General introduction
1.1 FROM EMBRYOLOGY TO THE FINAL STAGE OF MATURATION

Dental development is defined as a progressive and continuous process determined by epithelial-mesenchymal interactions and controlled by genetic, epigenetic and environmental factors over time. Various enzymes catalyze a cascade of signaling pathways important for the regulation of dental development. The human dentition consists of four different tooth types, incisors, canines, premolars and molars, which beside their own form characteristics will go through same developmental stages. A thickening of the oral epithelium at 6th week of gestation indicates the earliest histological sign of tooth formation. The initiation of dental development will be followed by the bud, cap and bell stages, displaying the anatomy of individual teeth. In the beginning of bud stage around the 8th week of gestation, the dental epithelium continues to invaginate into the dental mesenchyme forming the tooth germ. Then, during the cap stage at 9th-10th weeks of gestation, epithelium extends further into the mesenchymal tissue surrounding the dental papilla and forming the enamel organ. Around 11th-12th weeks of gestation the bell stage will follow, determining the type of tooth based on the specific cusp pattern: single-cusped teeth (incisors and canines) or multicusped teeth (premolars and molars). The end of the bell stage assigns the finalization of cyto-differentiation and the initiation of matrix secretion. The mineralization of dental hard tissues including enamel and dentin starts around the 14th-16th week of gestation with the initial calcification of deciduous incisors, while the mineralization of the permanent dentition initiates only at birth with the calcification of the first molars. The normal formation and mineralization of hard tissues will continue until the tooth reaches the final developmental stage, represented by the apical closure of the root canals.

The timeframe between the initial stage of formation and the final stage of development counts approximately a 10 year interval, independently ascertained for each group of teeth in maxilla and mandible. While stages of dental development become visible in dental panoramic radiographs (DPRs), developing teeth can be used to calculate dental age. As a result, standards of dental age calculation have been implemented in different populations. The age assessment methods use the identification of calcification stages based on radiographic images to assign a dental maturity score weighted by a dental age standard derived from a specific population. The reliability of age estimation is higher when applying methods based on the stages of dental development than when applying methods based...
on skeletal development, because the dental development is controlled more by genes than by environment.\textsuperscript{24,25}

After formation, hard tissues of teeth face the least turnover in their structure. The lack of remodeling implies that the alterations of dental development happen during the ontogenesis. Thus, early life determinants of a genetic, environmental or epigenetic origin will more probably indicate normal variations and disturbances of the developing dentition.

1.2 INDICATORS OF DISTURBED DENTAL DEVELOPMENT

The most recognized disturbances of dental development are congenital and/or of a genetic origin because tooth formation is determined during the intrauterine life and is mostly

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**Figure 1.1.** The principal stages of tooth formation

The figure is adapted from Jernvall J, Thesleff I. Tooth shape formation and tooth renewal: evolving with the same signals. Development. 2012;139(19):3487-97.
controlled by genes. Mutations of genes that are directly involved in tooth formation and maturation can lead to fatal phenotypes of the dentition with failure of tooth formation from one missing tooth to lack of the whole dentition. As the number of missing teeth increases, the timing of maturation for the present teeth will exceed the normal variations and will be distinguished as disturbances in terms of delayed development. The environment plays a minor role during the initiation, however, severe conditions of a considerable magnitude such as systemic diseases, malnutrition, hormonal alterations, radiation or traumas can increase the importance of the environmental component. Because of the biologic response the duration of a force is more important than its magnitude. Hence after the initial formation of tooth germs, persistent factors can play a distinctive role on a continuous process such as dental development. Local factors such as forces generating from the dentofacial complex and dental diseases such as caries generating from the oral bacteria will affect the timing of dental maturation. Furthermore, general epigenetic factors including hormonal changes will become more important during the transitional dentition because of the coinciding time with puberty. Even though the above mentioned factors play a distinctive role in particular time instants of maturation, they should be considered during the whole process of dental development.

1.2.1 Genetic factors

In the past few years research has confirmed that genetic factors contribute to the variation of dental development. Over 300 genes have been identified as being involved in the key stages of odontogenesis, including initiation, morphogenesis and differentiation. Common underlying mechanisms involving genes are likely to be the cause of dental anomalies such as absent or extra teeth, representing the extreme disturbances of the dentition. The analysis of genetic data in humans mainly involved the whole genome linkage analysis, mutation analysis of specific genes or association analysis of suggestive candidate genes. Thus, as a result of genetic wide association studies (GWAS), several genes were identified as linked to dental development including \(EDA\), \(HOXB2\) and \(IGF2BP1\). Other studies have suggested the association of ectodysplasin encoders \(EDA\) and \(EDAR\) with dental phenotypes. Specific gene analysis and experimental studies mainly performed in mice have shown that genetic mutations of \(MSX1\), \(PAX9\), \(AXIN2\), \(EDA\), \(EDAR\), \(EDARADD\) and \(WNT10A\), are responsible for hypodontia (congenital absence of 1-5 teeth) and oligodontia (congenital absence of 5
or more teeth), concluding that the normal expression of these genes is important for the formation of the tooth germ. As the tooth represents a complex organ with both ectodermal and mesenchymal origin, the same genes are implicated in the development of the other tissues of ectodermal origin. In addition, factors that perturb the expression of the responsible genes affect not only dental development but also the development of the other ectodermal organs such as hair, nails, skin and glands. As a result of, disturbances of dental development such as oligodontia belong to the phenotype of many ectodermal dysplasia syndromes such as Witkop syndrome (OMIM 189500), Schopf-Schulz-Passarge syndrome (OMIM 224750), Oligodontia-colorectal cancer syndrome (OMIM 608615), Otedental dysplasia (OMIM 166750), Dermoodonto-dysplasia (OMIM 125640), Odontomicronychial dysplasia (OMIM 601345) Turphpenny type (OMIM 601345) etc. Whether oligodontia can be distinctively isolated without other additional abnormal physical features underlies another question: Is non-syndromic oligodontia just a mild expression of ectodermal dysplasia?

Although numerous genes and genetic linkages that control dental development have been unraveled, the literature still lacks an explanation of the mechanisms that keep in balance such a complex network of genes from the earlier stage to the finalization of dental development. The need to bridge the gap between molecular events during odontogenesis and variations or disturbances of human dentition still remains a remarkable question.

1.2.2 Environmental factors

The function of our dentition had to adapt to the environmental changes. As a result of the dietary changes, the velocity of dental development in modern humans have decreased compared to our ancestors. On the other hand, daily diets poor in nutrients and rich in sugar have increased the prevalence of dental caries, recognized as the most common dental disease. What we eat will partly determine the way we chew. The pressure against the jaws and teeth could affect the growth of jaw and development and eruption of teeth. Malnutrition leads to adverse outcomes in growth and development, not excluding the teeth. However, even if the diet is adequate, a deficiency in one or two essential nutritional components can affect growth and development. Teeth are the most mineralized organs of our body, hence sufficient supply of micronutrients such as calcium, phosphorus and vitamins are essential for dental development and to reduce the risk of dental diseases. Vitamin D is important for calcium and phosphorus homeostasis, which are essentially needed to form the hydroxyapatite crystals of enamel and dentin. Other vitamins of the B complex such as folate (vitamin B9) and cyanocobalamin (vitamin B12) can alter the comfort and function of the surrounding tissues, including periodontium and gingiva. Furthermore, folic acid may play a role in the etiology of cleft lip and/or palate, which is highly suspected to share similar causes with disturbances of dental development with abnormalities in number, size, shape and timing of formation. Thus, altered nutrition during pregnancy can influence the size of teeth, time of eruption and enamel mineralization inducing developmental disturbances. Environmental changes relate to the ecological characteristics as well. The diversity in climate, latitude and altitude in different geographical areas, corresponds also
to dental variations in populations including not only variations in the morphology and anomalies of teeth but also in timing of development\textsuperscript{21,71-74}.

Although environment is considered to play a minor role on dental development, particular exposures in early life such as systemic diseases or malnutrition can disturb the formation and maturation of teeth leading to dental anomalies. Furthermore, environmental characteristics can partly explain normal variations in the timing of dental development.

\textbf{1.3 ACCELERATION OF DENTAL DEVELOPMENT: NORMAL VARIATIONS AND DISTURBANCES}

The biologic clock of different individuals is set differently, hence some children mature early, other develop slowly and so appear to be behind even though later in time, they will catch up and can pass children who were advanced at a certain time point\textsuperscript{74}. Timing variations in growth and development are particularly evident in adolescence because children undergo a spurt of growth, shown to be associated with an acceleration in maturation of teeth\textsuperscript{33}. In an ongoing process such as dental development certain patterns are already known and used as proxy of normal dental development. To assess dental development for each participant, we identified the developmental stages of the left mandibular teeth from the DPRs as described by Demirjian (Figure 1.3)\textsuperscript{75}. In case any permanent tooth in the left mandible was congenitally missing, the stage of development was assessed from the corresponding tooth in the right mandible. If the corresponding tooth was missing too, regression equations which take into account the development of the remaining teeth in the lower left quadrant and age of a child, were applied to assess the stage of development for the agenetic tooth\textsuperscript{76}. In addition, we calculated dental age for each individual by testing several dental age standards\textsuperscript{17,19-21,75}.

According to Demirjian, the calculated dental age using developmental stages of mandibular teeth, represents the development of the whole dentition. For example, at dental age 10 years approximately half of the roots of mandibular canine and mandibular first premolar

\begin{figure}[h]
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\includegraphics[width=\textwidth]{Figure1.3.png}
\caption{Developmental stages of the left mandibular quadrant (DPR of a 9 year old individual)}
\end{figure}
are completed representing a stage 7 of development; nearly half of the root of upper first premolar is completed representing a stage 6 of development and there is still significant root development of the mandibular second premolar, maxillary canine and maxillary second premolar, representing a stage 5 of development in a DPR. By dental age 11, the roots of all incisors and first permanent molars should be well completed representing a stage 8 of development. In the same way, dental ages from 3 to 16 years can be described.

As part of the physical growth, dental development is characterized by timing that arises from normal variation to disturbance. The normal timing variation of dental development is difficult to be defined, however an approximation of 6 months in terms of delay or advance should be considered within the normal range of maturation of the dentition. Beyond normal variations, disturbances of the developing dentition are more often recognized. The most common disturbances of dental development arise congenitally during the initial stages of tooth formation and mostly affect the number of teeth. Hypodontia, the congenital absence of 1 to 5 teeth, is the most common dental anomaly. Whereas, both oligodontia, the congenital absence of 6 or more teeth and anodontia, the absence of all permanent teeth are rare findings. Oligodontia can be displayed as an isolated (OMIM 616724), or non-isolated or syndromic trait often associated with ectodermal dysplasia (OMIM 305100). As part of a syndrome, oligodontia presents an extensive phenotype including various dental and craniofacial malformations that require special treatment by an interdisciplinary team of orthodontists, maxillofacial surgeons and prostodontists. Differentiation of syndromic oligodontia from isolated oligodontia still remains a clinical challenge. Genetic tests are necessary to determine diagnosis, however in unknown cases, the definition of a genetic spectrum can also be a challenge itself. Thus, assessing the phenotype of dental development in terms of timing and morphology, such as size and shape of teeth, will help clinicians to distinguish syndromic patterns from isolated cases of oligodontia and plan special treatment in each case.

In general, the main disturbances of human dentition such as tooth agenesis including hypodontia and oligodontia, supernumerary or malformed teeth, trauma or tooth loss due to caries will cause mechanical interference leading to delayed or ectopic eruption, which will then contribute to malocclusion and altered development of the overall dentition. Consequently, the timing of orthodontic treatment need will be affected. Thus, investigating factors that relate to the normal variations of the developing dentition and the most common disturbances affecting teeth will provide more insight and a better understanding for clinicians.

1.4 HYPOTHESIS OF THIS THESIS

Dental development in children is disturbed by genetic and environmental factors acting between the initial stage of tooth formation to the prior stage of final calcification.
1.5 OBJECTIVES

1. To assess whether early life determinants indicate variations of dental development in childhood
   1.1. Investigating the role of ancestral background on dental development, in a geographic and genetic perspective of ancestry
   1.2. Studying the influence of maternal folic acid, vitamin B12 and homocysteine on dental development in children
   1.3. Examining the association of maternal and fetal vitamin D with dental development in late childhood

2. To study the role of the most common dental related problems on the developing dentition
   2.1. Investigating the association of hypodontia with dental development in children
   2.2. Studying the relation of dental caries with dental development in children and adolescents

3. To examine the direct and indirect genetic implications in disturbed dental development
   3.1. Investigating the association between WNT10A mutations and dental development in patients with isolated oligodontia
   3.2. Examining the distinction in dental development phenotype between oligodontia as part of ectodermal dysplasia and isolated oligodontia

1.6 STUDY DESIGN AND DATA COLLECTION

The manuscripts of this thesis are conducted in the general and clinical population. Data in the general population was collected from two cohorts, the Generation R Study and the Nijmegen Growth Study. The Generation R Study is a population-based prospective cohort study from fetal life until young adulthood established in the city of Rotterdam in the Netherlands. The cohort is designed to identify early environmental and genetic causes that lead to normal and abnormal growth, development and health from fetal life, childhood and young adulthood. In total 9,778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study. Data collection in children and their parents include questionnaires, interviews, detailed physical and ultrasound examinations, behavioral observations, magnetic resonance imagining and biological samples (Figure 1.6.1). For the current thesis, the measurements of dental development were ascertained from dental panoramic radiographs, taken in 4,561 children at around the age of 10. An assessable and clear radiographic image was obtained in 4,447 DPRs which were scored using the Demirjian method to identify the developmental stages of each mandibular tooth in the left quadrant.

The Nijmegen Growth Study is a mixed-longitudinal, interdisciplinary population-based cohort study in healthy Dutch children conducted from 1971 to 1976 at the Radboud University Medical Centre in Nijmegen, the Netherlands. Children were enrolled at 4, 7 and 9 years of age and followed until 9, 12 and 14 years and general, physiological, dental and
anthropometric measurements of children were collected. We measured dental development using the Demirjian method in 452 DPRs taken at around the age of 10 in participants born between 1960 and 1968.

To extend our research on disturbances of dental development, we collected data from clinical samples (Figure 1.6.2). The individuals included in our studies visited the Department of Oral & Maxillofacial Surgery, Special Dental Care and Orthodontics in Erasmus University Medical Center (UMC), Rotterdam, the Netherlands; the Department of Medical Genetics of the University Medical Center Utrecht, Utrecht, the Netherlands; the Department of Special Dental Care and the Department of Orthodontics and Craniofacial Biology in Radboud University Medical Centre, Nijmegen, the Netherlands; Heerenveen Orthodontic Practice (OP), Heerenveen, the Netherlands and Dent-Ital clinic, Durrës, Albania.

Figure 1.6.1. Design and selected data-collection in the Generation R Study

<table>
<thead>
<tr>
<th>Total Cohort (N=9,778)</th>
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<td>Enrolment during pregnancy (N=8,880) and at birth (N=898)</td>
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<tr>
<th>Measurements during pregnancy (N=8,880)</th>
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<tr>
<td>Questionnaires: maternal diet, parental health, socio-demographic and lifestyle factors</td>
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<td>Biological samples: maternal blood samples (in early and mid-pregnancy)</td>
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<th>Measurements at birth (N=9,749)</th>
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<td>Midwife and hospital data: pregnancy and neonatal complications, birth outcomes</td>
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<td>Biological samples: cordblood samples</td>
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<th>Measurements at preschool period (N=7,893)</th>
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<td>Physical examinations: child anthropometrics, body composition</td>
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<td>Biological samples: child blood samples</td>
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<tr>
<th>Measurements at childhood period (N=8,305)</th>
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<td>Dental development (N=4,447): Stages of development for the left mandibular teeth, dental age</td>
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Figure 1.6.2. Data collection from the medical centers and dental clinics

<table>
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<th>Measurements of dental development in children and adolescents (6-16 years old)</th>
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<td>Isolated oligodontia</td>
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<td>Radboud UMC (N=43)</td>
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<td>Ectodermal dysplasia</td>
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<td>Radboud UMC (N=12)</td>
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<td>Dental Caries</td>
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<td>Dent-Ital clinic (N=118)</td>
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REFERENCES

42. Tucker AS, Headon DJ, Courtney JM, Overbeek P, Sharpe PT. The activation level of the TNF family receptor, Edar, determines cusp number and tooth number during tooth development. Dev Biol. 2004;268(1):185-94.