Department of Forensic Medicine
Faculty of Medicine
Doctoral Programme in Population Health
University of Helsinki

FORENSIC AGE ASSESSMENT IN FINLAND,

AND DENTAL DEVELOPMENT OF SOMALIS

Mari Metsäniitty

SUPERVISORS

Docent Janna Waltimo-Sirén, DDS, PhD
Department of Oral and Maxillofacial Diseases, University of Helsinki
Helsinki, Finland

Professor Emerita Helena Ranta, DDS, PhD
Department of Forensic Medicine, University of Helsinki
Helsinki, Finland

REVIEWERS

Professor Emeritus Pekka Karhunen, MD, PhD
Department of Forensic Medicine, University of Tampere
Tampere, Finland

Professor Emerita Maija Laine-Alava, DDS, PhD
Institute of Dentistry, University of Eastern Finland
Kuopio, Finland

OPPONENT

Professor Pertti Pirttiniemi, DDS, PhD
Department of Oral Development and Orthodontics, University of Oulu
Oulu, Finland

Dedicated to Iines, Anni, and Aku

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

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The publications are referred to in the text by their Roman numerals.

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ABBREVIATIONS

ABFO	American Board of Forensic Odontology
AGFAD	Study Group on Forensic Age Diagnostics
ANOVA	Analysis of variance
BC	Belgian Caucasian
BMI	Body mass index
CEAS	The Common European Asylum System
CFR	Charter of Fundamental Rights of the European Union
CI	Confidence interval
CRC	United Nations Convention of the Rights of the Child
CT	Computed tomography
CVM	Cervical vertebral maturation
C3	Third cervical vertebra
DA	Dental age
DNA	Deoxyribonucleic acid
EASO	European Asylum Support Office
EU	European Union
F	Female
FDI	The World Dental Federation
FELS	Fels longitudinal study, a method for assessing skeletal maturity of hand and wrist
FO	Forensic odontologist
GH	Growth hormone
G-P	Greulich and Pyle
ICC	Intraclass correlation
IGF-I	Insulin-like growth factor-I
IOFOS	International Organization for Forensic Odonto-Stomatology
LB	Lower boundary
M	Male
MAE	Mean absolute error
ME	Mean error
MEN2B	Multiple endocrine neoplasia, type 2B
MRI	Magnetic Resonance Imaging
mRNA	Messenger ribonucleic acid
NNL	Neonatal line
OV	Olli Varkkola
PT	Permanent teeth
RMSE	Root mean square error
RUS	Radius, ulna and short bones
SA	Skeletal age

SAB	South African Black
SM	Somali model
STUK	Radiation and Nuclear Safety Authority
THL	National Institute for Health and Welfare
TM	Third molars
TWI, TW2, TW3	Tanner and Whitehouse methods
UB	Upper boundary
UDI	The Norwegian Directorate of Immigration
UN	United Nations
WHO	World Health Organization
WM	Willems et al. model
3T MRI	3 Tesla Magnetic Resonance Imaging
ΔMAE	Difference in mean absolute error
ΔME	Difference in mean error
ΔRMSE	Difference in root mean square error
μSv	microSievert

1 ABSTRACT

Assessment of the age of an unknown person often occurs in forensic medicine. Besides victim identification, age assessment procedures have become more and more frequent in assessing adult versus minor status of asylum seekers or assessing the age of an unaccompanied minor. Age assessment is largely based on the evaluation of markers of biological maturity. The methods used in Finland comprise bone age assessment from hand–wrist radiographs as well as dental age assessment, normally from dental panoramic radiographs. The accuracy of these methods is largely dependent on the validity of reference data. Collection of radiographic reference data today is largely restricted due to ethical reasons, and it is necessary to rely on historical samples. Radiographic data, even if available on clinical grounds, from developing countries for ethnicity-specific reference tables is hampered by the fact that the chronological age of the subjects is often unknown or unreliable. That underlies today's situation where age assessments of African asylum seekers in particular are performed without normative values of the same ethnicity.

The purpose of this study was to critically analyse and possibly increase the quality of forensic age assessments. This was addressed by 1) assessment of the present validity of the historical method for age assessment by Greulich and Pyle (G-P) (1959), and its comparison with other skeletal X-ray and dental methods; 2) qualitative and quantitative analysis of the forensic age assessments of asylum seekers in Finland, and a description of the Finnish legislation that outlines the process; 3) creation of a Somali dental development model on lower left permanent teeth (PT) from 31 to 37 and to compare it with the previously validated Willems et al. model (2001); and 4) exploration of whether addition of information of third molar (TM) development increases the accuracy of age assessment of young Somalis.

The radiographic data from Finnish child victims (N=47) of the Southeast Asian Tsunami in 2004 was analysed using three dental methods: 1) eruption of the teeth, 2) mineralisation of PT, and 3) mineralisation of TM. The three skeletal methods of the hand and wrist included 1) the G-P method (1959), 2) the radius, ulna, and short bones (RUS) method, and 3) the 20-bones method (TW2) (Tanner et al. 1983). The second study was based on information collected from Finnish legislation texts, EU statistics and public statistics on asylum seekers in Finland by the Immigration Authorities. The study also included an analysis of the forensic age assessments performed in Finland for asylum seekers in 2015. The dental development of a unique sample, Somalis born and living in Finland with known chronological age and ethnic background, was analysed using panoramic radiographs taken on clinical

grounds. PT development was staged according to Demirjian et al. (1973) and all available TM were staged according to Köhler et al. (1994). First, the Willems et al. model (WM) (2001) on PT was validated on a Somali sample comprising 635 individuals (324 males, 311 females) in the age range between 4 and 18 years. A Somali-specific age estimation model (SM) based on the WM was then established. Secondly, on 803 Somalis (397 males, 406 females) aged 3–23 years the age prediction performances of PT and TM development were validated, separately and combined, using a Bayesian approach.

When comparing dental and skeletal methods, the dental methods showed the smallest deviation from chronological age, and the highest average intraclass correlation (ICC) values were obtained using the development of PT (0.994). Of all 3,024 asylum-seeking minors in 2015, 149 underwent forensic age assessment and they originated from 11 countries altogether, most often Afghanistan, Iraq, and Somalia. For age assessment, information from both dental panoramic radiographs and hand–wrist radiographs were utilised, according to the Finnish legislation, using previously obtainable reference data. Regarding the dental development of Somalis, the WM validated on the Somali sample resulted in a statistically significant difference in chronological age only for females with a mean error (ME) of 0.20 years (p=0.0006). When the performances of the two models (WM and SM) were compared, small but statistically significant differences (p<0.0001) in ME were detected: -0.07 years in males and 0.16 years in females. The approach combining PT and TM overestimated age by a ME of 0.031 in males and 0.011 years in females, showing the best age prediction performance of all three approaches.

In conclusion, with the exception of assessment based on only TM, dental methods proved to be more accurate than skeletal methods. In line with that result, the current Finnish legislation on forensic age assessment has been successfully implemented and approved by forensic odontologists and immigration authorities. The legislation justifies dental panoramic radiographs for the purpose of age estimation. In assessment of the age of young individuals, the widely used WM, based on a Belgian population, performs well for Somali children, and the performance of SM was only slightly better. These findings support the universal application of the WM for forensic age assessment. The age prediction performance in Somali children, born and living in Finland, is improved by combining the information of PT and TM, especially in the age groups of 12 to 15 years, when both PT and TM are still developing.

2 INTRODUCTION

In forensic medicine, an essential question often concerns the age of an individual. In situations when the chronological age of the individual is unknown, biological methods of age estimation are widely used. They are, for instance, based on the size of the individual, the occurrence of secondary sex characteristics, maturation of bone, or dental development (European Asylum Support Office 2018; Schmeling et al. 2016). Using the latter method, estimation of dental age is advocated for by the fact that 1) it covers the time period from prenatal to late puberty or early adulthood, when the last apices of the wisdom teeth are closed, 2) the development of teeth is strongly genetically regulated, and the influence of environmental factors is minimal, 3) the method is considered fairly reliable, 4) even though ionising radiation is used, the radiation exposure is small, and 5) the psychological burden of the method is minimal (Schumacher et al. 2018). Based on these facts, dental age estimation has been selected as a key method in forensic age assessment in Finland. Forensic age assessments are performed in Finland by dentists with special competence in forensic odontology.

Performing age estimation by using any of the previously mentioned biological age-estimation methods requires comprehensive knowledge of the normal timetable of development of the population and the normal variation within the population. The timing of dental development has been comprehensively studied in the Finnish population (Chaillet et al. 2004a; Kataja et al. 1989; Nyström et al. 2007). The reliability of the methods increases when the control material is from the same ethnic group, because of the possible differences between the populations (Cavrić et al. 2016a; Liversidge 2008; Olze et al. 2006; Phillips and van Wyk Kotze 2009). Even though increasing scientific evidence exists that ethnic differences are negligible for age estimations based on tooth development (Elamin et al. 2017; Esan and Schepartz 2018a; Kihara et al. 2017; Liversidge et al. 2010 and 2017; Thevissen et al. 2010a; Willems et al. 2017), additional evidence regarding populations of African descent is needed to confirm this. Other factors that increase the reliability include the topicality of the control material because of possible secular changes between generations (Birchler et al. 2018; Eskeli et al. 2016; Sasso et al. 2013; Vucic et al. 2014), and consistency of results from different methods.

Extensively used methods in forensic age estimation are based on skeletal and dental development. The atlas of Greulich and Pyle, "*Radiographic Atlas of Skeletal Development of the Hand and Wrist*" (1959), forms the basis of a widely established method of skeletal age estimation based on hand and wrist radiographs. The validity of the atlas has nevertheless been frequently questioned because the reference population is Caucasian from North America

from dating back to 1931–1942. Ethnic differences have been indicated to exist when using the method of Greulich and Pyle (G-P) (Loder et al. 1993; Ontell et al. 1996), whereas van Rijn et al. (2001) showed that G-P is still useful for Dutch Caucasian children. Correlation between skeletal age and dental age is an important topic of research in order to improve the methods used for forensic age estimation and to guarantee the legal protection of examinees.

Within the concept of dental age estimation, contemporary research aims at an increased understanding regarding ethnic variation in tooth development, and in this thesis, especially of the timing of dental development of Somalis. The dental maturity status is vastly used in forensics to estimate age (Schmeling et al. 2016), as well as in orthodontics and paediatric dentistry for treatment planning and as a diagnostic tool (Celikoglu et al. 2011). In orthodontics, dental age assessment is beneficial for optimising the onset of treatment with fixed appliances, although it has been shown that dental age, like chronological age, is a poor predictor of pubertal growth spurt, the timing of which is generally assessed for optimising treatment with functional appliances (DuPlessis et al. 2016; Litsas and Lucchese 2016). Scientifically this research is important, since few studies on dental development of sub-Saharan populations exist.

The reliability of forensic age estimation is essential when performing age assessments for asylum seekers. The topic has become even more important due to the high numbers of asylum seekers arriving in Europe (Eurostat; The Finnish Immigration Service(a)) and the consequent exponential increase in the number of forensic age assessments performed during recent years. This is an international phenomenon, but there is no international consensus on the grounds and methods for age estimation, nor common legislation that regulates the process. Age estimations can reliably be performed by experienced professionals and by using validated methods and best available reference data. Good practice includes an analytical approach identifying areas still requiring improvement, providing the motivation for the following study on forensic age assessment in Finland and the evaluation of the timing of dental development in ethnic Somalis.

3   REVIEW OF THE LITERATURE

3.1   FORENSIC AGE ESTIMATION

The age of the individual is an essential factor of identity and the question of age often arises in forensic medicine. Early research in age estimation was often based on research of the deceased, while age estimation in living individuals is a relatively recent research area within the forensic sciences (Black et al. 2011). The first forensic age assessment cases of the living in Finland were in the 1990s and related to international adoptions. The need for forensic age assessments increased notably since 2009 due to the rising number of immigrants and unaccompanied minor asylum seekers coming to Finland without a certificate of identity (Parsons 2010). According to the Aliens Act (301/2004) and the amendments of the Act (549/2010, 501/2016), immigration authorities have the right to refer asylum seekers to the National Institute of Health and Welfare (THL) for age assessment in cases when the real age of the immigrant is unknown. In other countries, forensic age assessment can also be requested by courts and other government authorities (Schmeling et al. 2016). These assessments are especially performed to clarify if the person is of major age, legal adult age, the cut-off being 18 completed years. In recent years, in Finland, the majority of minor asylum seekers referred by the authorities to forensic age assessment had reported that they were 15–17 years old and a similar trend was seen in other parts of Europe as well (Eurostat). Unaccompanied minor asylum seekers may arrive without documents for identification, because in part of the world no official system for registering births exists or registration may be delayed due to infrastructural, cultural or geographical reasons (Pelowski et al. 2016; Timme et al. 2017a).

Upon arrival authorities may have set the age incorrectly, and sometimes the age of a minor immigrant needs to be re-evaluated later. The Finnish Government's Proposal (Hallituksen esitys eduskunnalle laiksi ulkomaalaislain muuttamisesta 240/2009) states that age estimation examinations performed upon ones own proposal and at one's own or at other authorities' cost must fulfill the same demands of reliability as those performed by request of Immigration authorities and paid for by the government. To guarantee the quality of the examination methods and that the use of ionising radiation fulfills the national legislation, these private examinations must be performed by two forensic odontologists (FO), one of them being affiliated with the University of Helsinki. The FOs performing private age estimations are co-workers in the age estimations of THL, which guarantees the quality and consistent procedures and methods of the examination. An individual's correct age is important regarding procedural

privileges and social benefits, for instance, the right to education, shelter, and child care services (Schmeling et al. 2016). A minority status guarantees a wider range of rights and legal safeguards according to the United Nations (UN) Convention of the Rights of the Child (CRC) and the Common European Asylum System (CEAS) (Schumacher et al. 2018). An incorrect age-group affiliation may have severe consequences, such as problems caused by inappropriate accommodation of the unaccompanied minor with adults in the reception center, or the opposite (European Asylum Support Office 2014 and 2018).

Forensic age assessment is used in criminal proceedings as well. The age limits of relevance in different European countries in criminal proceedings may vary from 14 to 21 years (Criminal Code of Finland Act 39/1889 and the amendment 515/2003; Schmeling et al. 2003 and 2016).

Forensic age assessment is sometimes applied in competitive sports. For instance, the age range to compete in Youth Olympics is 14–18 years. When an individual claims to be younger than their chronological age, age falsification can give an athlete advantages by unfair physical superiority. Problems may also arise when an over-aged athlete participates in contact sports and thus increases the risk of injuries to other, younger athletes. Another important issue is to protect the mental and physical health of under-aged athletes by excluding them from adult sports. Not unreasonably, no legal or ethical basis to use radiological examinations for age assessment in competitive sports exists (Timme et al. 2017a).

Forensic age assessment in the asylum process includes ethical challenges such as conflicting goals, equality and fairness, autonomy and informed consent, and, especially, whether the asylum seeker's consent to the procedure is genuinely voluntary. A sufficiently accurate age assessment can help to overcome these challenges (Aarseth and Tonsaker 2018; Malmqvist et al. 2017), and a medico-legal evaluation of asylum seekers improves the outcome of the asylum-seeking process (Franceschetti et al. 2018).

3.2 METHODS OF FORENSIC AGE ESTIMATION

In situations when the chronological age of the individual is unknown, non-medical age assessment methods, like further assessment of documentary evidence, an age-assessment interview or psychosocial assessment, as well as medical methods of age estimation are widely used (European Asylum Support Office 2018; Schumacher et al. 2018). Medical assessment is based on the height and size of the individual, the occurrence of secondary sex characteristics, maturation of bone, and/or dental development.

The risks of medical age assessment include that a child could be falsely assessed as an adult, or the assessed age of the child could be too high. Moreover, the individual undergoing medical age assessment may experience degrading or inhumane practices, for instance nudity, during examination (Schumacher et al. 2018), or become exposed to ionising radiation. In some European countries, there is no legal framework to perform radiological examinations for forensic age assessment (Timme et al. 2017a). Children and adolescents who are often involved in forensic age-estimation proceedings are substantially more sensitive to the risks of ionising radiation than adults, and therefore the criticality of any radiation exposure needs to be evaluated on an individual basis and the radiographs must be sufficiently optimised (Pakbaznejad Esmaeili et al. 2016).

The doses from radiographic examinations in forensic age assessments vary from less than 0.0001 mSv (left hand and wrist X-ray) to 0.026 mSv (dental panoramic radiograph) or to 0.6–0.8 mSv (computed tomography of the clavicle) (Ramsthaler et al. 2009). In particular, in computed tomography (CT), the level of exposure exceeds the magnitude of half the annual exposure to natural radiation, which is 1-5 mSv, whereas in radiography of the hand and wrist, knee, and TM, the amount of radiation is up to twice the daily dose of natural radiation (Schumacher et al. 2018). Age assessment is recommended to start with hand and wrist and dental radiographs, where the dose of radiation is low. Only if these age markers do not provide enough information of the age or they suggest being of adult age should markers involving CT of the clavicle be considered (Schumacher et al. 2018). Anthropometric data and information on developmental disorders can be collected instead, or dental examination can be performed by recording dental eruption and status (Timme et al. 2017a). These methods cannot be implemented in all countries, however (Aliens Act). It should be emphasised that the accuracy of age diagnostics will diminish without the use of imaging procedures (Timme et al. 2017a). Therefore, non-ionizing radiation methods such as magnetic resonance imaging (MRI) (De Tobel 2017; Ebner et al. 2014; Guo et al. 2015; Hojreh et al. 2018; Krämer et al. 2014a and 2014b; Serin et al. 2016; Vieth et al. 2018) and ultrasound methods have been developed (Mentzel et al.2005; Schulz et al 2008; Utczas et al. 2017). DNA-methylation in forensic age estimation is currently studied, and, in the future, it might be possible to obtain age assessment from a sample of blood or saliva (Parson 2018). Here focus is on medical forensic age assessment methods.

3.2.1 MEDICAL HISTORY AND PHYSICAL EXAMINATION

According to the recommendations of the Study Group on Forensic Age Diagnostics (AGFAD) age assessment procedures should begin with an interview of medical history of possible illnesses and medication affecting

growth (Schmeling et al. 2016), including any deficiencies in nourishment (Schmeling et al. 2000). Anthropometric data, meaning the measurement of an individual, like height, weight with clothes on, and body type (body mass index=BMI) are recorded. Medical assessment is needed to identify developmental disorders. Diseases may lead to developmental delay and underestimation of age, which can be legally beneficial for the asylum applicant, whereas accelerated development may lead to overestimation of age, which should be avoided (Schmeling et al. 2016). Differences exist between sexes as well: females start their growth spurt at younger age than males (Tanner et al. 1983). During puberty, oestrogen induces the stimulation of growth hormone (GH) and insulin-like growth factor-I (IGF-I), which are the main stimulators of longitudinal bone growth (Shim 2015).

Growth retardation results from complex socioeconomic factors during important periods of growth in children, malnutrition being the most common reason (Hoffman and Klein 2012). Chronic diseases, psychosocial growth disorder, hypothyroidism, deficiency in GH, excess cortisol as well as syndromes, such as Down's syndrome, and Turner syndrome cause delay in growth (Barstow and Rerucha 2015; Blodgett et al. 1956; Kerrebijn and de Kroon 1968; Tanner et al. 1983).

More rarely, diseases accelerate statural growth either directly or through their medical treatment. For instance, hyperthyroidism, GH substitution, precocious puberty, obesity, some rare hormonal developmental disorders like gigantism and acromegaly, and syndromes such as Marfan syndrome, Sotos syndrome, Beckwith–Wiedemann syndrome, Klinefelter syndrome, and multiple endocrine neoplasia, type 2B (MEN2B), accelerate statural growth (Barstow and Rerucha 2015; Carel et al. 2004; Coutant et al. 2017; Kamp et al. 2002; Krekmanova et al. 1999). Hyperthyroidism is the most common finding of the previously mentioned disorders in asylum applicants, and when undetected, might lead to over-estimation of age (Rudolf et al. 2015). Schmeling et al. (2016) estimate that due to findings of medical interview or physical examination, age assessment is not possible in 1% of cases.

3.2.2.1 SEXUAL MATURATION

Gonadal and adrenal stimulation during puberty will lead to sexual maturity. Female maturation starts with breast development, and the stage of maturation is estimated from the degree of breast development as well as the degree of pubic and underarm hair. Timing of menarche with regard to secondary markers of sexual maturity is irregular (Tanner 1976). For estimation of male maturation, the enlargement of the testes, scrotum, and penis is evaluated as well as the degree of underarm, pubic, and facial hair (Schmeling et al. 2016; Tanner 1976). Since the beginning of menstruation

relies on individual memory, it has a limited value in age assessment. The development of external genitalia can be visually or metrically assessed, but the problem in collecting this information is both ethical and social (Black et al. 2011). The most commonly used method for clinical evaluation of secondary sexual characteristics is the "Tanner secondary sexual development" staging (Tanner and Whitehouse 1976), and the criteria are given in the following Table 1. Any age assessments based on these criteria should be done with caution due to the large normal variation of approximately four years. The method is very inaccurate and when it includes assessment of sexual maturation serious ethical considerations exist. The method requires nudity or the examination of genitalia and is therefore not recommended by EASO (European Asylum Support Office 2018; Schumacher et al. 2018), and is not used in Finland.

Table 1. Tanner secondary sexual development staging for females and males adapted from Tanner and Whitehouse (1976) and Black et al. (2011).

Stage	Breasts (Female)	Genitals (Male)	Pubic Hair (Female and Male)
I	No glandular tissue; areola follows the skin contours of the chest	Testicular volume less than 1.5 ml; penis of 3 cm or less	No pubic hair
II	Breast bud forms, with small area of surrounding glandular tissue; areola begins to widen	Testicular volume 1.6−6 ml; skin on scrotum thins; penis length unchanged	Small amount of long, downy hair with slight pigmentation at the base of penis (M)/ on the labia majora (F)
III	Breast begins to become more elevated, and extends beyond the borders of the areola, which continues to widen but remains in contour with surrounding breast	Testicular volume 6−12 ml; scrotum enlarges further; penis begins to lengthen to about 6 cm	Hair becomes coarser and curlier, begins to curve laterally
IV	Increased breast size and elevation; areola and papilla form a secondary mound projecting from the contour of the surrounding breast	Testicular volume 12−20 ml; scrotum enlarges further and darkens; penis increases in circumference and length to 10 cm	Adult-like hair quality, extending across pubis but sparse on the medial aspect of the thighs
V	Breast reaches final adult size; areola returns to contour of the surrounding breast, with a projecting central papilla	Testicular volume greater than 20 ml; adult scrotum and penis of approx. 15 cm in length	Hair extends to medial surface of the thighs

3.2.2 SKELETAL AGE

Skeletal age is based on radiographic detection of first the appearance of primary and secondary ossification centres, followed by the beginning of epiphyseal fusion and the different stages of epiphyseal fusion and finally the completion of epiphyseal fusion (Hackman 2012). The stage of biological maturity of an individual is compared with a standard and then translated into a probable chronological age (Black et al. 2011). Females have an accelerated skeletal maturation compared to males, and reach the final ossification stage earlier (Molinari et al. 2004).

Several atlases have been created to assess the stage of skeletal maturation or skeletal age. A standard radiographic image represents a child at a certain age; when performing age estimation, the radiograph is compared to the image in the atlas. The atlases present standards of different ossification centres of the body: hand and wrist (Cameriere et al. 2006; Gilsanz and Ratib 2005; Greulich and Pyle 1959; Gòk et al. 1985; Tanner et al. 2001; Thiemann and Nitz 2006), knee (Pyle and Hoerr 1969), foot and ankle (Hoerr and Francis 1962), and elbow (Brodeur et al. 1981; Dimeglio et al. 2005). Hackman (2012) tested six of these previously mentioned methods of age assessment for living individuals in modern populations from Scotland and India: hand and wrist by Greulich and Pyle (1959) and Tanner et al. (2001), knee by Pyle and Hoerr (1969), foot and ankle by Hoerr and Francis (1962), and elbow by Brodeur and Gravis (1981) and Dimeglio et al. (2005), and found that only the G-P atlas allows for age estimation up to 18 years of age in females. In the living individuals, other ossification centres can be used as well (Table 2) (Black et al. 2011). Development of the cervical vertebrae has been suggested for age estimation (Litsas and Lucchese 2016), but preferably combined with dental development due to problems caused by large biological variance of the shapes and difficulties in separating adults from children (Gelbrich et al. 2017; Thevissen et al. 2012a).

3.2.2.1 SKELETAL DEVELOPMENT

Moderate exercise is essential for healthy skeletal development, especially during growth, in order to improve bone mass and density, as well as to prevent later osteoporosis (Shim 2015). In skeletal maturation, girls are advanced compared to boys (Molinari et al. 2004). Several paracrine and endocrine factors affect the normal skeletal development. GH, oestrogens, androgens, and IGF-I are the most important hormones controlling skeletal maturation. Longitudinal bone growth is mainly influenced by GH and IGF-I through their direct effect on chondrocytes in the growth plate, whereas bone maturation is mainly affected by androgens or testosterone. Paracrine factors, such as parathyroid-hormone-related protein, bone morphogenetic protein, and fibroblast growth factor, stimulate differentiation of chondrocytes during bone formation and growth (Shim 2015). The fusion of growth plates of long bones can be enhanced by elevated levels of serum estradiol in men, and lead to premature ossification of growth plates (Timme et al. 2017a).

Table 2. Skeletal age estimation in the living, and approximate time for complete fusion of epiphyseal ossification centres. Adapted from Black et al. (2011).

Bone	Females/years	Males/years
Humerus		
Distal fusion	11-15	12-17
Medial epicondyle	13-15	14-16
Proximal fusion	13-17	16-20
Radius		
Proximal fusion	11-13	14-17
Distal fusion	14-17	16-20
Ulna		
Proximal fusion	12-14	13-16
Distal fusion	16-18	17-20
Hand		
All epiphyses	13-15	15-17
Femur		
Head fusion	12-16	14-19
Greater trochanter	14-16	16-18
Lesser trochanter	16-17	16-17
Distal fusion	14-18	16-20
Tibia		
Distal fusion	14-16	15-18
Proximal fusion	13-17	15-19
Fibula		
Distal fusion	12-15	15-18
Proximal fusion	12-17	15-20
Foot		
Calcaneus	15-16	18-20
Distal and mid-phalanges and metatarsal heads	11-13	14-16
Proximal phalanges and base of metatarsal one	13-15	16-18
Medial clavicle	28	28
Iliac crest	24	24

Hypothyroidism, cortisol, and systemic diseases cause delay in skeletal maturation (Kerrebijn and de Kroon 1968; Tanner et al. 1983), whereas precocious puberty (Carel et al. 2004), adrenogenital syndrome, hyperthyroidism (Schmeling et al. 2016), and high-dose growth hormone treatment (Kamp et al. 2002; Krekmanova et al. 1999) can lead to accelerated skeletal maturity (Schmeling et al. 2016). Symptoms of accelerated hormonal development affecting skeletal maturation are the same that affect statural growth (see paragraph 3.2.1). In an optimal environment, meaning high

socioeconomic status and individual wellbeing, the genetically determined potential of skeletal maturation independent of place of birth or ethnic background leads to normal skeletal maturation whereas a poor economic environment with malnutrition, poor hygiene, and a high morbidity rate may induce retardation of skeletal maturity (Schmeling et al. 2000).

3.2.2.2 HAND AND WRIST

A radiograph of the hand and wrist (left, traditionally) is recommendable, because potentially harmful ionising radiation can be minimised by isolating the hand from the body. The hand and wrist bones also contain multiple ossification centres. The appearance, morphological changes, and fusion of different centres follow an established pattern (Figure 1). The epiphysis of the distal radius fuses last, and at a relatively late age (Table 2) (Hackman 2012).

The G-P atlas (Greulich and Pyle 1959) is widely used to assess bone age. The atlas is based on children in 1930s North America. The atlas presents standard hand and wrist radiographs of children at 30 points of different maturity stages, girls and boys separately. The researcher compares the examinee's X-ray to the standards and chooses the best fitting one with a given age. For females 31 and for males 27 standards exist. The weakness of the method is the subjectivity of the technique, because the different bones of the hand and wrist may develop at different pace and a perfect match is often difficult to find. However, each standard picture includes an explanation to guide in choosing the correct maturity stage (Greulich and Pyle 1959). In younger age groups greater weight should be given to the developmental stages of the metacarpals and phalanges because concentrating on carpal ossification centres leads to underestimation of age (Hackman 2012). When using the G-P atlas, the minimum age for complete skeletal maturation of distal forearm and hand bones as evaluated from a standard hand and wrist radiograph is 16 years for both sexes (Tisé et al. 2011). Prediction intervals (95%) are large after 10 years of age: around 4 years. In forensic age estimation, individual variability must be taken into account when using G-P (Chaumoitre et al. 2017). Among different skeletal atlas methods, G-P is the only one that allows age estimation up to 18 years of age, except for males since their growth period is generally longer (Hackman 2012).

Figure 1. Hand and wrist radiograph of a child for forensic age assessment.

The most widely used single-bone methods are the Tanner and Whitehouse methods TWI, TW2, and TW3 (Black et al. 2011). The TW3 is based on a more contemporary European population (Tanner et al. 2001), but since new editions of the sold-out publication have not been printed, the TW2 method is applicable for forensic age estimation (Black et al. 2011). For the TW2 method hand and wrist radiographs were taken every 6 months over a period of up to 12 years from 2702 (1385 males and 1317 females) healthy British children aged 1 to 21 years in a longitudinal study from different samples during 1946–1972 (Tanner et al. 1983). Successive changes in the shape and density markings of each bone were recorded from first appearance until adult stage, and the continuous change was divided into eight or nine different stages of maturation from A (not visible in the X-ray) to H or I (fully developed), depending on the area. The stages are presented as standard radiographs as well as illustrations and text. Three different scoring systems were created 1) radius, ulna and short bones (RUS), 2) carpals only, and 3) both RUS and carpals combined (TW2 20-bones). The RUS score includes 13 bones, carpal score 7 bones, and the TW2 20-bones score 20 bones. The weighted bone maturity scores are presented in tables separately for boys and girls, and the total maturity score is the result of adding the maturity scores together. The maturity scores can be expressed in bone age by using centile standard curves for all three methods. The standard deviation of RUS bone age is approximately 1 year between ages 5–16 in males and ages 5–14 in females, and almost the same is expected for carpal bones.

Other methods exist as well for evaluating the maturation of the hand and wrist, namely, the Fishman's method (Fishman 1982), the FELS method (Fels longitudinal study, a method for assessing skeletal maturity of the hand and wrist) (Chumela et al. 1989), and the method by Cameriere and co-authors (2006). The Gòk (Gòk et al. 1985) and Thiemann-Nitz atlases (Thiemann and Nitz, 2006) have limited used since they were published in their creators' native languages, Turkish and German, respectively, but have been shown to be suitable for forensic age assessment due to contemporary reference populations (Büken et al. 2007 and 2009; Schmeling et al. 2006). Gilsanz and Ratib (Gilsanz and Ratib, 2005) have created a digital atlas and application for the computer, which is freely available on the internet. It includes artificial, idealised, sex- and age-specific images of skeletal development. This digital atlas has limited use in criminal proceedings due to overestimation of age (Schmidt et al. 2009). Recently, a biometric method to estimate age on hand radiographs has been developed based on width and length measurements of metacarpals and proximal phalanges (Remy et al. 2017). Cameriere et al. (2018) presented a Bayesian approach on carpals and epiphyses of radius and ulna as age indicators using longitudinal data from the Burlington Growth Centre. The areas of epiphyses of the radius and ulna and carpal bones were defined by a polygonal lasso instrument, the pixel areas were calculated and added to yield values for bone areas.

Since single-bone methods are time-consuming and do not offer improved prediction accuracy, atlas methods such as G-P (Greulich and Pyle 1959) and Thiemann-Nitz (Thiemann and Nitz, 2006) are recommended for assessing skeletal maturation of the hand and wrist (Schmidt et al. 2013a). RUS bone age has other benefits, because it can also be used for predicting the adult height of the child by using equations including height, chronological age, RUS bone age, and a constant (Tanner et al. 1983).

BoneXpert is an automated computer method for assessing skeletal age (Thodberg et al. 2009). From X-rays of the hand and wrist, BoneXpert reconstructs the borders of bones automatically, counts intrinsic bone ages, and changes them into TW or G-P bone ages. It contains models for both females and males with extended age ranges up to 18 years for females and 19 years for males (Thodberg et al. 2017). It is a valid method for assessing skeletal age of Finnish children. The mean difference between the values for ages from G-P and BoneXpert were for females 0.13 years and for males 0.028 years, smaller than in previous studies which validated BoneXpert (Lammert 2015).

Non-invasive imaging techniques have been developed to avoid radiation exposure during age assessment, and MRI shows the most promising results (Schmidt et al. 2015; Serinelli et al. 2015). When using hand MRI and G-P atlas criteria in skeletal age assessment of adolescents, no significant average

difference in assessed ages was discovered (Hojreh et al. 2018). Recently, the ability of MRI scans of the hand to discriminate the age of 18 years has been studied (Serin et al. 2016; Timme et al. 2017b). Serin and co-workers (Serin 2016) used a three-stage scoring system analysing the distal epiphysis of radius and ulna and the base of the first metacarpus in hand and wrist. They recommended that this method should not be used alone but rather in combination with other age assessment techniques. Timme and co-workers (Timme et al. 2017b) used the same clavicular ossification staging system as Schmeling et al. (2004) in conventional radiography and Kellinghaus et al. (2010a) in thin-slice multidetector CT for distal radial epiphyses with stages from I to V including several substages. Stage V, when the epiphyseal cartilage has fully ossified and the epiphyseal scar is not visible, was present for females at 22.3 years of age at the youngest and 23.1 years for males. Whether or not stage IVb could be used to discriminate 18 years of age, needs further studies (Timme et al. 2017b). In the future, automated bone age estimation from MRI may be possible and techniques have been developed (Ebner et al. 2014; Urschler et al. 2015).

Skeletal maturity of the hand and wrist has also recently been assessed by ultrasonic methods (Mentzel et al. 2005; Schmidt et al. 2013b; Utczas et al. 2017). When ultrasound waves hit high-density bone tissue, they are reflected and bone appears white in the ultrasonic receiver. When they hit a low-density cartilage tissue, the waves spread in the medium and cartilage appears black in the ultrasonic receiver (Schulz et al. 2008). Benefits of the method include objectivity, the method is free of ionising radiation, and it is easy to access. For small children difficulties may cause a lack of co-operation and an inability to keep still during the examination. Both Mentzel et al. (2005) and Utzcas et al. (2017) showed a high correlation between traditional radiograph of the hand analysed with the G-P method and an ultrasonic method. The ultrasound method was suggested to be used in epidemiological studies for 8- to 15-year-old children (Utczas et al. 2017). Ultrasound use in age estimation is, however, not recommended by the EASO (European Asylum Support Office 2018) because of deficiencies in the identification of phases with reasonable precision.

3.2.2.3 CLAVICLE

The clavicle is the last bone to ossify in the human skeleton, and in the medial clavicular epiphysis, which is used for age assessment, epiphyseal ossification starts in both sexes at age 16–21 years (Black et al. 2011). The staging by Schmeling et al. (2004) for clavicular ossification in conventional radiography includes 1) non-ossified epiphysis, 2) discernible ossification centre, 3) partial fusion, 4) the visibility of the epiphyseal scar, and 5) disappearance of the epiphyseal scar following total fusion (Figure 2). Stage 4 was detected in

females at age 20 years, and in males at 21 years, and stage 5 for both at 26 years at the earliest. Kellinghaus et al. (2010a and 2010b) later used similar staging adapted after Kreitner et al. (1997), but added several substages in thin-slice multidetector CT. For assessing age through medial clavicular ossification, CT is currently suggested as the method of choice (Houpert et al. 2016; Schmeling et al. 2016) because of the difficulties in the assessment of the clavicular development stages in prospective posterior–anterior chest radiographs (Cameriere et al. 2012a; Wittschieber et al. 2015). Also, intra-individual stage discrepancies are generally moderate in thin-slice CT imaging (Rudolf et al. 2018).
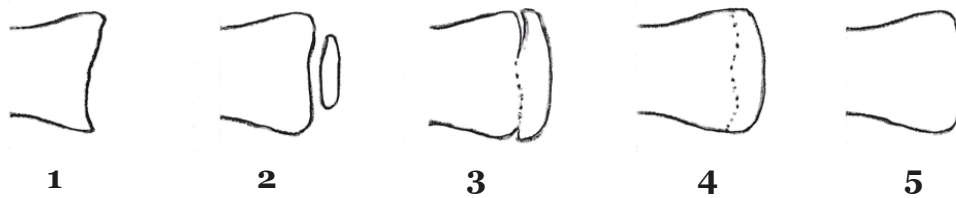

Figure 2. Main stages of clavicular ossification stages. Adapted from Schmeling et al. (2016).

MRI examination of the medial clavicular ossification by a modified protocol provides an ionising radiation-free method for forensic age assessment (Schmidt et al. 2007). MRI is nowadays more easily available since MRI scanners are not situated only in large hospitals anymore, and examinations are not so time-consuming and expensive as previously. In MRI the ossification stages by Schmeling et al. (2004) and Kellinghaus et al. (2010a) for medial clavicular cartilage are also used. Inclusion of the sub-stages (Kellinghaus et al. 2010a) also provides a more precise method to discriminate the age of 18 years in males and females (Schmidt et al. 2016). Stage 3c for both sexes means that age 18 years has been reached, and stage 4, age 21 years (Schmidt et al. 2017). Hillewig et al. (2017) showed that 3T MRI (3 Tesla MRI) provides higher resolution and more accurate age estimates of bone age than conventional radiography, and the examination with an MRI scanner takes a short time, only four minutes. Vieth et al. (2010) compared projection radiography, CT, and MRI and showed that imaging technique affects the estimation of the ossification stage of the medial clavicular cartilage.

Quirmbach et al. (2009) and Schulz et al. (2008) were the first pioneers in staging the clavicular ossification with ultrasound for a radiation-free forensic age assessment purpose. They used a four-stage method for the ossification of medial clavicular epiphysis. The minimum age for stage two was 17.1 years, for stage three 16.7 years, and for stage four 22.5 years (Schulz et al. 2008).

Although economic and fast to use, this method has proven to have difficulties in reliably differentiating between stages 1–4, and it is not yet a valid alternative to CT (Gonsior et al. 2016).

3.2.2.4 KNEE

The Pyle and Hoerr (1969) atlas method for the knee is based on finding the best possible match between the radiographic image of the examinee and the series of radiographs presented in the atlas. The material for the atlas was collected in North America from Caucasian children of high socioeconomic class since 1926. During the longitudinal study radiographs were taken from birth through 21 years of age every 3 months. In the atlas, each radiograph was assigned skeletal ages separately for males and for females. Because of overlaying structures in radiographs, any age estimation must involve two images of the knee: antero-posterior and lateral, which will increase the dose of radiation (Table 3) (Hackman 2012).

Table 3. Skeletal areas of the knee joint used for age assessment in the atlas of Pyle and Hoerr (1969). Adapted from Hackman (2012).

	Skeletal area			
View	Distal femur	Proximal tibia	Proximal fibula	Patella
Anterior posterior	Visible	Visible	Visible	Not visible due to overlaying
Lateral	Visible	Visible	Not visible due to overlaying	Visible

Hackman (2012) studied the usability of the Pyle and Hoerr atlas (1969) for contemporary Scottish children and adolescents. In females, the ossification of epiphyseal plates of the knee joint had reached full maturity by the age of 16 years and in males by the age of 19 years. In Hackman's study, a strong relationship was found between chronological age and estimated age for both sexes. The standard deviations were 9.86 months for females and 10.75 months for males. For a contemporary Scottish population, the Pyle and Hoerr atlas (1969) provides repeatable and reasonably accurate results for children and it could be used as an alternative to the hand and wrist radiographs, but the main limitation is the upper age limit of 16 years for females. The changes between epiphyseal plates of the knee can be subtle, and hence this method requires prior studying and experience (Hackman 2012; Hackman and Black 2013).

Cameriere et al. (2012b) developed a method to assess age 18 years from antero-posterior X-rays of the knee. Three stages are evaluated from the distal femur, proximal tibia, and proximal fibula. Stage 1 is defined as epiphysis not fused, stage 2 epiphysis fully ossified with epiphyseal scar visible, and stage 3 as fully ossified epiphysis but epiphyseal scar is not visible. The scores for stages 1, 2, and 3 are 0, 1, and 2, respectively. They are counted together, and the cut-off value suggested for discriminating the age of 18 years in an Italian population was a score 4 for males and 5 for females, but the method needs further studies in other populations to validate its usefulness (Galić et al. 2016). Also, on frontal radiographs, the persistence of epiphyseal scar of the three epiphyses of the knee was not a marker of a recent fusion since it was visible up to 40 years of age and therefore not correlated to skeletal age (Faisant et al. 2015). The scientific literature suggests that there are a significant number of cases where the development of the knee joint is complete before the age of 18 years for both sexes. Therefore, the skeletal age of the knee is not sufficient to safely exclude minors (Schumacher et al. 2018).

MRI in forensic age estimation has been applied for the knee as well. The epiphyses of the distal femur (Krämer et al. 2014a) and proximal tibia (Krämer et al. 2014b) were studied with 3T MRI applying a combination of the staging method introduced by Schmeling et al. (2004) and adapted by Kellinghaus et al. (2010a). Females did not reach ossification stage IV in the distal femur and proximal tibia before age 14 years, whereas for males, respective cut-off ages were 18 years and 16 years (Krämer et al. 2014a and 2014b). Recent studies show that forensic age assessment is feasible by using MRI of the knee (Auf der Mauer et al. 2018; Ottow et al. 2017; Saint-Martin et al. 2015). The age groups 14 and 16 years can be determined for both sexes, but the bony fusion does not discriminate the age of majority (Auf der Mauer et al. 2018; Ottow et al. 2017). In the Knee Bone Age Atlas using MRI (Pennock et al. 2018), the patella provided the best age assessment in early childhood, whereas the femur worked better in later skeletal maturation. Application of MRI of the knee is not easy, and the influence of different MRI hardware is unclear. The results need to be validated by other research groups (Vieth et al. 2018).

3.2.2.5 ILIAC CREST

The suitability of conventional radiography of iliac crest apophysis of the pelvis for forensic age diagnostics has been validated. The epiphyseal ossification starts in both sexes at ages 17–20 years (Black et al. 2011). Wittschieber et al. (2013a) used classification from 1 to 4, and sub-classifications in stages 2 and 3 ranging from a to c modified after Kreitner et al. (1997) and Kellinghaus et al. (2010a). Stage 4, corresponding to full ossification of the apophyseal cartilage, is reached in females at the age of 16 and in males at 17 years. Risser sign grading is another age assessment method for pelvic radiographs. The

method was originally developed to evaluate the risk of progression of spinal deformity with scoliosis patients (Risser 1958). Two Risser sign grading systems exist, namely American (US) and French, both including five stages. Both sexes reached stage 5 at age 14 years at the earliest in the US grading, whereas in the French grading this stage was reached at the earliest at the age of 16 in females, 17 years in males (Wittschieber 2013b). A later study by Bartolini et al. (2018) showed, however, that the staging technique modified after Kreitner et al. (1997) and Kellinghaus et al. (2010a) is easier to use than the Risser method, since it is not affected by variations of ossification. Wittschieber et al. (2013c) validated a modified method originally developed by Cameriere et al. (2006) on iliac crest apophysis using means of area measurements. The method showed only moderate correlation with chronological age (Bartolini et al. 2018). Due to the high effective dose of radiation in the gonadal area of the body, iliac crest methods are not recommended for age estimation of living individuals unless pelvic radiographs or CT were previously taken for medical reasons (Ekizoglu et al. 2016; Lottering et al. 2017; Wittschieber et al. 2013a).

MRI offers a radiation-free method for the evaluation of the ossification of the iliac crest. However, due to the limitations of MRI, further sub-classification of the four stages is not possible. Reliable stage determination can be achieved with MRI; further research is recommended, however, especially for ages 10 to 20 years (Wittschieber et al. 2014a).

Ultrasound diagnostics of the iliac crest using the four-stage method were validated in two studies by Schmidt et al. (2011 and 2013c); ossification stage 4 was reached in females at the earliest at 17.1 and 17.9 years, whereas in males at 17.4 and 18.0 years, respectively in the two studies. The sonographic examination could improve the results of age diagnostics when integrated with other methods. The examination is, however, highly dependent on the level of experience of the examiner (Schmidt et al. 2013c).

3.2.2.6 ELBOW

Anatomically, the junction of the distal humerus, proximal radius, and proximal ulna form the elbow joint. The Brodeur et al. atlas (Brodeur et al. 1981) of the elbow was designed to complement the hand and wrist atlas. It is based on cross-sectional data (Hackman 2012). It includes both an anterior–posterior and a lateral image of the elbow, presenting all of the epiphyses at different ages, as well as abnormalities of the elbow. The maturation of the elbow is complicated, and overlying structures in elbow radiographs may hamper the analysis. The estimated age is presented as an age range. Standard radiographs are presented for both sexes separately between six-month

intervals, up to the age of 16 years 6 months, which is a limitation of the method in forensic age estimation (Hackman 2012).

The Sauvegrain method (Sauvegrain et al. 1962), developed in France, utilises four anatomical landmarks of the elbow: proximal radial epiphysis, olecranon apophysis, the lateral condyle, and trochlea. From antero-posterior and lateral elbow radiographs 27 points of the structures are scored, and then the scores are summed for a total score. A graph presenting the scores for girls and boys is used to determine the skeletal age, with a score of 27 representing full maturity. The most commonly used variation is the modified method of Sauvegrain et al. (1962) by Dimeglio et al. (2005) in which they proposed intermediate scores for certain elbow growth centres, improving the accuracy of the method. Both methods are useful for the assessment of skeletal age from elbow radiographs during the 2 years of the pubertal growth spurt, because between 11 and 13 years of skeletal age in girls and between 13 and 15 years of skeletal age in boys clear morphological development the olecranon apophysis occurs, and the methods are simple and reproducible. Since the age group is restricted, it has limited use in forensic age estimation (Canavese et al. 2008 and 2014; Dimeglio et al. 2005; Hackman 2012).

MRI of the elbow is in clinical medical use (Abdullah et al. 2018), but it has not been, to the author's knowledge, studied as a forensic age estimation application.

Ultrasound of the olecranon was validated on a four-stage classification system. Full maturity was first noted at the age of 12.3 years in females and 13.7 years in males, but further studies are needed on other samples (Schulz et al. 2014).

3.2.2.7 FOOT–ANKLE

Forensic age estimation in living individuals based on the ossification of the foot is not commonly applied (Hackman 2012). The foot–ankle atlas of Hoerr and Francis (1962) is based on the same data collected from 4,483 children from Cleveland as the G-P atlas for the hand and wrist (Greulich and Pyle 1959) and Pyle and Hoerr (1969) for the knee. The foot–ankle atlas presents two radiographs for each developmental stage: an anterior–posterior radiograph and a dorsoplantar radiograph for both sexes with two skeletal ages, a female age and a male age. All of the bones of the foot as well as the distal tibia and fibula are included in the final comparison. Each pair of X-ray plates is accompanied by a written description of the changes and maturity indicators, which are important in identifying a particular stage of skeletal maturity. However, not all of the bones and epiphyses available are used. Full maturity for the female foot in the atlas is 15.2 years of age, and for males 17.5

years (Hoerr and Francis 1962). Hackman (2012) found that there were difficulties in using the atlas because of the deficient quality of the radiographs due to overexposure and imprecise pictures and because age estimation required both views of the foot–ankle. Yet, she showed for both females and males a strong relationship between chronological age and estimated age from the foot–ankle using the Hoerr and Francis atlas (1962) in the Scottish study population.

Whitaker et al. (2002) developed a simple scoring system which allowed the estimation of chronological age from the radiographs of the developing bones of the foot. Scales of assessment include three centres of ossification: the primary, secondary, and state of fusion. Since the estimated age ranges are wide, the Whitaker method is not recommended in forensic age assessment (Davies et al. 2013).

MRI of the distal tibia and calcaneus was studied in the Turkish population applying the three-stage method of the ossification introduced by Saint-Martin et al. (2013). The last stage (stage three) of ossification first occurred at the age of 14 years in females and 16 years in males, therefore, this method has limited use in forensic age estimation (Ekizoglu et al. 2015a). An automated MRI method of the distal tibia was developed based on the analysis of variation in grey levels in the epiphyseal-metaphyseal junction, and the method classified 97.4% of males and 93.9% of females correctly to be at least 18 years of age in a sample of 80 males and 80 females, age range 8–25 years and mean age of 17.1 years (Saint-Martin et al. 2014).

3.2.2.8 CERVICAL VERTEBRAE

In dental practice, cephalometric radiographs are frequently used in orthodontics for treatment planning, and the skeletal development of cervical vertebrae can be evaluated from these X-rays. The main goal of using the estimation of the maturation of cervical vertebrae in lateral cephalometric radiographs is to define the pubertal growth spurt during orthodontic treatment (Hassel and Farman 1995).

The cervical vertebral maturation (CVM) method by Baccetti et al. (2005) was originally developed for the assessment of the optimal timing of orthodontic treatment with functional appliances. They presented six maturational stages of skeletal development for cervical vertebrae C2, C3, and C4. The stages are presented as schematic drawings for each vertebra. Seedat and Forsberg (2005) suggested assessment of the development of C3 to evaluate skeletal age and growth maturation instead of hand and wrist radiographs. They validated maturation of the body of C3 from lateral cephalograms applying six developmental stages by Hassel and Farman (1995), which are presented as

illustrations. They detected morphological changes consistent with skeletal maturation. Later, Thevissen et al. (2012a and 2013) showed that development of the cervical vertebrae C2, C3 and C4 contained the most age-related developmental information, and the registration techniques by Baccetti et al. (2005) (the CVM method) and Seedat and Forsberg (2005) were recommended. The CVM method shows a high level of correlation with the hand and wrist skeletal age method in the age range of 3–18 years (Szemraj et al.2018). Computer-assisted analysis for CVM age has also been developed (Paula de Caldas et al. 2010).

3.2.2.9 OTHER SKELETAL METHODS

Other skeletal age estimation methods have also been studied. In CT scans, the fusion of spheno-occipital synchondrosis of the cranial base is completed in both sexes by 17 years of age (Can et al. 2014), and a weak correlation between the fusion of the spheno-occipital synchondrosis in the cranial base and age has been reported (Bassed et al. 2011). Due to the large radiation dose on a sensitive area of vital organs in the head, the method is suitable for medico-legal death investigations, but not for the living. Instead, the use of MRI on the fusion of the spheno-occipital synchondrosis avoids this problem, and a preliminary study showed an average age for total fusion of 17.78 ± 2.20 years in females and 18.43 ± 1.84 years in males (Ekizoglu et al. 2015b).

Rai et al. (2008) developed a method utilising mandibular body length, mandibular length, and mandibular height to assess the utility of these three linear parameters for age estimation. This method, however, is not recommended for forensic age estimation since it contains very little information on age and explains only 3% of the variability of age (Thevissen et al. 2012a). In another study of the mandible, ramus length was compared to chronological age in order to predict whether the individual is 18 years or older (de Oliveira et al. 2015). It showed that when ramus length is at least 7 cm there is an 81% chance that the individual has reached the age of majority, whereas the mandibular ramus length predicted sex with an accuracy of only 54%. According to Nanda et al. (2012), facial width in children predicts higher age-related growth rate than facial height or depth, and therefore the mandibular and facial width could be used as potential age predictors. Kumagai et al. (2018) studied two skeletal age-related variables, namely cranial and mandibular widths, from frontal radiographs. The mandibular width had a strong correlation with the chronological age, whereas for the cranial width and chronological age the correlation was weak.

Ekizoglu et al. (2018) studied the growth and development of the proximal humerus (shoulder) with MRI. They used a scoring system following the

Schmeling et al. (2004) and Kellinghaus et al. (2010a) methods. The earliest ages for closed epiphysis was 18 years for males and 17 years for females.

3.2.3 DENTAL AGE

In forensic sciences and clinical dentistry, tooth formation is examined mainly by using panoramic radiographs. Dental age is based on different stages of tooth development. First, the dental follicle appears, then calcification centres begin to form in the superior level of the crypt. Mineralisation starts at the tip of the cusps. The calcification centres fuse to form an occlusal surface of the crown and later the root begins to form. Dental development ends with the radiological closure of apex of the root and the periodontal membrane reaching a uniform width around the apical root (Demirjian et al. 1973, Nanci 2017). Enamel and dentine are radiographically apparent when they are sufficiently mineralised. (Figure 3). Dental development and mineralisation are explained in more detail in paragraphs 3.2.3.1 and 3.2.3.3.

Figure 3. Dental radiograph of an 8-year-old girl. On the left tooth 37 the crown is fully mineralised, and the roots have started to form. On the right tooth 38 the cusps have merged. Both teeth are surrounded by a dental follicle.

The dental age can also be evaluated from the eruption of teeth, but it is not as accurate as the mineralisation status of the teeth and can only be used during active eruption periods (Paragraph 3.2.3.2) (Haavikko 1970; Nyström et al. 2001). The stage of biological maturity of an individual is compared with a standard atlas or tables and then translated into a probable chronological age (AlQahtani et al. 2014; Moorrees et al. 1963; Schour and Massler 1941). Dental maturation is less affected by variation in nutritional and endocrine status

than skeletal maturation (Kullman 1995), but inherited disorders like osteogenesis imperfecta may affect the timing of dental development (Vuorimies et al. 2017).

3.2.3.1 DENTAL DEVELOPMENT

Human dentition consists of two sets. The primary or deciduous dentition ("milk teeth") consists of 20 teeth in total, and includes incisors, canines, and molars, which are smaller-sized than the PT. The deciduous dentition, which is radiologically about half-formed at birth, becomes complete by the age of 2½ years, then is replaced by PT gradually, starting normally at age 5 or 6 years. The permanent or secondary dentition consists of 32 teeth, including incisors, canines, premolars, and molars (Hillson 1996). The World Dental Federation (FDI) proposed a numbering system for teeth, which was adopted by the World Health Organization (WHO). Each tooth is allocated a two-digit number; the left designates quadrant and the right designates the tooth order (Table 4) (World Dental Federation).

Table 4. A numbering system for teeth proposed by the World Dental Federation (FDI).

FDI Two-Digit notation															
Permanent teeth															
Upper right								Upper left							
18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
Lower right								Lower left							
Deciduous teeth (milk teeth)															
Upper right								Upper left							
			55	54	53	52	51	61	62	63	64	65			
			85	84	83	82	81	71	72	73	74	75			
Lower right								Lower left							

Tooth formation starts prenatally at around 6 weeks for primary teeth and around 16 weeks for PT. The enamel organ forms from dental lamina, a thickening on the oral epithelium in the embryo's jaws, and reciprocal interactions between the epithelial layer and underlying jaw mesenchyme are fundamental in the process of dental morphogenesis. Molecular mechanisms regulate the stages of tooth formation by signalling networks. The initial phase is called the bud stage, followed by the cap stage, and finally the bell stage. Variations in development are larger in the permanent than the primary dentition. The signalling system has an important role in the maintenance of dental stem cells, which support the continuous growth of teeth (Balic and Thesleff 2015; Hillson 1996). Beneath the gradually thickening and cervically

extending layer of enamel, dentin matrix is laid down and mineralises forming hydroxyapatite units and completing the crown formation. The subsequent root formation is also due to the odontoblast-derived dentin formation and mineralisation, and eventually the root becomes covered by a thin layer of cementum – the third mineralised tooth-tissue type. In the final stage of dental development, radiological closure of the apex follows, even though circulation and nerves pass through apex (Demirjian et al. 1973, ; Nanci 2017). In the incremental pattern of enamel and dentin, a differential incremental line, the neonatal line (NNL), was recently studied by Hurnanen et al. (2016). The NNL forms at birth and can be used as evidence of live birth.

3.2.3.2 ORAL INSPECTION AND ERUPTED TEETH

Clinical tooth eruption refers to the emergence of teeth through the gingiva and is a single event for each tooth. Assessment of eruption status of teeth is suitable for age estimation during the active period but has limited use during inactive periods. Many tables and graphic presentations are available to evaluate the dental age from eruption status, but the large variation in ages must be taken into account (AlQahtani et al. 2010; Haavikko 1970; Nyström et al. 2001). Bengston (1935) has divided the eruption of the tooth into four stages. The occlusal or incisal surface: 1) is covered entirely by bone, 2) breaks through the crest of the alveolar bone, 3) is midway between the alveolar bone and the occlusal plane, and 4) is in the occlusal plane. The first two stages can only be detected with radiographs. Pahkala et al. (1991) used four stages for the emergence of PT: 0 = tooth not visible in oral cavity, 1 = at least one cusp visible in the oral cavity, 2 = entire occlusal surface or mesiodistal width of tooth visible, and 3 = tooth in occlusion or at the occlusal level if antagonist tooth not fully erupted. Later, Olze et al. (2007) developed eruption staging for TM. The stages are as follows A) occlusal plane covered with bone; B) alveolar emergence, complete resorption of alveolar bone over occlusal plane; C) gingival emergence, penetration through gingiva by at least one cusp of the tooth; and D) complete emergence of the tooth in occlusal plane. In TM in particular, there are difficulties in assessing eruption from radiographs, such as visualising the alveolar bone level, defining gingival or partial eruption, and recognising full eruption (Kjaer 2014). Population-specific data is recommended for forensic age estimation when using assessment of dental eruption (Eskeli et al. 1999; Olze et al. 2007). Orthodontic treatment and premature extraction of primary teeth may affect the path and timing of the emergence of PT (Hadler-Olsen et al. 2018; Posen et al. 1965), therefore hampering age estimation. Eruption may be used as a supplement to other dental age estimation methods (Schmeling et al. 2016), but not for distinction between minors and majors (Gambier et al. 2019).

Moorrees et al. (1963) have also defined the stages of root resorption of the primary teeth when PT are erupting in single and multirooted teeth, from apex closed to gradual resorption of the root until the final stage of resorption when three-quarters of the root is resorbed. A modification of the method with only four root resorption stages was published by Vuorimies et al. (2017). This method is not common in forensic age estimation (European Asylum Support Office 2018).

3.2.3.3 MINERALISATION STATUS OF THE TEETH

Dental maturity can be registered and used to indicate dental age since previous studies have demonstrated significant correlations between consecutive stages of dental development and chronological age (Olze et al. 2006 and 2007). In forensic sciences, the dental maturation status is most frequently applied to assess chronological age (Schmeling et al. 2016) and dental age is commonly assessed for victim identification from dental panoramic radiographs (Figure 4). Several dental age estimation techniques
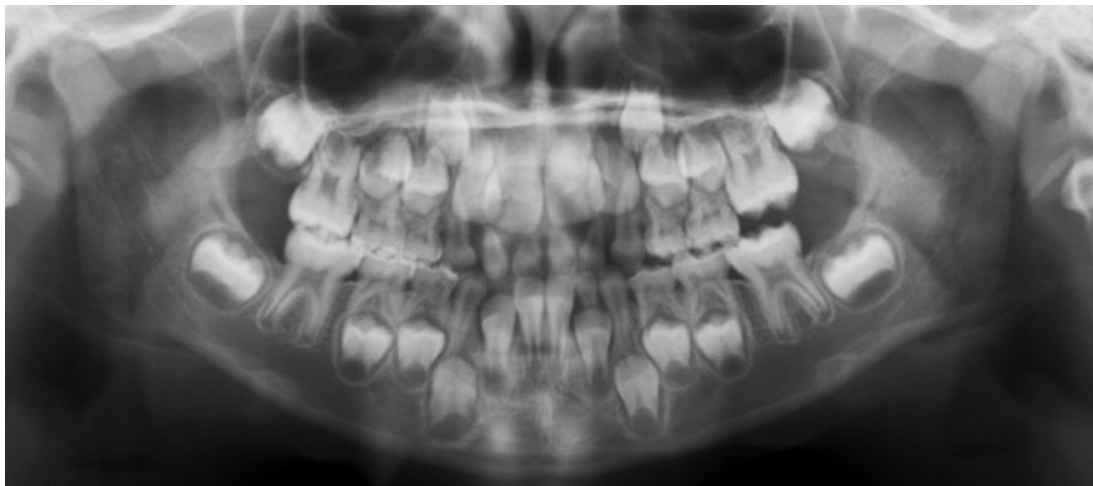

Figure 4. Dental panoramic radiograph of a 7-year-old girl with early mixed dentition. All the permanent teeth except the wisdom teeth are developing.

for the deceased exist, but these methods demand extraction of a tooth, which is not possible for the living (Bang and Ramm 1970; Dalitz 1962; Gustafson 1950; Johanson 1971; Lamendin et al. 1992; Solheim 1993; Solheim and Kvaal 1993). Another major forensic indication to use dental age is to estimate the age of young individuals with uncertain identity, typically for asylum seekers. An important indication in paediatric dentistry and orthodontics is to apply dental development as a diagnostic tool as well as in treatment planning (Celikoglu et al. 2011). Especially in orthodontics, estimation of dental age is beneficial for optimising the onset of treatment with fixed appliances (Litsas

and Lucchese, 2016). In these cases, radiological methods using the mineralisation status of the teeth are most often applied. Examples of dental mineralisation staging techniques from radiographs are presented in Table 5. The number of stages in the presented techniques varies from 4 to 15 (Anderson et al. 1976; Chaillet and Demirjian 2004; Demirjian et al. 1973; Demisch and Wartmann 1956, Engström et al. 1983; Garn et al. 1958; Gleiser and Hunt 1955; Gustafson and Koch 1974; Haavikko 1970; Harris and Nortjé 1984; Komínek et al. 1975; Kullman et al. 1992; Köhler et al. 1994; Liliequist and Lundberg 1971; Moorrees et al. 1963; Nolla 1960; Nortjé 1983). Several modifications have been presented of these staging methods (Kataja et al. 1989; Mincer et al. 1993; Nyström et al. 2007; Orhan et al. 2007; Smith 1991; Willems et al. 2001 and 2017). De Tobel et al. (2017a) created a staging system for TM in MRI.

Dental age estimation charts or diagrams of formation and eruption have been presented by, for instance, Schour and Massler (1941), Ubelaker (1999), and AlQahtani et al. (2010). The London Atlas, developed by AlQahtani et al. (2010), is also available as a free software program providing an accurate and fast method for age estimation and showing best performance of the three diagrams mentioned above (AlQahtani et al. 2014).

In the Demirjian et al. (1973) method, which is universally applied on PT (Flood et al. 2013; Maber et al. 2006), the development of the seven left mandibular PT (FDI 31 to 37) are staged. The method is based on a French-Canadian reference sample (F=1482, M=1446). In cases with one or more missing index teeth, the contra-lateral homologous teeth can be used instead. The eight stages are named with letters from A to H, from the first appearance of calcified points to the closure of the apex (Figure 5). For each stage there are one to three written criteria for each stage that must be considered. In cases of doubt, the earlier stage is always assigned. After staging the development of the seven left mandibular PT (FDI 31-37), maturity scores are summed up and compared to reference tables or graphs for girls and boys separately, giving a maturity score which is then transformed to an estimate of chronological age (Demirjian et al. 1973).
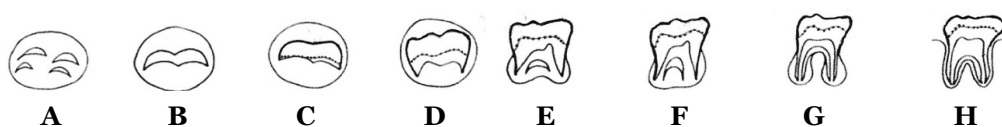

Figure 5. Demirjian stages for permanent teeth, adapted from Demirjian et al. (1973).

Table 5. Dental developmental staging techniques in age estimation, presented in chronological order.

Staging Technique (year)	Examined teeth (FDI)	Number of stages	Age range* (years)	Sex	Origin of subjects	Included subjects	Presentation of results
Gleiser and Hunt (1955)	46	15	0-10	F/M	American	50	Tables
Demisch and Wartmann (1956)	38, 48	7	8-16	F/M	American	151	Tables
Garn et al. (1958)	34-38 44-48	5	2-15	F/M	American	255	Tables
Nolla (1960)	18-48	11	2-23	F/M	American	50	Tables
Moorrees et al. (1963)	38-48	13/14	0-early adulthood	F/M	American	380	Graphs
Haavikko (1970)	18-48	12	2-21	F/M	Finnish	1,162	Tables
Liliequist and Lundberg (1971)	17-27 37-47	8	6.5-14.5	F/M	Swedish	287	Tables
Demirjian et al. (1973)	31-37	8	2-20	F/M	French Canadian	2,928	Tables and graphs
Gustafson and Koch (1974)	17-47 55-85	4	3-13	F/M	Swedish	41	Schematic presentation
Komínek et al. (1975)	18, 28, 38, 48	7	NA	F/M	Czech	NA	NA
Anderson et al. (1976)	18-48	14	3-21	F/M	Canadian	232	Tables
Engström et al. (1983)	38-48	5	Young individuals	F/M	Swedish	211	Regression analysis
Nortjé (1983)	48	8	15-21	F/M	South African	500	Tables
Harris and Nortjé (1984)	48	5	15-21	F/M	South African	407	Tables
Kullman et al. (1992)	38, 48	7	<15-25	F/M	Swedish	677	Tables
Köhler (1994)	18, 28, 38, 48	10	15-25	F/M	German	938	Tables
Demirjian 2 (Chaillet & Demirjian, 2004)	31-38	10	2-18	F/M	French	1,031	Tables and graphs
De Tobel et al. (2017a)**	18, 28, 38, 48	9	14-26	F/M	Belgian	309	Bayes rule

*Age range of the study sample

**Magnetic resonance imaging technique

***Abbreviations: F female; M male; NA Not available

The age prediction performances of Demirjian's method have been tested in different populations and shown to result in an overestimation of chronological age (Ambarkova et al. 2014; Cavrić et al. 2016a; Feijóo et al. 2012; Flood et al. 2013; Maber et al. 2006; Willems et al. 2001). Willems et al. (2001) adapted Demirjian's method using a large Belgian Caucasian (BC) reference sample and simplified the method by avoiding calculation of maturity scores. The Willems et al. model (WM) has been validated in several populations (Bangladeshi, British Caucasian, Bosnian-Herzegovian, Belgian Caucasian, Chinese, Macedonian (Former Yugoslav Republic of Macedonia), Kosovar, Malaysian, Serbian, Turkish) and showed better performance than Demirjian's method (Akkaya et al. 2015; Ambarkova et al. 2014; Djukic et al. 2013; Galić et al. 2011; Kelmendi et al. 2018; Liversidge et al. 2010a; Nik-Hussein et al. 2011; Onat Altan et al. 2016; Shi et al. 2018; Willems et al. 2001; Ye et al. 2014). Under- and overestimations were within a mean error range of 0.39 to -0.58 years. The Willems method, when all the PT (31-37) are mature, gives an estimate of the minimum age of 15.79 years for girls and 16.03 years for boys (Willems et al. 2001).

When all the PT 31-37 are mature, the TM development is used in dental age estimation. Olze et al. (2005) validated common classification systems: Demirjian et al. (1973), Gleiser and Hunt (1955), Gustafson and Koch (1974), Harris and Nortjé (1984), and Kullman et al. (1992) for assessing the mineralisation of TM. The most accurate results were obtained with the staging system of Demirjian et al. (1973). The advantage of the staging system is that a sufficient number of stages exist without speculative estimations of root length (Olze et al. 2005). Mincer et al. (1993) used Demirjian staging for TM to evaluate the accuracy of age estimation for American Caucasian and African Americans. The method is called ABFO after the Committee of American Board of Forensic Odontology. They also used regression formulas and empirical probabilities to assess whether the individual has reached the age of 18 years. The original 15-point staging technique developed by Gleiser and Hunt (1955) and modified by Köhler et al. (1994) has been shown to be most suitable for age predictions in the late developmental stages of TM (Thevissen 2013). The Köhler staging includes three stages for crown formation and seven for root formation in TM evaluated from dental panoramic radiographs, ten stages altogether (Figure 6). Köhler et al. (1994) found an accelerated development of TM in males, and the fully completed roots appear at the age of around 23 years, with a deviation of ±4 years in both sexes. In cases of impaction of the TM caused by the ascending branch of the mandible or by the second molar, development is delayed (Köhler et al. 1994; Olze et al. 2012).
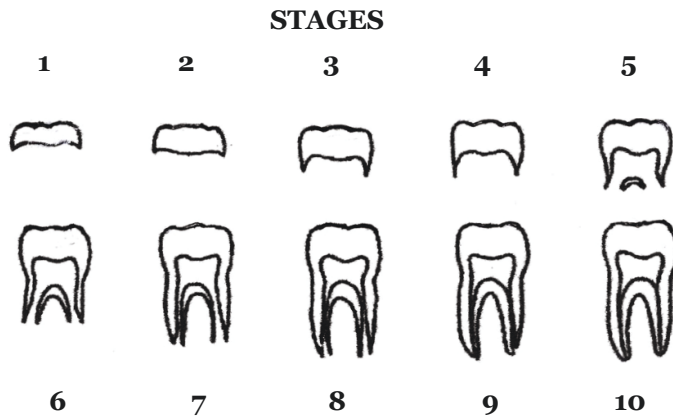
Figure 6. Köhler staging of third molars adapted from Köhler et al. (1994): 1) Crown ½ 2) Crown ¾ 3) Crown completed 4) Root initial 5) Root ¼ 6) Root ½ 7) Root ¾ 8) Root completed 9) Apex ½ 10) Apex completed.

When TM are applied in forensic age estimation, large confidence intervals in their development are problematic (Liversidge and Marsden 2010; Tangmose et al. 2015). Rather than a population-specific reference sample, reference data with a wide age range with uniform age distribution and a Bayesian statistical approach are recommended (Liversidge et al. 2017; Tangmose et al. 2015; Thevissen et al. 2009, 2010a and 2010b). In the Bayesian method, the age indicators are imported for age estimation and it is used to calculate point estimates like mean error (ME) (chronological age minus predicted age), mean absolute error (MAE), and root mean squared error (RMSE) as well as prediction interval of the estimated age. The approach takes into consideration correlation between the age indicators and the problem of missing indicators (Boldsen et al. 2002; Fieuws et al. 2016). The fact that the Bayesian approach does not show a tendency towards under- or overestimations of the age variable is beneficial (Sironi et al. 2018). The method has been applied in children and sub-adults in PT and TM development as combined predictors (Altalie et al. 2014; Franco et al. 2013; Ramanan et al. 2012; Yusof et al. 2014).

The method by Kvaal et al. (1995) measures the apposition of secondary dentine in the PT. Sometimes when all the PT, including the TM, have completed root formation, periapical intraoral X-rays are needed, for instance in cases where the age of an adult asylum seeker has been incorrectly evaluated upon arrival (Cameriere and Ferrante 2007; Sharma and Srivastava 2010). In sub-adult ages, these regressive changes are minimal, and Kvaal's method does not improve the age prediction in this age group (Thevissen 2013). The application of the Kvaal method on direct digital dental panoramic radiographs (tomograms) is not recommended (Landa et al. 2009), and it should be applied in single periapical X-rays as in the original description of the method.

The radiographic visibility of the periodontal ligament and the root pulp in the lower TM has been suggested as a method to evaluate whether the individual is at least 18 years old (Olze et al. 2010a and 2010b). The visibility of the periodontal ligament is divided into four stages. The results of the usability of the Olze method (2010a) are somewhat controversial, however (Chaudhary and Liversidge 2017; Guo et al. 2018; Timme et al. 2017c), because in many cases the visibility stage in lower TM cannot be reliably estimated due to either fused roots or narrowed furcations. Therefore, this method is of limited value for age estimation.

Dental age estimation with MRI has been tested in several studies (Baumann et al. 2015; De Tobel et al. 2017b; Guo et al. 2015). Demirjian et al. (1973) and Mincer et al. (1993) staging were applied in these studies. Because the appearance of the teeth in the magnetic resonance images differs greatly from the appearance on dental radiographs, new staging techniques needed to be invented for TM in order to apply the method in age estimation. De Tobel et al. (2017a) developed a nine-stage technique with descriptive criteria and schematic drawings for TM in MRI and achieved comparable reproducibility and performance compared to traditional radiographic methods. There are different MRI protocols, and in order to increase the ability to assess TM apices more accurately a higher in-plane resolution is more important than a smaller slice thickness (De Tobel et al. 2018).

An automated dental age estimation technique could overcome the problem of individual variation in stage allocation. The automated technique requires software to recognise the teeth of interest in radiographs or in MRI images, classify the degree of dental development, and estimate the age of the subjects based on the classified stages and information based on the age of the reference population. Unterpirker et al. (2015) developed automatic software for the localisation of TM for MRI. De Tobel et al. (2017c) developed an automated technique to stage lower TM development in dental panoramic radiographs for age estimation using a modified Demirjian's staging (1973) and found that the overall performance was comparable with staging by observers. So far, a fully automated dental age estimation method is not available.

Contemporary reference material is recommended for the dental age estimation since there is a secular trend of acceleration of tooth development meaning that the children born in the beginning of the millennium 2000 reach the same dental maturity earlier than the children born 30 or 40 years earlier (Sasso et al. 2013; Vucic et al. 2014). However, Rautman and Edgar (2013) found no evidence of positive secular trend in dental development in the study population. Indeed, differences in dental development between generations exist (Birchler et al. 2018). Eskeli et al. (2016) studied secular trends in the emergence of PT, and found a positive secular trend in the first phase of mixed

dentition eruption, but for the second phase of mixed dentition a negative secular trend was detected.

Other factors may also affect the timing of tooth development; impaction of mandibular TM causes delay in mineralisation compared to non-impacted lower TM (Olze et al. 2012). Caries is an important factor because it accelerates the emergence and development of PT (Brin and Koyoumdijsky-Kaye 1981; Leroy et al. 2003 and 2009) and affects the emergence sequence of the PT (Leroy et al. 2009). Ethnic differences in dental and skeletal development are discussed in paragraph 3.2.6.

3.2.4 COMBININING DENTAL AND SKELETAL METHODS

Improved age-estimation results are obtained by combining diverse age predictors, frequently the dental and skeletal age (Bassed et al. 2011; Cameriere et al. 2012c; Department of Forensic Medicine, Oslo University Hospital; Gelbrich et al. 2015; Kumagai et al. 2018; Schmeling et al. 2016; Thevissen et al. 2012a).

Combining the information from the third cervical vertebra (C3) in lateral skull radiographs and TM clearly improved the age estimation results, especially during the early phase of TM development (Thevissen et al. 2012a). In a recent study, information from mandibular second and TM in panoramic dental radiographs was combined with C3 automatic geometric measurements by a semi-automated method with the result that shape change of C3 could, as an additional method, discriminate whether the person is under or at least 18 years of age (Mânica et al. 2018). The effective dose of an additional lateral skull radiograph varies between two and three micro Sievert (μSv).

The BioAlder method combines information from the development of the hand and wrist and the left lower third molar from X-rays (Department of Forensic Medicine, Oslo University Hospital). In a study by Bassed et. al. (2011) information from CT scans combining TM and the medial clavicle improved the performance of age estimation compared to application of any of these markers alone, whereas the fusion of spheno-occipital synchondrosis did not contribute to the age estimation model.

Kumagai et al. (2018) validated the radiographic information of two dental and four skeletal predictors in age estimation. They combined PT, TM, the hand and wrist, cervical vertebrae, the skull, and the mandible. Even though the best performance was achieved by combining all the variables, due to high radiation dose, the combination of teeth and hand and wrist information is recommended for forensic age estimation. The risks involved with multiple

radiation exposures in forensic procedures for the living must be taken into account (Thevissen et al. 2012b).

Methods to automatically combine multi-factorial skeletal and dental development data have been developed. An automatic age estimation method (Štern et al. 2018) is based on the MRI data of three different anatomical sites: the hand and wrist, clavicle, and teeth. The method applies a deep convolutional neural network which is trained on a dataset.

3.2.5 DNA METHYLATION

DNA methylation is an epigenetic process in which methyl patterns change in the DNA molecule during the lifespan in response to environmental factors. Methylation can cause changes in the activity of a DNA segment without changing the sequence. DNA methylation is essential for normal development and is also associated with the process of aging (Horvath 2013). Abundant international research on DNA methylation is currently taking place on age estimation (Bekaert et al. 2015; Huang et al. 2015; Parson 2018; Smeers et al. 2018; Spólnicka et al. 2018). Benefits of the method are that the analysis can be done from a sample of saliva or blood and there is no upper age limit, as in dental and skeletal development.

The methylation-sensitive analysis of carefully selected DNA markers (CpG sites) has brought promising results by providing prediction accuracies of ±3–4 years (Vidaki et al. 2017) and better age correlation than other biomarkers such as messenger RNA (mRNA) (Zubakov et al. 2016). The forensically oriented methylation-based studies to estimate chronological age demonstrate that sequence analysis of bisulfite-converted DNA currently seems to be the most promising technique, albeit one that requires higher DNA amounts than are available in typical forensic samples (Parson 2018). A challenge in the DNA methylation method is that prediction intervals become larger with increasing age because of individual variation in epigenetic ageing rates (Smeers et al. 2018).

Utilisation of the age-associated DNA methylation markers combined with skeletal age (SA) and dental age (DA) has been validated by Shi et al. (2018). They measured SA with G-P (Greulich and Pyle 1959), TW3-RUS, and TW3-Carpal methods (Tanner et al. 2001) and DA with Demirjian et al. (1973) and Willems et al. (2001) methods. After validation, they chose four age-associated CpG sites for boys and five CpG sites for girls. DNA methylation markers in combination with SA and DA greatly improved the accuracy of age estimation in Chinese children. The method is, however, time-consuming, complex, and expensive. So far, DNA methylation is not applied in forensic age estimation in Europe (European Asylum Support Office 2018).

3.2.6 ETHNIC DIFFERENCES IN DENTAL AND SKELETAL DEVELOPMENT

Differences exist between populations in skeletal maturation (Cole et al. 2015; Mansourvar et al. 2014; Zabet et al. 2015), but nutritional and socioeconomic status have the greatest effect. In lower socioeconomic groups, skeletal maturation is expected to be delayed when compared to a reference group of higher socioeconomic status (Schmeling et al. 2000).

The study by Hackman (2012), testing the G-P atlas on a Scottish population showed a strong correlation between estimated age and chronological age. Similar results in different populations exist (Büken et al. 2009; Gȍk et al. 1985; van Rijn et al. 2001). On the other hand, research (Büken et al. 2007; Loder et al. 1993; Ontell et al. 1996; Tisé et al. 2011) suggests that G-P should be applied with population-specific versions. Combination of G-P with other age-estimation techniques increases the accuracy of age assessments (Bassed et al. 2011; Blankenship et al. 2007; Cameriere et al. 2012c; Garamendi et al. 2005; Gelbrich et al. 2015; Kumagai et al. 2018; Schmeling et al. 2016; Thevissen et al. 2012a).

The dental development and eruption of different populations have been extensively studied in recent years (Acharya 2011; Akkaya et al. 2015; AlQahtani et al. 2014 and 2017; Altalie et al. 2014; Ambarkova et al. 2014; Arge et al. 2018; Baylis and Bassed 2017; Birchler et al. 2018; Blenkin and Taylor 2012; Bolaños et al. 2003; Chaillet and Demirjian 2004; Chaillet et al. 2004 and 2005; Djukic et al. 2013; Eskeli et al. 1999; Feijóo et al. 2012; Flood et al. 2013; Franco et al. 2013; Galić et al. 2011; Gambier et al. 2019; Ghafari et al. 2018; Lewis and Senn 2010; Liversidge 2008; Liversidge et al. 2006; Mesotten et al. 2002 and 2003; Nyström et al. 2001 and 2007; Olze et al. 2007; Orhan et al. 2007; Ramanan et al. 2012; Roh et al. 2018; Rozkovcová et al. 2012; Solari and Abramovitch 2002; Thevissen et al. 2010a; Vidisdottir and Richter 2015; Willems et al. 2001 and 2017; Yusof et al. 2014). In many populations, Demirjian's method (1973) showed an overestimation of chronological age (section 3.2.3.3) (Ambarkova et al. 2014; Cavrić et al. 2016a; Esan and Schepartz 2019; Feijóo et al. 2012; Flood et al. 2013; Maber et al. 2006; Willems et al. 2001), whereas the Willems et al. (2001) method performed better (Akkaya et al. 2015; Ambarkova et al. 2014; Djukic et al. 2013; Galić et al. 2011; Kelmendi et al. 2018; Liversidge et al. 2010; Nik-Hussein et al. 2011; Onat Altan et al. 2016; Shi et al. 2018; Willems et al. 2001; Ye et al. 2014).

In the literature, controversies still exist regarding possible differences in dental development between African Black and other populations (Cavrić et al. 2016a; Liversidge 2008; Olze et al. 2006; Phillips and van Wyk Kotze 2009). Several studies on the dental development of sub-Saharan populations have been performed lately on the development of PT and TM

(Angelakopoulos et al. 2018; Cavrić et al. 2016a and 2016b; Davidson and Rodd 2001; Dardouri et al. 2016; Elamin et al. 2017; Esan and Schepartz 2018a and 2018b, 2019; Garamendi et al. 2005; Kihara et al. 2017; Liversidge et al. 2017; Olze et al. 2006; Phillips and van Wyk Kotze 2009; Uys et al. 2018). Regarding Somalis, there is only one study where Davidson and Rodd (2001) compared dental age and chronological age between Somali and Caucasian children on a relatively small sample. According to previous research, children of African origin are slightly more advanced in dental development than other groups (Blankenship et al. 2007; Harris and McKee 1990; Liversidge et al. 2010 and 2017; Mincer et al. 1993; Olze et al. 2004 and 2006; Uys et al. 2018; Willems et al. 2017). Willems et al. (2017) found in their study on South-African Black children that the children in the age cohort 15.00–15.99 years showed fully developed PT 31–37, indicating a more advanced tooth development in the sampled population. Nevertheless, there is more evidence that suggests similarity in the dental development of children and adolescents of African origin to other ethnicities (Elamin et al. 2017; Esan and Schepartz 2018a; Kihara et al. 2017; Liversidge et al. 2010 and 2017; Thevissen et al. 2010b; Willems et al. 2017).

3.2.7 FORENSIC AGE ESTIMATION METHODS IN EUROPE

In different countries even within Europe and the European Union, the variation of methods used for forensic age assessment is extensive (European Asylum Support Office 2014 and 2018; Separated Children in Europe Programme 2012; Solheim and Vonen 2006). The use of the methods is regulated by individual national policies and there are at present no common standards (Council of Europe 2017). In 2016, the following methods of forensic age assessment were applied in European countries (number of countries): hand and wrist (23), dental X-rays (19), clavicle X-ray (12), dental observation (16), sexual maturation (7), and physical development assessment (11). Non-medical methods like submitted documents (27) and an interview (17) for estimating age were used in most European countries, and some countries used social services assessment (11) and estimation based on psychosocial assessment (6). Ultrasound was not applied in any European countries for forensic age assessment (European Asylum Support Office 2018). In recent years, many age assessment procedures have changed, and even in different parts within a country, variable methods are applied (Department of Forensic Medicine, Oslo University Hospital; European Asylum Support Office 2018; Pradella et al. 2017; Rättsmedicinalverket).

In Finland, the forensic age assessment is largely based on dental development. Forensic age assessment is always performed by two FOs, who are dentists with special competence in forensic odontology — a specialised training organised in collaboration by the Finnish Dental Association and the

University of Helsinki. There are at present two FO posts in THL in Helsinki, and other FOs (N=20) practice in addition to their daily dental work. The examinee is first interviewed by an FO or other medical professional. The FO chooses the appropriate methods to estimate age. Usually forensic age assessment includes radiographic examinations of the dentition and the hand and wrist: a dental panoramic tomogram and an X-ray of the left hand and wrist, sometimes periapical intraoral radiographs are incorporated. With these examinations –or any others,– an exact chronological age cannot be determined, since normal biological variation must be taken into account.

In Nordic countries, especially in Sweden and Norway, the legitimacy of forensic age assessment has raised discussion recently (Aarseth and Tonsaker 2018; Malmqvist et al. 2017). In May 2016 in Sweden, the National Board of Forensic Medicine was commissioned by the government to create a new system for forensic age assessment. Presently, the Swedish forensic age estimation of an asylum seeker includes a dental panoramic radiograph where the stage of development of TM is evaluated, as well as MRI of the knee taken for the evaluation of skeletal age. For the age limit of 21 years, a complimentary examination of the clavicle is included. For the analysis, a standardized matrix is used to evaluate if the person is under or over the important legal age thresholds 15, 18 and 21 years. (Rättsmedicinalverket).

In Norway, the responsibility of forensic age estimation was transferred to the Department of Forensic Medicine of University Hospital of Oslo on 1.1.2017. BioAlder (since 19.06.2017) was developed to aid in the assessment of age for young unattended asylum seekers. It is a statistical calculation tool based on studies of hand bones and lower left wisdom teeth of 14,207 (6,867 girls and 7,340 boys) young persons of known chronological age. BioAlder developmental stages of hand bones and teeth are evaluated from radiographs, and the model gives an estimate of the age span. The results are presented as 95% and 75% prediction intervals as well as a percentage of individuals under the ages 16 and 18 years (Bleka et al. 2018a and 2018b; Dahlberg et al. 2018; Department of Forensic Medicine, Oslo University Hospital). The information of sex, estimated Greulich and Pyle (1959) skeletal age and/or estimated Demirjian's staging (1973) in the lower left wisdom tooth are combined to generate a report, and it is delivered to the Norwegian Directorate of Immigration (UDI) (Bleka 2018a; Department of Forensic Medicine, Oslo University Hospital).

In Denmark, important legal age limits are 15 and 18 years. The forensic age estimation includes physical examination by a forensic pathologist (Larsen et al. 2015). Height and weight are measured, secondary sexual maturation is visually evaluated according to Tanner (1962), and medical history is recorded. A radiologist estimates skeletal age from X-ray of the left hand according to Greulich and Pyle (1959), and a forensic odontologist estimates

the dental age from panoramic dental radiograph and intraoral X-rays of TM according to Köhler et al. (1994), Haavikko (1970), and Mincer et al. (1993). An age range is given with the range ±2 years to the counted mean age (Larsen et al. 2015).

In Iceland, age estimation for asylum seekers is most often determined from tooth formation, mainly TM. At least three dental methods are used for age estimation, to increase the accuracy of the analysis (Vidisdottir and Richter 2013).

In Germany, the important age limits are 14 and 18 years (Schmeling et al. 2016). In Berlin, the international and multidisciplinary AGFAD was established in March 2000. AGFAD has issued recommendations for forensic age assessment of children, adolescents, and young adults (Schmeling et al. 2007), and it has 134 members from 18 countries (Schmeling et al. 2016). AGFAD also organises annual proficiency tests in order to improve the consensus of the experts on age and the quality of age assessment reports (Mansour et al. 2017). The recommended methods include physical examination consisting of anthropometric data, status of sexual maturation, and information on developmental disorders, an X-ray of the left hand and wrist, a dental examination, and a dental panoramic radiograph. If hand and wrist bones are fully developed, a thin-slice CT of the clavicle is recommended to distinguish the age limit of 21 years. Radiological examinations in forensic age assessment are regulated by legislation. In Germany the number of age assessments is not registered nationwide, but in Berlin 157 examinations were performed in 2014 (Schmeling et al. 2016), and in Hamburg 699 between 1990 and 2000 (Mansour et al. 2017).

In Austria, the protocol involves, as a recommendation, radiographs of the hand and wrist and TM. Only if minority or majority cannot be safely excluded, a CT of the clavicle would be considered. During 2014–2016 in Austria, around 4,700 age assessments were performed for unaccompanied minors. Around 46% of cases resulted in the exclusion of minority. The reported costs were around 1,200 EUR per age assessment (Schumacher et al. 2018).

In Belgium, when the age of the asylum seeker is unknown or if the alleged age is questioned, Belgian law prescribes that the Guardianship Service orders a medical test to confirm if the person has reached the age 18 years or not (Wetgeving 2002). At the University of Leuven, a protocol for age assessment —the triple test– has been developed (Thevissen 2013). The procedure includes clinical dental examination and dental panoramic radiograph. The Willems method (2001) is used for left permanent mandibular teeth. If they are mature, TM developmental stages are registered, and a Bayesian method developed by Thevissen et al. (2009) is applied to estimate whether the person is younger or older than 18 years. A hand and wrist radiograph is taken from

the non-dominant side for analysis using G-P (1959). When hand bones are mature, a radiograph of the medial part of both clavicles is included using Schmeling's method (2004) for age assessment (Thevissen 2013).

In Italy, the important legal age thresholds are 14 and 18 years (Focardi et al. 2014). According to the new Italian law (Gazzetta Ufficiale della Repubblica Italiana 2017), the age of an asylum seeker shall be determined based on administrative and other documents and a psychosocial interview. Only if there are reasons to doubt the age of the unattended minor, medical age assessment is possible, and this concerns criminal cases as well. Very few age assessments have been performed since the new law. They are requested by the Government's local offices and ordered by Public Prosecutors. Methods include left hand and wrist X-ray (Greulich and Pyle 1959), and sometimes dental panoramic radiograph. Combination of these two methods has been used in a few cases of illegal immigration. No data is available on the frequency of evaluation of physical and sexual maturation, but probably these are used only rarely (Pradella et al. 2017).

In the United Kingdom, important legal age thresholds are 16, 18, and 21 years (Hackman 2012). Age assessment methods include documents, interview, and estimation based on physical appearance. When two independent officers of the Home Office estimate an applicant to be an adult, she/he can approach social services for an age assessment (European Asylum Support Office 2014 and 2018). A voluntary X-ray pilot for asylum seekers was proposed in 2012 but was not permitted because of ethical concerns (United Kingdom: X-ray pilot for child asylum-seekers sparks outrage 2012).

In France, important legal age thresholds are 13, 18, and 21 years. Until the age of 21 years an asylum seeker cannot be evicted from the French territory and is eligible for specific support (Remy et al. 2017). Evaluation of sexual maturation is forbidden by French law, but otherwise AGFAD recommendations are followed: dental panoramic radiograph, left hand and wrist, and CT of the clavicle (European Asylum Support Office 2018).

Schumacher et al. (2018) proposed the establishment of a comprehensive, scientifically sound, agreed upon, and regularly updated catalogue of age markers, as well as the development of an Age Marker Assessment Protocol that describes how the minimum and maximum age of an individual can be concluded from a set of images. They also suggested the establishment of Age Marker Training capabilities in order to distribute knowledge on age markers and qualify specialists, or to establish an Age Marker Diagnostics Centre in the EU with permanently available specialists in the age diagnostics of juveniles. It would enable medical age assessment in all Member States and its uniform application.

3.3 FORENSIC AGE ESTIMATION REPORT

An age assessment report is a summary of all findings of the interview, background information, and age estimation examination, and it should include clear statements on the age assessment reliability in order help the decision making of authorities (Schmeling et al. 2016). The age estimation investigation should be performed by two independent forensic specialists. In the forensic age estimation report, the findings of different methods are combined by a co-ordinating expert, in Finland the FO employed by THL, but the report should be signed by both investigators (IOFOS 2018; Schumacher et al. 2018).

Numerous reference studies have demonstrated a correlation between consecutive stages of each previously subscribed age assessment method and chronological age (Kellinghaus et al. 2010a; Olze et al. 2006 and 2007; Schmeling et al. 2006; Thevissen et al. 2010a and 2010b; Tisé et al. 2011; Willems et al. 2001 and 2017). The methods and reference studies chosen need to have a large enough sample, preferably of the same origin, and the study sample should have a good age and sex distribution. Good reproducibility of the used parameter registration technique is needed, as well as sound and reproducible scientific statistics in the study. The reference study outcome needs to be validated. In the report, all methods used need a reference described in the scientific literature (IOFOS 2018).

Only appropriately trained forensic specialists should do the staging of developmental phases and two forensic specialists should do an independent assessment. In case of doubt regarding which of the two phases should be assigned for the developmental phase, the earlier one should be chosen. In case of disagreement, a third specialist should be involved in the assessment (Schumacher et al. 2018). The report should include, according to International Organization for Forensic Odonto-Stomatology (IOFOS) recommendations, which teeth are clinically present, specific characteristics of certain teeth staged according to referred reference scales such as the occlusion, the degree of attrition, the colour of the teeth, the staining and calculus, the periodontal condition, the dental hygiene status and any pathology possibly influencing the age assessment. The possible influence of these factors on the age estimation outcomes should be reported, when oral inspection is performed (IOFOS 2018). Abnormal developments need to be documented and should be part of the training of specialists (Schumacher et al. 2018).

When the question concerns whether the person has reached a legally significant age with the highest standard of proof, the minimum age principle is used. The decisive factor is the minimum age at which a specific feature has attained the relevant stage in the reference study used. The minimum age is

the youngest age of the person in the reference population having attained the corresponding developmental stage. When several features are studied, the minimum age is the highest of all the minimum ages (Schmeling et al. 2016). When intraindividual stage differences exist in medial clavicular ossification, the minimum age of the more advanced side is chosen (Rudolf et al. 2018). Use of the minimum age principle guarantees that the estimated age is not an overestimation, but rather an underestimation of chronological age. When all the features have reached the stage of final development, it is possible to express the most likely age period (Schmeling et al. 2016). The conclusion of the report should contain a minimum age and/or an estimated age including a measure of its uncertainty, and an answer to the request in the age estimation assignment. Use of averages should be avoided. In cases of disagreement, a consensus between both specialists should be obtained. The margin of error must be clearly stated in the age estimation report. If any doubt exists, the minor age is presumed, and the benefit of doubt applied. The report should be signed by both investigators (IOFOS 2018; Schumacher et al. 2018). According to AGFAD protocol, the only age indicator at present to detect adults without reasonable doubt is the classification of medial clavicular ossification (Rudolf et al. 2018).

3.4 ASYLUM SEEKERS AND THE PROCESS OF ASYLUM SEEKING

The current world population in 2017 of 7.6 billion is expected to reach 9.8 billion in 2050, meaning an over two-billion increase, and over 90% of this population growth is predicted to take place in Africa and in Asia. Between 2017 and 2050, the populations of 26 African countries are projected to expand to at least double their current size, which is expected to lead to large movements of migrants toward high-income EU countries (United Nations 2017). In 2017, 68.5 million people had to forcibly leave their homes due to wars, violence, and persecution (UNHCR).

Recently, wars, conflicts, famines, and nature catastrophes in different parts of the world have led to an increase in the numbers of asylum seekers coming to Europe and other industrialised countries. A clear change was noted already in 2003, when the number of pending asylum applications in industrialised countries reached 505,000 (The Finnish Immigration Service (a)). During 2015, the peak thus far, altogether over one million asylum seekers came to Europe over the Mediterranean Sea, most from Syria and Afghanistan. The portion of Sub-Saharan Africans was about 10%, and Eritrea, Nigeria, and Somalia were most often the countries of origin. In 2016 in EU countries, asylum seekers for the most part originated from Syria, Afghanistan, and Iraq, Somalia being the tenth most common country of origin. A similar trend was

seen in Finland, where first-time asylum applications from non-EU countries came mostly from Iraq, Afghanistan, Syria, and Somalia (Eurostat).

In 2015, in relative terms, the largest increase in the number of first-time applicants was recorded in Finland, with a more than nine-fold increase from 2014. A clear change occurred between 2015 and 2016, when Austria, the Netherlands, Slovakia, Belgium, Denmark, Finland, Hungary and Sweden reported less than half the number of first-time asylum applicants compared to the previous year, and Norway also recorded a large drop. In the EU between 2015 and 2016 large increases in the number of first-time asylum applicants were reported in Germany, as well as in Greece and Italy (Eurostat).

From 2015 to 2016, applications from unaccompanied minors in EU countries declined from 88,700 to 63,300. In 2017, there were 728,470 applications for international protection in the EU, Switzerland, and Norway, representing a decrease of 44% compared to 2016, but remaining at a higher level than prior to the refugee crisis starting in 2015. In 2017, half of the applicants were in the age category between 18 and 35 years old, and almost a third were minors. In 2016, of all the applicant minors, 16% were unaccompanied, 13 % in 2017. An unaccompanied minor is defined as a person less than 18-years-old who arrives without an adult responsible for the child, or a minor who is left unaccompanied after arriving in the country. In Europe among asylum seekers there are more males than females, and especially in the younger age groups there is a significant gender inequality. In 2016 and 2017, in the age group 14–34 years around 75% were male among the first-time asylum applicants (Eurostat; European Asylum Support Office 2018).

A well-founded fear of being persecuted in one's home country or in the country of permanent residence due to ethnic origin, religion, nationality, membership in a certain social group or political opinions, constitutes grounds for asylum in Finland. Another requirement is that the protection by the authorities in the country of origin is not guaranteed. The Finnish Immigration Service will evaluate whether the applicant meets the provisions for asylum (The Finnish Immigration Service (b)). The criteria for asylum are defined in the Finnish law and international agreements that Finland has ratified (Aliens Act). Upon arrival in Finland, the applicant must immediately inform the border control or the police authorities of the application for asylum. The person is registered as an asylum seeker. Biometric identifiers, such as fingerprints, signature and photograph, are gathered as well as basic details from the information registers, and the person is directed to a reception centre. Finnish District Courts assign a legal representative to each unattended minor asylum seeker arriving in Finland. The duty of the guardian is to ensure the child's best interests in all situations. The Finnish Immigration Service is responsible for covering expenses of the representative (The Finnish

Immigration Service (b)). In the asylum interview, the immigration authorities decide if a forensic age estimation is needed.

3.5 LEGISLATION

3.5.1 INTERNATIONAL LEGISLATION

Several human rights and fundamental freedoms treasured by the Convention on the Rights of the Child (CRC) (United Nations 1989) and the Charter of Fundamental Rights of the European Union (CFR) (2000) are relevant in the age assessment process. The best interests of the child (Article 3 CRC and Article 24 CFR) must be considered in all actions concerning children. Until conclusive results of age assessment indicate that the individual is an adult, she/he must be considered as a child. The Articles (Article 2 CRC and Article 21 CFR) of right to non-discrimination require that every person is treated objectively and individually regardless of origin or ethnical background. All individuals have the right to identity (Articles 1, 7 and 8 CRC) and the correct age is an essential factor of an individual's identity. Age determines the rights and obligations of an individual as well as the state's obligations towards the individual such as protection, education, and healthcare. Any deviation from chronological age may have serious effects, such as problems caused by incorrect accommodation of the unaccompanied minor with adults at the reception centre, or vice versa. Every child has a right to express their views freely and a right to be heard (Articles 12 and 14 CRC and Articles 24 and 41 CFR) during age assessment process. The right to information is essential to understand the age assessment process, as well as the rights and obligations that the process induces. When consent is required, it should be given based on accurate information and free will.

The age assessment process must respect the applicant's dignity and physical integrity (Articles 3 and 37 CRC and Articles 1, 3 and 5 CFR). Examinations requiring nudity are highly intrusive and must be avoided. It is especially traumatic to applicants with a cultural background different from the host country and the victims of human trafficking or persecution. Interference by authorities into one's personal life needs to be justified as stated in the Articles (Article 16 CRC and Articles 7 and 8 CFR) concerning the respect for private life and protection of personal data. According to EU law, personal data can only be legally collected and kept under strict conditions, including the consent of the person or of his/her representative or guardian. Children and their guardians or legal representatives should be informed about the data that is collected under the respective countries' national legal framework. The right to an effective remedy (Articles 12 and 47 CFR) implies that the outcomes of the process can be challenged, and the applicant should not be responsible for

the financial costs of the legal procedure (European Asylum Support Office 2018; Separated Children in Europe Programme 2012; Schumacher et al. 2018). The benefit of the doubt is a key principle in age assessment since none of the current methods are able to determine a specific age with total certainty. Also included in the anti-trafficking directive (EU 2011) is that the benefit of the doubt should be applied when the age is uncertain (European Asylum Support Office 2018).

In different countries, even within Europe and the EU, the methods for forensic age assessment vary due to differences in national legislation. Each EU Member State may decide how to transpose the considerations of the respective Directives into national legislation. A proper EU-common formal regulation concerning age assessment procedures is still lacking. In Germany, for instance, there are three legal contexts allowing forensic age assessment including ionising imaging: Social Code Book VIII 2015, Residence Act 2007, and one for criminal proceedings (Council Directive 2005/85/EC 2005; Schmeling et al. 2016). In Austria as well, the forensic age assessment has been regulated in detail since the year 2010 for all age controversies affecting asylum seekers (Rudolf 2015). However, many countries still lack legislation concerning forensic age assessment (European Asylum Support Office 2018; Separated Children in Europe Programme 2012).

3.5.2 FINNISH LEGISLATION

At present, according to the Aliens Act (301/2004) and the amendments of the Act (549/2010 and 501/2016 Section 6a and 6b), the Finnish Immigration Service has the right to refer asylum seekers to the THL, for age assessment in cases of reasonable doubt for questioning the reliability of the information the person has given on her/his age. If the date of birth has not been registered, or if the physical appearance, behavior, or linguistic expression indicate an older person, the immigration authorities will initiate the forensic age assessment process.

Two experts perform the forensic age assessment and jointly sign the statement, and at least one of them must be an employee of the THL. An expert may be a certified medical practitioner or a certified dentist with the prerequisite special competence in forensic odontology, in other words, a forensic odontologist (FO). Actions required for the examination may also, at the request of the THL, be performed by central hospitals, municipal health centres, or private health care units. Health care professionals perform the radiographic examination. At the moment, the interview is done by the local FO in four different locations: Helsinki, Lappeenranta, Oulu, and Turku.

Identity of the person to be examined must first be confirmed by an official from the Finnish Immigration Service. The parent, guardian or other legal representative of the child has the right to be present during the examination. The person has to give her/his written consent for the radiographic and other examinations, and if registered under 18, the District Court-imposed representative must also sign the consent. In advance, the applicant and the guardian or legal representative are informed of the importance of the age assessment, the methods used, potential health effects, and the consequences of undergoing, and of refusing, an examination in the native language of the applicant or in a language that he or she sufficiently understands. Often an interpreter is needed during the examination. If the person refuses the examination, the applicant is considered an adult in case no reasonable grounds for refusal exist. Refusal, on the other hand, does not constitute grounds for rejecting an application for international protection.

Other laws and restrictions need to be taken into account in forensic age assessment concerning radiation and its use (Decree on use of radiation; Radiation Act (592/1991) and the amendment of the Act (1142/1998 Chapter 10 39§); Radiation and Nuclear Safety Authority (STUK)), as well as how to save and conserve the examination documents (Laki viranomaisten toiminnan julkisuudesta (621/1999)).

In Finland, the important legal age thresholds are 15, 16, 18, and 21 years (Criminal Code of Finland, Act 39/1889 and the amendment 515/2003). An important indication of forensic age assessment is to determine if the unattended asylum seeker is of criminally responsible age (15 years) or if the individual has reached the age of majority (18 years), when adult criminal law is applicable (Schmeling et al. 2008). In Finland, the Coercive Measures Act (806/2011) defines the inspection of a person suspected for a crime, and a written referral for a person's inspection must be given by the police authority before the forensic age assessment. When the Police Authorities refer a victim of a crime with a previous immigrant status to the age assessment, the procedure is similar as in the age assessments referred by the Immigration Authorities and an informed consent has to be signed by the examinee and/or by the guardian or the legal representative. The Convention on the Rights of the Child (United Nations 1989) postulates that governments act in the best interest of the child without specifically defining the age assessment procedure.

4 AIMS OF THE STUDY

In forensic age assessment skeletal and dental radiographic methods are applied. The aim of this thesis was to discover whether the commonly used methods are applicable in modern Finnish children, how the legislation concerning forensic age estimation in Finland works, and to study dental development in a rare sample of young Somalis of known chronological age.

The following specific aims were set for the present study:

I   To assess the present validity of the historical method for age assessment by Greulich and Pyle (1959) and to compare it with other skeletal X-ray and dental methods in modern Finnish children and sub-adults.

II   To qualitatively and quantitatively analyse the forensic age assessments of asylum seekers in Finland and to describe the Finnish legislation that outlines the process.

III To collect data on the development of permanent teeth (from 31 to 37) of Somalis in order to create a Somali dental development model and to compare it with the previously validated Willems et al. model (2001).

IV   To add the age-related Somali information of development of the wisdom teeth to the information of the development of other permanent teeth, since a combined model of permanent teeth and wisdom teeth has been validated before.

The hypotheses were:

I   The Greulich and Pyle atlas (1959) is still valid in modern Finnish children and dental radiographic methods are more accurate in age estimation than skeletal methods.

II   The present Finnish legislation concerning forensic age assessment (Aliens Act) is fulfilling its expectations.

III The Willems et al. model (2001) performs well in the Somali population.

IV   Combining the information of the development of permanent teeth and third molars improves age estimation outcomes.

5 STUDY SUBJECTS, MATERIALS, AND METHODS

5.1 STUDY SUBJECTS AND MATERIALS

Study I (Age assessment by skeletal X-ray and dental methods): Analysis of the dental and skeletal radiographic data from Finnish child victims of the Southeast Asian Tsunami.

Study II (Forensic age assessment of asylum seekers in Finland): Collection of information from the Finnish legislation texts, EU statistics and the public statistics on asylum seekers in Finland by the Immigration Authorities.

Studies III and IV (Dental age estimation in Somali children): Analysis of dental panoramic radiographs taken of young Somalis, born and living in Finland, and statistical analysis of the material.

5.1.1 AGE ASSESSMENT BY SKELETAL AND DENTAL X-RAY METHODS (STUDY I)

In the Southeast Asian Tsunami on 26 December 2004, of all 5,395 victims who perished in Thailand, 178 were Finnish citizens; of the 174 identified Finnish victims, 56 were children under 18 years old. The victims were identified in Thailand and then repatriated to Finland. According to Finnish law on the investigation of the cause of death (2009/1065), when a person dies in an accident a complete forensic autopsy must be performed. In addition, DNA testing and post mortem dental examination were conducted for identification verification. Age assessment was performed from dental panoramic radiographs and from X-rays of the hand, wrist, and fingers. The final material consists of 47 Finnish children of known ages 0–17.9 years.

5.1.2. FORENSIC AGE ASSESSMENT OF ASYLUM SEEKERS IN FINLAND (STUDY II)

The material consists of Finnish legislation texts concerning forensic age estimation and methods as well as the analysis of the forensic age assessments performed in Finland for asylum seekers in 2015. Information was collected from the Finnish legislation texts: Act on the Status and Rights of Patients (785/1992), Aliens Act (301/2004), Coercive Measures Act (806/2011), Decree on use of radiation (423/2000), Radiation Act (592/1991), and Radiation and Nuclear Safety Authority (2014), as well as from EU statistics (Eurostat) and the public statistics of asylum seekers in Finland by the

Immigration Authorities (The Finnish Immigration Service (b)). The numbers and anonymous results of the forensic age assessments among asylum seeking minors were received from the forensic odontologist (FO) at the Department of Forensic Medicine, University of Helsinki.

5.1.3 DENTAL AGE ESTIMATION IN SOMALI CHILDREN (STUDIES III AND IV)

In dental clinics of health centres in the capital city of Helsinki, patient files represent approximately 30 nationalities. All individuals eligible to be included in the current study were born in Finland after 1.1.1980. Their parents were born in Somalia, their mother tongue was Somali, and their permanent address was in Helsinki. In the search, the upper age limit was set to 25 years, the age limit based on the full development of the dentition, giving highest consideration to inter-individual variation (AlQahtani et al. 2010; Mincer et al. 1993; Nyström et al.2007).

The research was a retrospective study which included collection of radiographic data of eligible young individuals of known gender and age, calculated to one decimal place, at the time of the exposure. According to the Finnish Population Register Centre in 2010, 2,115 persons fulfilled the above criteria. In order to collect as much study material as possible from the age groups 18±2 years, the original material search from 2010, which included dental panoramic radiographs of 489 young Somalis, was repeated in 2015. Retrospectively, 1,231 dental panoramic radiographs taken for the diagnosis and treatment planning of 811 Somali persons were collected from the files at the division of Oral Health Care of the Department of Social Services and Health Care in Helsinki, including both film and digital radiographs. Only one dental panoramic radiograph from each person was included in the study, aiming at a homogenous age distribution. Subjects were excluded from the study if they were found to have any medical abnormalities affecting dental development, reducing the sample to 808 persons.

The development of the left seven mandibular permanent teeth (PT; FDI 31 to 37) was staged according to Demirjian et al. (1973). If a tooth was missing, the contra-lateral homologous tooth was used instead. When all the PT (31 to 37) were mature (Demirjian stage H, N=120), or when one or more permanent mandibular tooth was missing (N=53), the radiograph was excluded. The latter exclusion was done because no age estimation can be performed in the presence of missing Demirjian stages by the Willems et al. method (2001). The final sample in the study III comprised 635 subjects (311 females, 324 males) in the age range between 4 and 18 years (Table 6). The material of Study IV comprised the previously mentioned data from left mandibular PT combined with the data from third molars (TM) and included dental panoramic

radiographs from 803 subjects (406 females, 397 males) in the age range from 3 to 23 years (Table 6). Sample selection and dental developmental staging were performed by the author MM.

Table 6. Age and sex distribution of the Somali sample.

Age (years)	STUDY III			STUDY IV		
	Female (%)	Male (%)	Total	Female (%)	Male (%)	Total
3-3.99	0 (0.00)	0 (0.00)	0	0 (0.00)	1 (0.25)	1
4-4.99	1 (0.32)	3 (0.93)	4	1 (0.25)	3 (0.76)	4
5-5.99	6 (1.93)	7 (2.16)	13	6 (1.48)	8 (2.02)	14
6-6.99	20 (6.43)	19 (5.86)	39	20 (4.93)	19 (4.79)	39
7-7.99	46 (14.79)	33 (10.19)	79	47 (11.58)	37 (9.32)	84
8-8.99	48 (15.43)	42 (12.96)	90	50 (12.32)	48 (12.09)	98
9-9.99	47 (15.11)	50 (15.43)	97	50 (12.32)	51 (12.85)	101
10-10.99	34 (10.93)	48 (14.81)	82	36 (8.87)	49 (12.34)	85
11-11.99	35 (11.25)	41 (12.65)	76	36 (8.87)	43 (10.83)	79
12-12.99	25 (8.04)	35 (10.80)	60	27 (6.65)	38 (9.57)	65
13-13.99	25 (8.04)	23 (7.10)	48	31 (7.64)	27 (6.80)	58
14-14.99	12 (3.86)	12 (3.70)	24	20 (4.93)	17 (4.28)	37
15-15.99	11 (3.54)	6 (1.85)	17	22 (5.42)	17 (4.28)	39
16-16.99	0 (0.00)	3 (0.93)	3	17 (4.19)	17 (4.28)	34
17-17.99	1 (0.32)	2 (0.62)	3	11 (2.71)	8 (2.02)	19
18-18.99	0 (0.00)	0 (0.00)	0	14 (3.45)	7 (1.76)	21
19-19.99	0 (0.00)	0 (0.00)	0	8 (1.97)	0 (0.00)	8
20-20.99	0 (0.00)	0 (0.00)	0	7 (1.72)	3 (0.76)	10
21-21.99	0 (0.00)	0 (0.00)	0	3 (0.74)	2 (0.50)	5
22-22.99	0 (0.00)	0 (0.00)	0	0 (0.00)	1 (0.25)	1
23-23.99	0 (0.00)	0 (0.00)	0	0 (0.00)	1 (0.25)	1
Total	**311**	**324**	**635**	**406**	**397**	**803**

5.2. METHODS

5.2.1. SKELETAL AGE ASSESSMENT METHODS (STUDY I)

The second edition of the Greulich and Pyle atlas (1959) was used for evaluation of skeletal development of the hand, wrist, and phalanges independently by two forensic odontologists (OV and MM) with special competence in forensic odontology. The inter- or intraobserver consistencies

were not tested. The observers discussed inconsistencies in evaluations until a consensus of the skeletal age was reached. Because many hands were injured, the skeletal age assessment was performed for 47 children: 23 girls and 24 boys. Due to severe injuries in the fingers in several cases, the Radius, ulna and short bones (RUS) and 20-bones methods (TW2)(Tanner et al. 1983) were applicable in only 34 children.

5.2.2 DENTAL AGE ASSESSMENT METHODS (STUDY I)

Dental age was assessed by dental eruption based on Finnish reference tables (Nyström et al. 2001) on only eight children due to advanced decomposition of soft gingival tissues. In 33 children, Demirjian's method (1973) based on radiographic mineralisation of seven left mandibular PT (FDI 31-37) was used. The mineralisation of TM was assessed by development tables by Mincer et al. (1993) in seven children. Dental methods were applied on 46 children (Table 7). The results were gathered from dental identification forms.

Table 7. The number of various age assessment methods

Age assessment method	Number
Skeletal	*47*
Greulich and Pyle (1959)	47
TW2, 20 bones (Tanner et al. 1983)	34
Dental	*46*
Demirjian et al. (1973)	34
Mincer et al. (1993)	7
Haavikko (1970)	1
Nyström (2001)	8

5.2.3 THE METHODS OF FORENSIC AGE ASSESSMENT USED IN FINLAND (STUDY II)

The number of asylum seekers and country of origin in Finland, from 2005–2015 were collected from the public statistics of the Immigration Authorities, as well as the number of forensic age assessments performed from the statistics of the FO (OV) at the University of Helsinki. All results were presented as tables in publication II.

5.2.4 METHODS OF DENTAL AGE ESTIMATION IN SOMALI CHILDREN USING THE WILLEMS ET AL. MODEL (STUDY III)

The development of the seven left mandibular PT (FDI 31 to 37) were staged according to Demirjian et al. (1973). The development of all present TM were staged according to the 10-point staging technique developed by Gleiser and Hunt (1955) and modified by Köhler et al. (1994) for future research (Study IV). Inter- and intra-observer reliability of the Demirjian et al. (1973) staging for PT was tested, as well as the Köhler et al. (1994) staging for TM for future research. Inter-observer reliability was tested by examining 37 panoramic radiographs by the principal investigator and a second examiner separately, and intra-observer reliability by re-examining 37 dental panoramic radiographs after two months by the principal investigator.

The WM (2001) was validated on the Somali sample and a Somali-specific age estimation model (SM) based on the WM was established. The results of the age prediction performances of both WM and SM were compared by determining the difference between true age and the predicted age (mean error=ME). The ME represented over- or underestimations (i.e., bias); the mean absolute error (MAE) and root mean squared error (RMSE) quantified the magnitude of the errors (i.e., accuracy). Both the MAE and RMSE reflect bias and lack of precision.

5.2.5 METHODS OF DENTAL AGE ESTIMATION IN SOMALI CHILDREN COMBINING PERMANENT TEETH AND THIRD MOLARS DEVELOPMENT (STUDY IV)

The staging of development of PT and TM as well as inter- and intra-observer reliability testing are presented in paragraph 5.2.4.

Three approaches were applied: using only the PT, using only the TM, and using both PT and TM. The analyses were performed on a dataset with all subjects having at least four of the seven permanent index teeth and at least one third molar. The age-estimation performances were quantified, calculating the ME (true age minus predicted age), the MAE and the RMSE. The coverage of the obtained 95% prediction intervals was tested. Results were calculated over the total age range and in 1-year age intervals.

5.2.6 STATISTICAL METHODS

The intra- and inter-observer agreements were quantified using Kappa and weighted Kappa statistics (Study III and IV). Age assessment methods,

excluding the method by Mincer et al. (1993), were tested for reliability with the Intraclass Correlation Coefficient using the SPSS 15.0 programme for Windows (Study I).

In Study III the SM was validated using leave-one-out cross-validation. The bias and the discrepancy between the age predictions of the WM and SM were evaluated using Wilcoxon signed rank tests. Spearman correlation between true age and the error in age estimation was used to evaluate if the direction and the magnitude of the difference depended on age.

In Study IV in all three approaches, PT, TM and PT+TM, a Bayesian age-estimation model for the multivariate distribution of the stages conditional on age (multivariate continuation ratio model) was fitted as described in Boldsen et. al (2002) and Fieuws et al. (2016). The three models were established for females and males separately and validated using 10-fold cross-validation (Study IV). All statistical analyses were performed using SAS software, version 9.4 of the SAS System for Windows (Studies III and IV). The described approach in Study IV was implemented with SAS software using PROC NLMIXED to fit the continuation-ratio models and to evaluate the conditional likelihoods.

5.2.7 ETHICAL CONSIDERATIONS

Ethical approval was granted by the Research Ethics Committee of Hjelt Institute, University of Helsinki (number 02/2010). In Study I, the material consisted of radiographs from the identification procedure of Finnish Tsunami victims. In Study II, only statistical data were applied. Panoramic and other radiographs are commonly taken on clinical dental reasons. For research ethical reasons, the study material of the Somali sample consisted of previously taken dental panoramic radiographs, and thus, the examinees were not exposed for extra ionising radiation. The permit for the study was received from Oral Health Care of the Department of Social Services and Health Care in Helsinki (Studies III and IV).

6 RESULTS

6.1. COMPARISON OF SKELETAL AND DENTAL FORENSIC AGE ESTIMATION METHODS (STUDY I)

The inter-examiner difference between the two FOs performing the radiographic age assessment analysis using G-P was, on average, 0.6 months. In the comparison of skeletal methods, G-P performed better in age estimation than TW2 (20 bones), showing average differences from the chronological age of 9.7 months and 10.3 months, respectively. For the dental methods, the average difference for the eruption of the teeth (Nyström et al. 2001) was 5.6 months, for the development of PT (Demirjian et al. 1973) 5.2 months, and for the TM (Mincer et al. 1993) 12.6 months from the chronological age. The dental methods except TM showed the smallest deviation from the chronological age (Figure 7). The highest average Intraclass Correlation Values were obtained using the dental methods based on eruption (0.990) and the development of PT (0.994). The key result was that dental methods based on erupted teeth and PT development proved to be more accurate than skeletal methods.
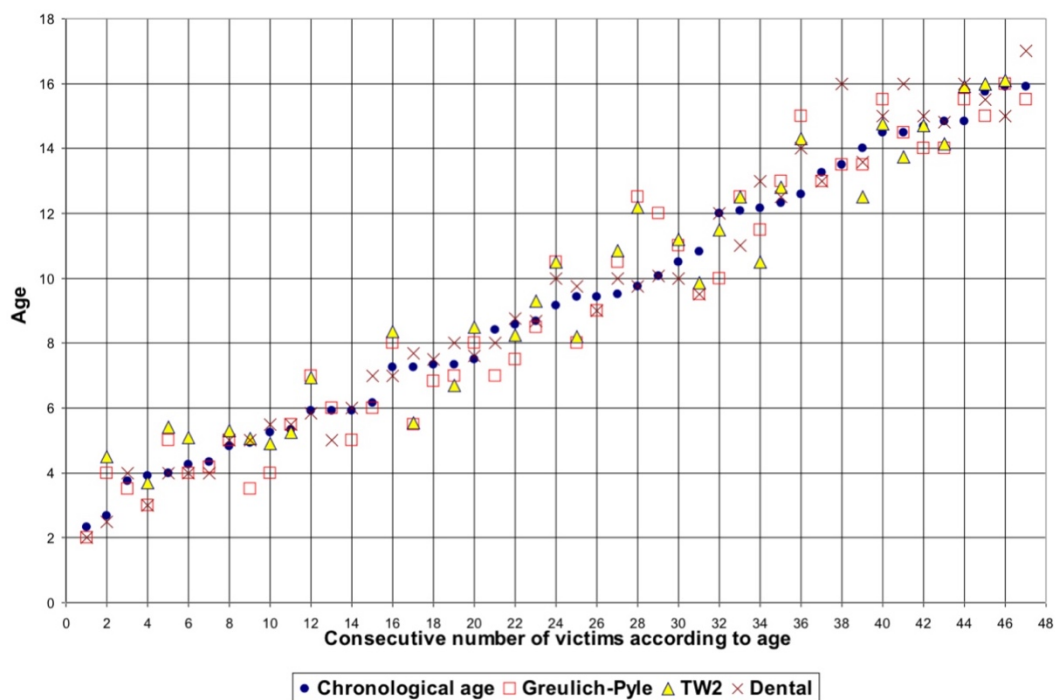
Figure 7. The results of age assessment of 47 children performed by three methods (Greulich and Pyle, TW2, and most age-appropriate dental) are presented in relation to the chronological age.

6.2 THE ANALYSIS OF FORENSIC AGE ASSESSMENTS PERFORMED IN FINLAND (STUDY II)

The study focused on Finnish legislation concerning forensic age estimation in 2005–2015 and methods as well as the analysis of the forensic age assessments performed in Finland for asylum seekers in 2015. In 2005–2015, variation in the number of forensic age assessments was observed, the peak being in 2015 (N=149). About one-third of the minor asylum seekers were referred to the forensic age assessment during the study period, but in 2015 only 5% of the 3,024 unaccompanied minor asylum seekers were examined (Figure 8). One of the focal results was that 60% (90/149) of the asylum seekers who notified their age under 18 years were assessed adults. In 2015, the three most frequent countries of origin were Afghanistan, Iraq, and Somalia.

The forensic age assessment in Finland in 2005–2015 included an interview of the examinee before taking radiographs. The important background information recorded was: sex, ethnic background, the examinee's notified age, and possible nutritional or medical factors affecting skeletal growth, as well as information of siblings. The weight and height were measured.

Since radiological methods are fundamental in forensic age assessment, and ionising radiation is used for non-medical purposes, the Radiation and Nuclear Safety Authority (STUK) issued an updated special permit for the Department of Forensic Medicine of the University of Helsinki for this purpose in 2014 (Radiation and Nuclear Safety Authority 2014). Dental panoramic tomogram and an X-ray of the left wrist, hand, and fingers were taken of the examinee. Periapical intraoral X-rays were taken when the age of an adult asylum seeker evaluated on arrival was later questioned (Cameriere and Ferrante 2007; Sharma and Srivastava 2010). After independent study of the radiographs the two forensic odontologists signed the joint forensic age assessment report.

The references of dental development chosen for each case depended on ethnic background and the maturity of the PT. If the development of teeth anterior to the TM was incomplete, three methods of the following reference tables were chosen: Demirjian et al. (1973), Kataja et al. (1989), Chaillet et al. (2005), Nyström et al. (2007), and Willems et al. (2001).
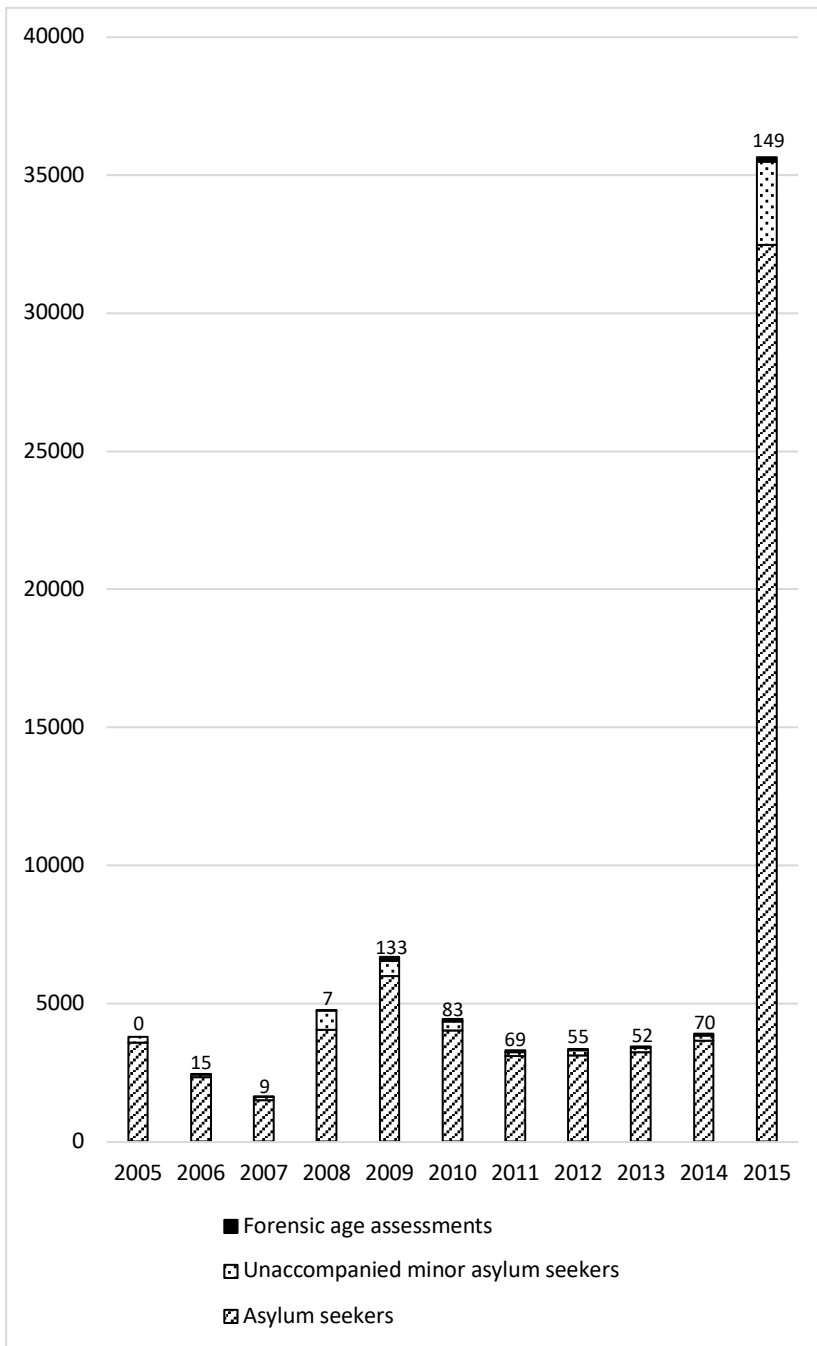
Figure 8. Number of asylum seekers (dashed area), unaccompanied minor asylum seekers (spotted area), and performed forensic age assessments (black area and numbers) in Finland 2005-2015.

All of these methods are based on Demirjian's staging technique from seven left permanent mandibular teeth (Demirjian et al. 1973). In tables by AlQahtani et al. (2010), tooth development was determined according to Moorrees et al. (1963) on all PT, TM included. In cases where TM were present, the references used were Mincer et al. (1993), based on American and African

American populations, and Orhan et al. (2007), based on the Turkish population, as well as previously mentioned tables by AlQahtani et al. (2010). As a progressive change, the size of the dental pulp diminishes gradually due to secondary dentin formation with increasing age. Thus, the age of an adult could be assessed on a fairly large range using the method of Kvaal et al. (1995) from intraoral periapical X-rays.

The method of Greulich and Pyle (1959) on the X-ray of the left hand, wrist and fingers was used for skeletal age assessment by comparing radiographs with the different age categories by sex in the atlas. Tiśe et al. (2011) have shown that the minimum age for complete skeletal maturation of the distal forearm and hand skeleton from a standard hand X-ray is 16 years for both sexes.

When the age of majority was in question, the probability of the individual to have reached the threshold age of 18 years (Mincer et al. 1993) was expressed in the forensic age assessment report with means and standard deviations of different references.

6.3 DENTAL DEVELOPMENT IN YOUNG SOMALIS (STUDIES III AND IV)

The intra- and inter-observer Kappa and weighted Kappa values (0.94–0.99) (Studies III and IV) revealed an excellent level of agreement for both PT and TM staging.

In Study III, the WM was validated on the Somali sample, a new SM was created, and their performances were compared. The result was considered statistically significant when the p-value was below 0.05. The WM validated on the Somali sample resulted in a statistically significant underestimation of age for females (ME=0.20 years; p=0.0006), whereas for the males the overestimation (ME=-0.02 years; p=0.27) and for the total sample the underestimation (ME=0.09 years; p=0.11) were not significant. The MAE values were 0.78 years for both females as well as for the total sample, and 0.77 years for the males. The RMSE values were 0.99, 1.02, and 1.01 for males, females, and the total sample, respectively (Table 2 in Study III).

The created SM slightly underestimated age in all three groups, the MEs varying from 0.04 to 0.05 years, the MAEs from 0.77 to 0.80 years, and RMSEs from 1.01 to 1.04 years (Table 2 in Study III). When the performances of the two models WM and SM were compared, statistically significant differences (p<0.0001) in ME were detected: -0.07 years in males and 0.16 years in females (Table 3 in Study III).

New dental maturity scores were developed for all seven left mandibular teeth in the Somali males and females of the dataset, where Individuals with all PT having stage H were excluded, based on a weighted analysis of variance (ANOVA) following the WM. Regression coefficients of the SM are reported in Table 8. Missing values correspond to developmental staging scores which did not appear in the dataset.

Table 8. Country-specific regression coefficients for males and females separately obtained from a fit of the model on the Somali dataset according to the Willems model (2001). Individuals with all permanent teeth stage H were excluded.

Tooth	B	C	D	E	F	G	H
M							
31	.	.	4.892	5.658	5.956	6.742	7.075
32	.	.	0.000	-0.437	-0.580	-0.365	0.140
33	.	0.000	-0.484	-0.673	-0.390	0.408	1.122
34	.	0.000	1.220	1.566	1.736	1.845	2.409
35	0.000	-1.020	-0.935	-0.752	-0.528	-0.646	0.609
36	.	.	.	0.000	0.946	1.205	1.542
37	0.000	0.000	0.508	0.658	1.345	1.386	.
F							
31	.	.	5.878	5.836	6.801	7.024	7.106
32	.	.	0.000	-0.678	-0.461	-0.334	0.040
33	.	.	0.000	0.143	0.341	0.675	1.045
34	.	0.000	0.138	0.443	0.818	1.724	2.523
35	.	0.000	-0.040	0.003	0.151	-0.494	0.204
36	.	.	0.000	0.000	-0.121	0.275	0.464
37	.	0.000	0.250	0.491	1.426	2.688	.

M male, F female, *B-H* tooth developmental stages by Demirjian et al. (1973)

In Study IV, the age prediction performance of PT and TM development was analysed, separately and combined, using a Bayesian approach. The overall age prediction performance was best in the combined approach PT+TM, overestimating age only by 0.031 years (11.3 days) in males and by 0.011 years (4.0 days) in females. Between approaches, all differences in ME ($p < 0.0001$), MAE, and RMSE were significant ($p < 0.05$) (Table 3 in Study IV).

The performances of PT, TM, and PT+TM combined per age category of 1 year were compared (Study IV). In the approach combining PT+TM, the ME was small and constant in the age groups from 4 to 15 years in males (-0.222–0.332 years) and from 7 to 16 years in females (-0.327–0.294 years). In the PT approach a large negative ME was observed in the age range from 15 to 17 years in males (-1.791 to -1.557 years) and in the 16-year-old category in females (-2.133 years), together with a sudden drop in MAE and RMSE in the 18-year-old age category in both sexes. In the TM approach, the ME values had

moderate variation in the age categories 9 to 21 years in males (-0.486–0.794 years), whereas in females more variation was present (-0.406–1.606 years) in these categories (Figure 1 in Study IV)

The coverage of the 95% prediction intervals is reported in Table 9. The smaller the prediction interval, the better, but this should not be at the cost of a decrease in coverage, which should be as close as possible to 95%. In the combined PT and TM approach, the optimal coverage result of 95% was observed in the age categories from 12 to 16 years in both sexes (Figure 2 in Study IV).

Table 9. The 95% coverage status for males and females in the model for permanent teeth, third molars, and combining permanent teeth and third molars.

Approach	95% Coverage status									
	Males					Females				
	Outside prediction interval		Inside prediction interval		Total	Outside prediction interval		Inside prediction interval		Total
	N	%	N	%	N	N	%	N	%	N
PT	28	7.05	369	92.95	397	29	7.14	377	92.86	406
TM	23	5.79	374	94.21	397	19	4.68	387	95.32	406
PT+TM	20	5.04	377	94.96	397	22	5.42	384	94.58	406

PT permanent teeth, *TM* third molars, *PT+TM* permanent teeth and third molars combined

7 DISCUSSION

7.1 COMPARISON OF SKELETAL AND DENTAL FORENSIC AGE ESTIMATION METHODS (STUDY I)

In forensic age assessment, the development of the hand and wrist and clavicle are most often applied (Schumacher et al. 2018). The last epiphyses to fuse are in the clavicle and iliac crest, maturing up to 28 and 24 years, respectively (Black et al. 2011). When using the G-P method, the minimum age for maturity in both sexes is 16 years (Tisé et al. 2011), which sets limitation for its usability in forensic age estimation. In the present study (Study I), the usability of the G-P method is put in a critical light showing an average difference of 9.7 months from the chronological age. Additionally, skeletal development is affected by nutritional, environmental, and socio-economic factors (Schmeling et al. 2000).

In young children, the use of dental eruption is possible in age assessment (Nyström et al. 2001), but the key issue in forensic medicine most often concerns the age of majority. In these cases, the use of PT and TM development are recommended. PT reach the final stage of development on average around the age of 16 years (Chaillet et al. 2005; Willems et al. 2001), whereas the development of TM is more variable, but reaches the final maturation, according to Mincer et al. (1993), at around 20 years. More variation was found in the study by Köhler et al. (1994), however. Fully completed roots appeared at the age of around 23 years with a deviation of ±4 years in both sexes. In the present study, the dental methods performed better in childhood than skeletal methods. PT showed the smallest average difference from the chronological age (5.2 months), but this can partly be due to the age distribution of the Finnish child victims of the tsunami, most of them being under 15 years of age.

Combining the information from the development of both teeth and the hand and wrist is recommended for forensic age estimation, since more accurate age-estimation results are obtained using different age predictors (Kumagai et al. 2018; Mânica et al. 2018). This is possible by applying a Bayesian approach and information from large datasets. The method BioAlder, combining developmental stages of hand bones and teeth, is already applied in Norway, providing an estimate of the age span and prediction intervals as well as a percentage of individuals under the ages 16 and 18 years (Bleka et al. 2018a and 2018b; Dahlberg et al. 2018; Department of Forensic Medicine, Oslo University Hospital).

7.2 THE FORENSIC AGE ASSESSMENTS PERFORMED IN FINLAND (STUDY II)

Many countries still lack legislation concerning forensic age estimation (European Asylum Support Office 2018). The situation was similar in Finland in 2009, when the numbers of forensic age assessments reached a peak (Figure 8), since no Finnish legislation existed concerning forensic age assessment. Therefore, the Ministry of the Interior started to prepare the Government's proposal for the Finnish Parliament to change the Aliens Act in 2009 (Hallituksen esitys eduskunnalle laiksi ulkomaalaislain muuttamisesta 240/2009). Based on the statement of the Parliamentary Ombudsman in the summer of 2009 the forensic age assessment was included in the amendment of the Aliens Act 301/2004. The amendment of the Act 549/2010 Section 6a and 6b was enacted in 2010. In 2016, new amendments, 501/2016 Section 6a and 6b (Aliens Act), were added transferring the responsibility of forensic age assessments from the University of Helsinki to the THL. Moreover, the Finnish Immigration Service is the only authority who has the right to refer asylum seekers to the forensic age assessment. Earlier, the Police Authorities and the Border Guard Authorities were authorised as well.

Benefits of the legislation include that the minor asylum seeker is accompanied with the District Court–ordered guardian. Additionally, the law protects the legal rights of the forensic odontologists performing forensic age assessments. Unfortunately, the law is not applicable to Finnish citizens having arrived earlier to Finland as asylum seekers and granted citizenship. In criminal cases, when age assessment is needed, the Coercive Measures Act (806/2011) and the amendments of the Act (1146/2013) are applicable.

Similarities in Finnish and EU legislation can be seen since sections 6a and 6b of the Finnish Aliens Act 2016 (Aliens Act) follow the respective regulations of EU-Procedure Directive 2005 (Article 17 para 5) (Council Directive 2005/85/EC 2005). For instance, informed consent of the examinee, the "ultima ratio" principle of medical expert opinion for the age assessment of an asylum seeker, and the assignment of qualified medical professionals are demanded. A special prerequisite from the Finnish Radiation and Nuclear Safety Authority (STUK) is that the dental radiographs of the forensic examination must be stored for possible later dental treatment, and the examinee must be informed of the need of dental care (Radiation and Nuclear Safety Authority).

Even though in Europe applications from unaccompanied minors in EU countries have decreased since 2015, the numbers are still higher than prior to the refugee crisis (European Asylum Support Office 2018; Eurostat). The peak in unaccompanied minor asylum seekers coming to Finland in 2015 was reflected in high numbers of forensic age assessments in 2016 (N=630). The

asylum-seeking wave is expected to continue in high numbers in Europe (United Nations 2017), therefore, legislation concerning forensic age estimation as well as uniform methods of age assessment are recommended (Schumacher et al. 2018).

7.3 DENTAL DEVELOPMENT IN YOUNG SOMALIS (STUDIES III AND IV)

In Study III, the purpose was to evaluate the ethnic variation in tooth development, especially the dental development of Somalis. This is important, since possible differences in dental development between Black African and other populations are disputed in the literature (Cavrić et al. 2016a; Liversidge 2008; Olze et al. 2006; Phillips and van Wyk Kotze 2009); although, recently, more evidence of similarity has appeared (Elamin et al. 2017; Esan and Schepartz 2018a; Kihara et al.2017; Liversidge et al. 2010 and 2017; Thevissen et al. 2010a and 2010b; Willems et al. 2017), as in the present study.

Recently, several studies on the dental development of sub-Saharan populations have been published (Angelakopoulos et al. 2018; Cavrić et al. 2016a and 2016b; Elamin et al. 2017; Esan and Schepartz 2019, 2018a and 2018b; Kihara et al. 2017; Liversidge et al. 2017; Olze, ym., 2006, Phillips and van Wyk Kotze, 2009; Uys et al. 2018; Willems et al. 2017). Information on Somalis indigenous to Northeast Africa is scarce and limited to the study by Davidson and Rodd (2001). Studies on dental development in Northeast Africa are few as well (Dardouri 2016; Garamendi 2005; Elamin et al. 2017). In the present study setting (Studies III and IV), reliable data on individuals of ascertained chronological age and ethnic background was gathered. This might not be the case in locally performed Sub-Saharan studies, due to the incomplete birth registers (Pelowski et al. 2016). In the future, inclusion of the country-specific regression coefficients for males and females from the Somali dataset based on the WM (2001) in forensic age estimation will enhance the age prediction performances in Somali children (Table 8).

In Study III, the WM only significantly underestimated the age for females, with an ME of 0.20 years (p=0.0006), whereas for other groups the ME differences from age were not significant. The constructed WM-based SM performed better than the WM on the Somali sample; however, although the differences between the two models were significant (p<0.0001), they were still clinically small: in males -0.07 years (26 days) and in females 0.16 years (58 days). This is in concordance with a recent study on South African Blacks (SAB) by Willems et al. (2017), where they built a similar WM-based SAB model and compared it with the WM. They found small but clinically insignificant differences in the validation of the two models. Previously, the WM has been validated on multiple populations and showed good

performance in age estimation (Akkaya et al. 2015; Ambarkova et al. 2014; Djukic et al. 2013; Galić et al. 2011; Liversidge et al. 2010; Nik-Hussein et al. 2011; Onat Altan et al. 2016; Willems et al. 2001; Ye et al. 2014). In the present study, the better performance could partially be explained by the large Belgian Caucasian sample size (N=2,116) when compared to the present SM (N=635). In larger samples, the sample means are closer to the population mean since they are reflecting the population more accurately.

In Study III, the age prediction performances were not constant over age; at younger ages there was a tendency of age overestimation, and at older ages a tendency of underestimation (Spearman correlation between the true age and the error in age estimation=0.36, p<0.0001). Although a classical finding in regression models (Aykroyd et al. 1997), the overestimation of age in younger children may be affected by the fact that the indications for taking dental panoramic radiographs vary in different age groups. For younger children, the indication is most often caries in primary teeth, which accelerates the development and emergence of PT (Brin and Koyoumdijsky-Kaye 1981; Leroy et al. 2003 and 2009) and affects the emergence sequence of the PT (Leroy et al. 2009), whereas for older children or adolescents the main indication is the need for orthodontic treatment.

In Study IV, the age prediction performance of PT and TM development, separately and combined (PT+TM) were analysed using a Bayesian approach. The combined approach PT+TM showed the best performance, overestimating age on average by only 0.031 years (11.3 days) in males and 0.011 years (4.0 days) in females. The combined PT+TM approach also yielded the lowest magnitude and variability of error (MAE and RMSE). ME provides information about the direction of the error; whether the model over- or underestimates the age of an individual. MAE quantifies the magnitude of the error and RMSE the variability in error, giving most weight to the largest errors. In the study sample (IV), the use of TM only for age estimation led to the biggest error and variability.

The performance of PT and TM combined was optimal between ages 13 to 14 years in males and 12 to 14 years in females (Study IV), most likely because both PT and TM are developing at these ages. According to the literature, the age at completed PT development (up to the second molars) is 16 years, on average (Chaillet et al. 2005; Demirjian et al. 1973; Willems et al. 2001). The present Somali data is in agreement since of the 16-year-olds, 80.7% of males and 96.4% of females had all mature PT. Since all PT can be mature at the earliest around 12–13 years (here the minimum in males was 13.39 years and in females 12.89 years), the use of PT+TM approach beginning from the age of 12 years is likely to be beneficial compared to PT approach. Indeed, the combined PT+TM approach showed its superiority regarding 95% coverage results in the age categories from 12 to 16 years in both sexes (Figure 2 in Study

IV). Consequently, in the practice of forensic age estimation, the most benefit of combining PT and TM information will be obtained in 12- to 15-year-olds. The oldest ages for immature PT were in males 17.37 and in females 17.97 years. After ages 17 years in males and 19 years in females, the TM model performs equally well (Figure 1 in Study IV).

Previous research shows that children of African origin are slightly more advanced in dental development than other groups (Blankenship et al. 2007; Harris and McKee 1990; Liversidge et al. 2010 and 2017; Mincer et al. 1993; Olze et al. 2004 and 2006; Uys et al. 2018). Our study was not performed in Somalia but in very different living conditions in Finland, therefore, possible environmental and nutritional factors influencing the outcomes were excluded. Studies show that no effect of malnutrition on dental development exists (Cameriere et al. 2007; Elamin and Liversidge 2013), but conversely, a few studies refer to advanced dental development in obese or overweight children (Hilgers et al. 2006) and in children with high-fat intake (Jääsaari et al. 2016). Even though in Finland the living conditions are better compared to Somalia, Somalis, being dark-skinned, often suffer from vitamin D deficiency because of the long dark period in Finland during the winter. This could affect the formation of dental enamel, but presumably not the timing of dental development. In Somalia fluorosis of the teeth is common, which is rare in children born in Finland.

7.4 LIMITATIONS OF THE STUDY MATERIAL

Staging of development of teeth as well as assessing growth of the hand and wrist as well as long bones is always prone to intra- and inter-examiner variability (Dhanjal et al. 2006; Lynnerup et al. 2008; Wittschieber et al. 2014b), to avoid this flaw automated methods to evaluate stage allocation and growth have been developed (De Tobel et al. 2017c; Thodberg et al. 2009). The aim of the automated age estimation technique is to achieve exact reproducibility. Software is needed for recognition of the desired region of the image, to assess the stage of development, and to estimate age according to a reference population (Thodberg et al. 2009). However, in the present study (III and IV) an excellent level of intra- and inter-observer agreement was achieved in stage allocation.

The research method in Study III skews the results in the age group 16–18 years, since complete PT maturation was an exclusion criterion. In these age groups only, individuals with advanced dental development were excluded, since in Study IV it was shown that all PT can be mature as early as 12 to 13 years of age.

7.5 FUTURE PERSPECTIVES

The methods of forensic age estimation are developing constantly. The possibility to implement BoneXpert on hand–wrist radiographs (Thodberg et al. 2009 and 2017) already exists in the University Hospitals of Oulu and Helsinki. Recently, BoneXpert G-P bone age rating was validated on healthy Finnish children (N=400), resulting in a mean difference between the chronological age and BoneXpert bone age of 0.13 years for females and 0.028 years for males: a good performance in the Finnish population (Lammert 2015). In the future, combining different dental and skeletal methods by applying a Bayesian approach (Bleka et al. 2018a and 2018b; Dahlberg et al. 2018; Kumagai et al. 2018) would enhance the age estimation outcomes. Collection of a reference database and the development of software for utilisation of both PT and TM data for the increased accuracy of age estimation of minors in practice is an important future goal. In discrimination between minority and majority problems arise when all TM are missing due to congenital hypodontia or extractions which may both be common. When MRI techniques improve, the use of ionising radiation in a non-medical examination could be avoided (De Tobel et al. 2018) and clavicular data could be used for assessment purposes. Hence, legislation should rapidly follow methodological improvements.

The methods for forensic age assessment vary even within Europe and the EU. Many countries still lack legislation concerning forensic age estimation (European Asylum Support Office 2014 and 2018; Separated Children in Europe Programme (SCEP) 2012; Solheim and Vonen 2006). Even though the asylum-seeking process is strictly regulated by international acts, each Member State may decide how to transpose the EU Directives into national legislation. It has been proposed that an Age Markers Diagnostics Centre should be established in Europe based on the expertise of specialists involved in AGFAD (Schumacher et al. 2018) to unite the European forensic age estimation procedure and methods. In Finland, national legislation improves the process and guarantees the unity of the methods applied.

8 CONCLUSIONS

The purpose of this study was to evaluate the Finnish forensic age assessment methods and related legislation, to compare common dental and skeletal methods of forensic age assessment, and to evaluate the ethnic variation in tooth development in a rare sample of Somalis with known age and ethnic background, as well as the performance of previously created PT and TM dental methods. Based on our findings, the following conclusions can be drawn:

I   The comparison of dental and skeletal age assessment methods indicated that the dental methods, especially the one based on development of PT 31 to 37, performed better than skeletal methods when predicting a child's age.

II   The current legislation concerning forensic age assessment in Finland has been well received among immigration authorities and by forensic odontologists.

III   The widely used WM based on the Belgian population performs well for Somali children, and the performance of the SM was only slightly better. These findings support the universal application of the WM for forensic age assessment.

IV   The age prediction performance in Somali children, born and living in Finland, improves by combining the information of PT and TM, especially in the age groups 12–15 years, when both PT and TM are still developing.

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