observed that advanced dental development was associated with increased sagittal and vertical facial growth. Further, an above-average advanced dental development was associated with a tendency for Class II malocclusion and incisor proclination. With regard to soft tissue parameters, children with advanced dental development displayed more prominent lips relative to the nose-chin line. We postulate that our findings could be explained by common genetic factors expressed during embryogenesis (Nanci 2007), as well as an adaptation of structures around the growing teeth (Moss 1997).

METHODOLOGICAL CONSIDERATIONS

Specific methodological considerations have been described in the studies presented in this thesis. In the following paragraphs, general methodological considerations regarding selection bias, information bias, and confounding are discussed.

Selection bias

Selection bias occurs when the association between the exposure and outcome of interest is impacted by sampling of study participants, such that a subset of individuals from the population are either over- or under-represented in the study sample. The results obtained are thereby not representative of the population intended to be analyzed. Of all children eligible at birth, the overall response to participate in the Generation R Study was 61% (Kruithof et al. 2014). This non-response at baseline is not likely random. For example, compared to those who enrolled in the study, non-participating parents and children more frequently were of non-Caucasian origin, had a lower socio-economic status, and more adverse birth outcomes, such as low birth weight, suggesting a selection toward a relatively healthier study population.

Another source of selection bias is a selective loss to follow-up. This type of bias occurs when the association between the exposure and outcome of interest is different between those participating in the study and those lost to follow-up. Of all children (n = 9,901) originally included in the Generation R study, 74.7% (n = 7,393) participated in the follow-up studies at age 9 years, and 62% (n = 4561) of them had a dental panoramic radiograph or a cephalogram (Kruithof et al. 2014). In the study published in this thesis, we investigated a potential bias related to the loss to follow-up by comparing demographic characteristics of children with and without dental radiographs. Mothers of children without dental radiographs were on average 2.05 years younger (95% confidence interval [CI], 2.26–1.85), were more likely to have a non-Dutch origin (59.4% vs. 40.6%, P < 0.001), were less educated (66.3% vs. 33.7% finished primary or lower education, P < 0.001), and were more likely to continue smoking during pregnancy (62.2% vs. 37.8%, P < 0.001). Selection towards a healthier population may have biased our effect estimates, however, this bias is difficult to estimate. To limit the bias related to the selective inclusion of participants, we applied a nonresponse analysis and adjusted analyses for a number of relevant confounding factors (Sterne et al. 2009).

Information bias

Information bias is a measurement error due to misclassification of participant data. In epidemiological research, there are two types of misclassification bias: differen-

tial and non-differential misclassification. Non-differential misclassification occurs when misclassification is unrelated to the occurrence or the presence of the exposure or outcome of the study. For example, this type of bias might have occurred as a result of examiner error when identifying the stage of tooth development of panoramic radiographs, or when positioning landmarks on cephalograms from which cephalometric parameters are calculated. To evaluate measurement error between two examiners, we calculated inter-rater agreement. Differential misclassification occurs when the misclassification is different for those with and without the exposure or outcome of interest. For example, adverse lifestyle variables such as smoking are usually underreported in epidemiologic studies. Still, most of the variables were directly assessed from medical records or standardized hands-on measurements, such as age, sex, height, weight and blood sample measurements, which are less prone to bias associated with the examiner.

Confounding

A confounding factor is a factor that is associated with both the exposure and the outcome, but not located in the causal pathway. If not taken into account, confounding may lead to biased effect estimates. We included covariates in analyses based on existing literature, or a change of more than 10% in effect estimates (Mickey and Greenland 1989). Although analyses were adjusted for multiple confounders, human growth and development is a complex process with numerous genetic, epigenetic, and environmental regulatory factors. For example, environmental factors such as general living conditions, nutrition, health status, and stressors have been strongly associated with growth and development status, including the development of teeth and facial features (Cameron and Bogin 2012; Dasgupta and Hauspie 2013). In some situations, we compensated for unmeasured covariates by including measured variables known to be highly correlated with the confounder of interest (i.e., proxy confounder). Still, residual confounding may be an issue due to imprecise and unmeasured variables, as in all observational studies.

CLINICAL IMPLICATIONS

The findings reported in this thesis are based on epidemiological studies conducted primarily in a healthy population which aids in the understanding of underlying mechanisms and factors associated with dentofacial development. The non-clinical nature of these studies may limit the ability of clinicians to draw conclusions relevant for their practice. Still, dental disturbances and malocclusions are highly prevalent in the general population. Thus, we can draw several conclusions from these

studies and begin to establish guidelines with respect to most common dentofacial disorders:

– The observed positive secular trend in accelerated dental development of children should be carefully considered in the context of when the norms were established, and that treatment decisions should be made using them solely as guidlines rather than rigid cutoffs.

– Identification of 3 novel genes associated with the dental development helps us in the understanding of disturbances associated with dental development. Genetic screening during pregnancy, which is in clinical practice commonly used for identification of severe congenital diseases, might be used in the future to calculate polygenic risk score for disturbances in dental development.

– A positive association exists between thyroid function and dental development. Optimal treatment of thyroid dysfunction could also have a beneficial effect on dental development, in addition to the primary goal of the treatment.

– The clinical challenge of treating patients with hypodontia has been well-documented in the past, however, our data showing that the rate of dental development is associated with craniofacial changes is relatively novel and interesting. Following this notion, the early assessment of the rate of dental development in a child can be used as a predictive factor for malocclusions and craniofacial problems, and it is a useful prognostic factor as it can aid in determining the optimal timing of an orthodontic treatment.

FUTURE DIRECTIONS

Future research should focus on replicating our findings, as well as expanding our knowledge about dental development and cephalometric morphology. The studies presented in this thesis are primarily cross-sectional studies embedded in the Generation R cohort study. The dentofacial developmental outcome variables were collected cross-sectionally when children were nine years old. Future studies with follow up data are important when studying the growth of human traits by taking into account the changes in growth velocity, due to puberty or catch-up growth (Rogol et al. 2002; Wit and Boersma 2002). For example, one area that would benefit from longitudinal data is the growth of mandible peaks during puberty. The follow-up collection of data would enable us to take into account subjects who have a sudden change in the velocity of dentofacial growth.

In addition, multiple genetic, epigenetic and environmental factors influence the complex process of dental and facial growth (Dixon et al. 1997; Guo et al. 2014). In this thesis, we investigated thyroid function and dental development. There are ongoing projects in the Generation R study to investigate the effect of nutrients such

as folic acid, vitamin B12, and homocysteine on dental development and hypodontia. Previous studies showed that the prenatal period is important for child growth outcomes later in life (Jaddoe et al. 2007; van den Broek et al. 2015). Future studies should also consider the influence of unhealthy habits such as smoking and alcohol use during pregnancy.

Finally, the recent scientific advancements in genetic studies enable us to expand our understanding of the genetic architecture underlying dentofacial outcomes. The GWA study presented in this thesis provides a solid basis for future genetic studies, as our results still require replication in other samples. In addition, to our knowledge, there are no large-scale GWA studies that investigated different craniofacial traits, such as sagittal jaw relationship, vertical facial dimensions, and teeth position. For example, identifying loci associated with a Class III malocclusion, a trait with high heritability (Nakasima et al. 1982; Xue et al. 2010), may increase understanding and improve treatment of this malocclusion. Therefore, exploring genetic background and clinical features of dentofacial abnormalities could also aid their treatment in the future.

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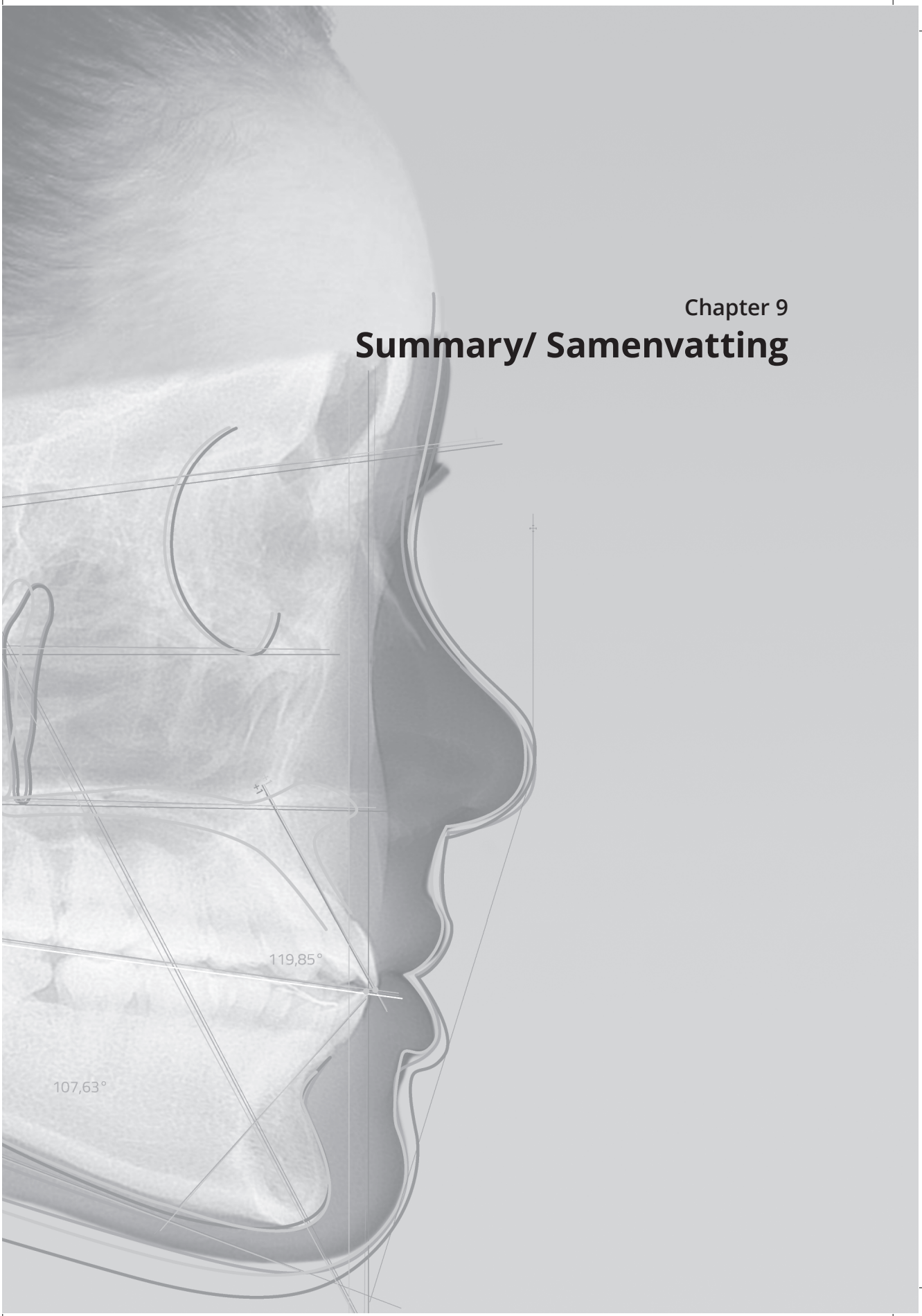


Chapter 9

Summary/ Samenvatting

SUMMARY

The relationship between dental development and facial morphology has been a point of interest for dental care professionals. In this thesis, we investigated the patterns of child's dental development by analyzing the effects of genetic, endocrine and other dental determinants. Further, we explored the influence of dental determinants on craniofacial morphology in children. All studies published in this thesis were embedded in the Generation R Study, a population-based prospective cohort from fetal life until young adulthood. The rationale and findings of the studies published in this thesis are organized into three parts.

Part I elaborates on the mechanism of dental and facial development in the intrauterine and postnatal period, and the factors which are influencing these growth changes. We also specify the aims of this thesis.

Part II of the thesis is focused on the characteristics and determinants of dental development. In **Chapter 2**, we report a 1.5 years advanced dental development in children born after the year 2000 compared to children born 40 years earlier; a result showing substantial secular changes of dental development in the Dutch population. These findings contradict previous studies which underline that dental development is a biologically stable process and independent of other environmental factors. **Chapter 3** reflects on the genetic background of dental development. We confirmed 9 loci which were previously associated with the eruption of teeth, and also confirmed 3 new loci: *IGF2BP3, IRX5*, and *PAX9*. In **Chapter 4** we expand the knowledge of the endocrine regulation of dental development by reporting an association between thyroid function and dental development. We observed that thyroid peroxidase antibody positivity in mothers during pregnancy as well as thyroid stimulating hormone at birth and during early childhood were inversely associated with dental development. These findings suggest that thyroid hormones might also regulate the function of tooth-forming cells. Concluding, in **Chapter 5**, we established a link between dental development and tooth agenesis, where we demonstrated that children with hypodontia had delayed dental development. Therefore, considering that tooth development is a polygenic trait, a potential underlying mechanism linking tooth agenesis and delayed dental development is the number of genetic variants present. For example, a smaller number of variants may cause delayed dental development, while a greater number of variants could cause tooth agenesis.

Part III describes the influence of hypodontia and dental development on the craniofacial morphology of 9-year-old children. In **Chapter 6** we observed a strong association between hypodontia and retroclination of incisors. Furthermore, hypodontia in the upper jaw was associated with the tendency toward a (pseudo)

Class III jaw relationship, while lower jaw hypodontia was associated with reduced vertical facial parameters in the posterior region. The underlying mechanisms explaining these associations were undeniably due to the compensational mechanism of the surrounding tissues towards the region of agenetic tooth. However, another underlying mechanism might be that genetic markers which are involved in tooth development also regulate facial growth. **Chapter 7** describes the altered cephalometric growth pattern of children with delayed dental development. We observed that advanced dental development was associated with an increased sagittal and vertical facial growth. Further, we observed that above-average advanced dental development was associated with a tendency for Class II malocclusion, incisor, and lip protrusion. Therefore, we can conclude that dental development, either portrayed as the change of the rate of dental development or tooth agenesis, influences craniofacial characteristics of a child. These findings might be useful for an orthodontist in early diagnosis of malocclusion and when assessing the prognosis of applied treatment.

Chapter 8 provides an insight into the main findings of the studies from previous chapters and reviews methodological considerations, clinical implications, and future directions.

SAMENVATTING

De relatie tussen tandontwikkeling en gezichtsmorfologie is een aandachtspunt voor professionals in de tandheelkunde. In dit proefschrift hebben we patronen van dentale ontwikkeling in kinderen onderzocht door de effecten van genetische, endocriene en andere dentale determinanten te analyseren. Verder hebben we de invloed van tandheelkundige determinanten op de craniofaciale morfologie bij kinderen onderzocht. Alle onderzoeken in dit proefschrift zijn ingebed in de Generation R studie, een prospectief cohortonderzoek van het foetale leven tot de jongvolwassen leeftijd. De achtergrond en bevindingen van studies gepubliceerd in dit proefschrift zijn georganiseerd in drie delen:

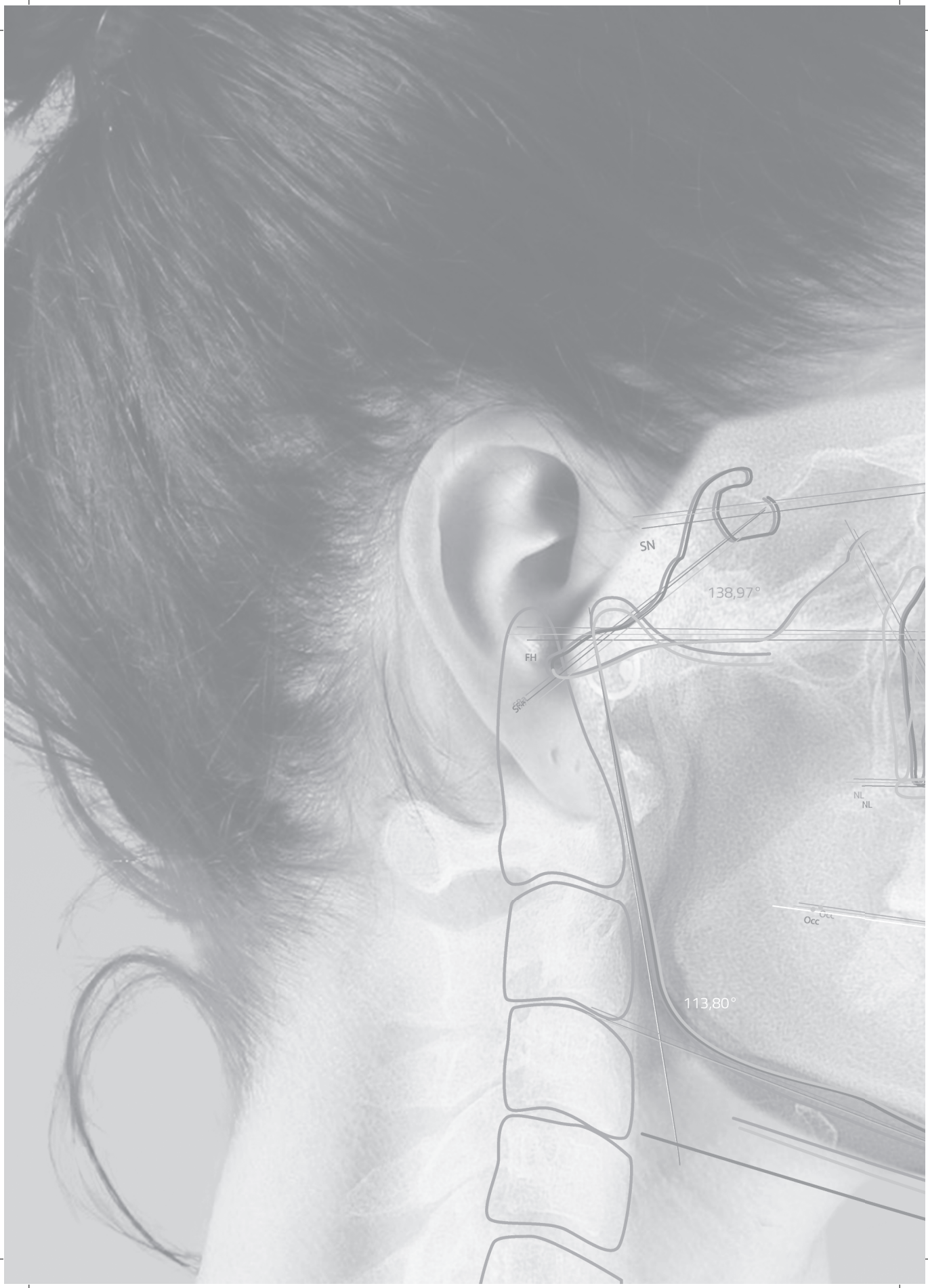
Deel I gaat in op het mechanisme van tand- en gezichtsontwikkeling in de intra-uteriene en postnatale fase, en bekende factoren die hierop van invloed zijn. We gaan ook in op de doelstellingen van het onderzoek in dit proefschrift.

Deel II van het proefschrift is gericht op de karakteristieken en determinanten van tandontwikkeling. In **hoofdstuk 2** is in kinderen versnelde tandontwikkeling van 1,5 jaar gevonden ten opzichte van 40 jaar eerder, die een grote impact op seculaire veranderingen van tandontwikkeling in de Nederlandse populatie hebben. Deze bevindingen zijn in tegenspraak met eerdere onderzoeken die benadrukken dat tandontwikkeling een biologisch stabiel proces is en onafhankelijk van andere omgevingsfactoren. **Hoofdstuk 3** reflecteert op de genetische achtergrond van tandontwikkeling. We bevestigden 9 loci die eerder werden geassocieerd met tanderuptie en we hebben 3 nieuwe loci ontdekt, *IGF2BP3, IRX5* en *PAX9*. In **hoofdstuk 4** breiden we de kennis van de endocriene regulatie van tandontwikkeling uit door het bevestigen van een associatie tussen de schildklierfunctie en tandontwikkeling. We observeerden dat thyroidperoxidase-antistoffen-positiviteit in moeders tijdens de zwangerschap en thyreoïdstimulerend-hormoon bij de geboorte en tijdens de vroege kinderjaren omgekeerd geassocieerd zijn met tandontwikkeling. Deze bevindingen suggereren dat schildklierhormonen ook de functie van de cellen betrokken bij de tandvorming zou kunnen reguleren. Ter afsluiting, in **hoofdstuk 5**, hebben we een verband tussen tandontwikkeling en agenesie aangetoond waaruit blijkt dat de gebitsontwikkeling bij de kinderen met hypodontie is vertraagd. Gezien het feit dat de tandontwikkeling een polygene eigenschap is, is een mogelijk onderliggend mechanisme dat tandagenesie en vertraagde tandontwikkeling verbindt, het aantal genmutaties. Als gevolg hiervan veroorzaken minder mutaties een vertraagde dentale ontwikkeling, terwijl bij een groter aantal mutaties tandagenesie optreedt.

Deel III beschrijft de invloed van hypodontie en tandontwikkeling op de craniofaciale morfologie van 9-jarige kinderen. In **hoofdstuk 6** zagen we een sterke associatie tussen hypodontie en retroclinatie van de incisieven. Bovendien was hypodon-

tie in de bovenkaak geassocieerd met de neiging tot een (pseudo) Klasse III relatie, terwijl de hypodontie van de onderkaak werd geassocieerd met een afname van de achterste gezichtshoogte. Deze afname zou kunnen worden veroorzaakt door verminderde alveolaire hoogte ter plaatse van agenetische elementen, hoewel het effect van veranderde gezichtsgroei bij kinderen met hypodontie ook niet kon worden uitgesloten. **Hoofdstuk 7** beschrijft het veranderde cephalometrische groeipatroon van kinderen met een vertraagde tandontwikkeling. We hebben vastgesteld dat versnelde tandontwikkeling werd geassocieerd met verhoogde sagittale en verticale gelaatsgroei met daarbij het ontstaan van een klasse II malocclusie, en protrusie van de snijtanden en lippen. Daarom kunnen we concluderen dat tandontwikkeling uitgedrukt in het tempo van tandontwikkeling of tandagenesie, de craniofaciale kenmerken van een kind beïnvloedt. Deze bevindingen kunnen nuttig zijn voor een orthodontist in vroege diagnose van malocclusie en bij de beoordeling van de prognose van de toegepaste behandeling.

Hoofdstuk 8 geeft een inzicht in de belangrijkste bevinding van de studies uit eerdere hoofdstukken en beschrijft methodologische overwegingen, klinische implicaties en toekomstige richtingen van onderzoek.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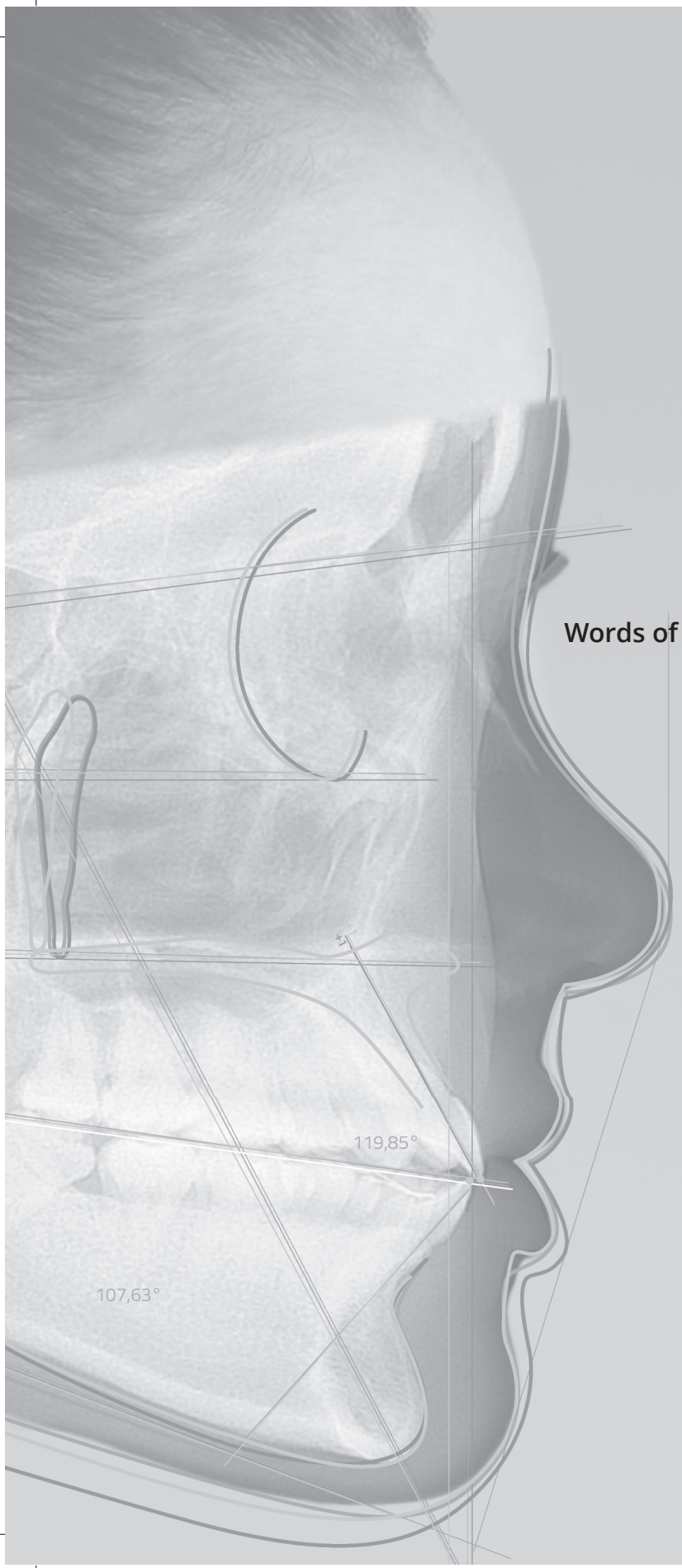

Appendix

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PUBLICATIONS

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SUBMITTED MANUSCRIPTS

Vucic S, Dhamo B, Jaddoe VWV, Wolvius EB, Ongkosuwito EM The association of dental development and craniofacial morphology in school-age children: The Generation R study

PORTFOLIO

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Research School:	Netherlands Institute for Health Sciences (NIHES)
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PhD training		
Master of Science in Clinical Epidemiology, NIHES, Rotterdam, the Netherlands		
Principles of Research in Medicine	2012	0.7
Clinical Decision Analysis	2012	0.7
Methods of Public Health Research	2012	0.7
Health Economics	2013	0.7
Cohort Studies	2013	0.7
Case-control Studies	2013	0.7
Social Epidemiology	2012	0.7
Markers and Prognostic Research	2012	0.7
Advances in Epidemiologic Analysis	2013	0.4
The Practice of Epidemiologic Analysis	2012	0.7
Logistic Regression	2013	1.4
Study Design	2012	4.3
Biostatistical Methods I: Basic Principles	2012	5.7
Clinical Epidemiology	2013	5.7
Methodologic Topics in Epidemiologic Research	2013	1.4
Biostatistical Methods II: Classical Regression Models	2012	4.3
Quality of Life Measurement	2013	0.9
From Problem to Solution in Public Health	2013	1.1
Public Health in Low and Middle Income Countries	2013	3.0
Courses for the Quantitative Researcher	2013	1.4
Doctor of Science in Clinical Epidemiology, NIHES, Rotterdam, the Netherlands		
History of Epidemiologic Ideas	2013	0.7
Conceptual Foundation of Epidemiologic Study Design	2014	0.7
Causal Inference	2014	0.7
Principles of Genetic Epidemiology	2014	0.7
Repeated Measurements	2014	1.4

Principles of Epidemiologic Data Analysis	2014	0.7
Bayesian Statistics	2014	1.4
Prognosis Research	2014	0.9
Missing Values in Clinical Research	2014	0.7
Extracurricular courses		
English Language C1.1	2012	1.4
Introduction to Medical Writing	2013	1.1
Research Integrity	2014	0.3
Stralingshygiëne 5R	2014	0.3
Seminars and Workshops		
Generation R research meetings	2013-17	1.0
Research meetings Department of Oral & Maxillofacial Surgery, Special Dental Care and Orthodontics	2013-15	1.0
Conferences and Presentations		
90th Congress of the European Orthodontic Society. Warsaw, Poland. Poster Presentation	2014	0.7
Nederlandse Vereniging voor Orthodontisten. Dalfsen, the Netherlands. Oral presentation	2015	0.5
Research meetings Department of Oral & Maxillofacial Surgery, Special Dental Care and Orthodontics. Oral presentation	2015	0.5
The International Association for Dental Research, General Session & Exhibition. Boston, United States. Poster Presentation	2015	0.7
Research meeting the Generation R Study. Oral presentation	2016-17	1.0
Research meetings MolEpi. Oral presentation	2017	0.5
Teaching activities		
Brunilda Dhamo, MSc student "The association between hypodontia and dental development"	2014	1.5
Rosalin W Drost, MSc student "Dentofacial trauma and players' attitude towards mouthguard use in field hockey: a systematic review and meta-analysis"	2015	1.5
Other activities		
Collaboration with the Injury Prevention Task Force within the Royal Dutch Hockey Association (KNHB)		
Completed exams of Dutch Ministry of Health, Welfare and Sport for dentists with a foreign diploma		

1 ECTS (European Credit Transfer System) is equal to a workload of 28 hours

WORDS OF GRATITUDE

Finally, after five years in the Netherlands, first as a Netherlands Institute for Health Sciences (NIHES) master student and then as an Erasmus–Western Balkans (ERAWEB) scholar and a Ph.D. student, I can begin writing the "Dankwoord" paragraph. The road towards my Ph.D. diploma has been an amazing journey, with many twists and turns. Now, looking back, I am happy that I am finishing this chapter of my thesis.

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