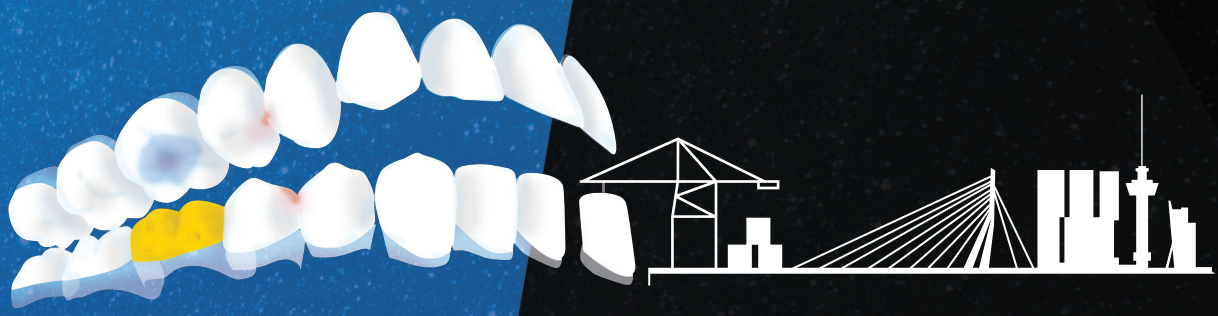
Advances in Epidemiological Research of Dental Enamel Hypomineralization and Dental Caries

J.T. van der Tas



Advances in Epidemiological Research of Dental Enamel Hypomineralization and Dental Caries

Justin van der Tas

Acknowledgements

The general design of the Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam, the Netherlands. Organization for Health Research and Development (ZonMw), the Netherlands Organisation for Scientific Research (NWO), the Ministry of Health, Welfare and Sport and the Ministry of Youth and Families.

The work presented in this thesis was conducted in the Generation R Study Group, in close collaboration with the Departments of Epidemiology and the Department of Oral and Maxillofacial Surgery, Special Dental Care and Orthodontics, Erasmus University Medical Centre Rotterdam, The Netherlands.

Printing and distribution of this thesis was kindly supported by the Generation R Study, ChipSoft B.V., Nederlandse Vereniging voor Mondziekten, Kaak- en Aangezichtschirurgie and Materialise NV.

MKA

Cover	Paul Stoutjesdijk
Layout	Renate Siebes | Proefschrift.nu
Printing	Proefschriftmaken.nl
ISBN	978-94-6380-948-1

Advances in Epidemiological Research of Dental Enamel Hypomineralization and Dental Caries

Vorderingen in epidemiologisch onderzoek van tandglazuur hypomineralisatie en tandcariës

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. R.C.M.E. Engels

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op
woensdag 4 november 2020 om 11:30 uur

door

Justin Thomas van der Tas

geboren te Rotterdam

Erasmus University Rotterdam

Erasmus

Promotiecommissie

Promotoren:	Prof.dr. E.B. Wolvius Prof. dr. F. Rivadeneira Ramírez
Overige leden:	Prof.dr. J.M. ten Cate Prof.dr. V.W.V. Jaddoe Prof.dr. H. Raat
Copromotor:	Dr. L. Kragt

Paranimfen:	Bart van Dijk Jeffrey Hoek

Contents

1

General introduction

Oral health is determined by multiple factors and biological processes, initiating from embryonic development and extending throughout the life course. Teeth already start to develop in an embryo of approximately 45 days old [1]. Before eruption of the first teeth at the age of six months, the teeth undergo several stages in which dental enamel is formed [2]. Dental enamel is a calcified tissue consisting of hydroxyapatite crystals and is the hardest tissue in the human body [1]. Despite its hardness, however, dental enamel may be affected by several entities. Molar Incisor Hypomineralization (MIH) and Hypomineralized Second Primary Molars (HSPM) for example, are two diseases in which the dental enamel shows mineralization defects after eruption. To date, there is limited knowledge about the etiology of MIH and HSPM. They both lead to more vulnerable dental enamel which leads to a higher risk of caries development [3, 4]. Dental caries is the other, much more common, disease of the enamel in which demineralization by external influences is the problem. Unlike MIH and HSPM, the etiology of dental caries has been well established, which have led to the development of effective preventive strategies such as fluoridation of toothpastes or the Nexø-method in which the focus lies on the patient's responsibility for self-care and the interval between periodic visits is based on the patient's level of self-care, the eruption period of the permanent teeth and the caries progression within the dentition and especially in the permanent first molars [5, 6]. However, up to now the prevalence of caries remains relatively high, with approximately 24% of all five-year-old children in the Netherlands [7]. Identification of risk groups and new risk factors may be a promising approach to facilitate more effective prevention. This thesis will focus on MIH, HSPM, and dental caries, tooth disorders in which the dental enamel plays a central role.

MIH and HSPM

Generally, MIH can be seen as enamel hypomineralization of the first permanent molars and/or incisors and HSPM as enamel hypomineralization of the second primary molars [8-10]. In some cases, the tips of permanent canine cusps and/or permanent premolars are affected as well. Enamel hypomineralization of these other sites, however, is little studied. Therefore, MIH is still defined as "enamel hypomineralization from systemic origin of one to four first permanent molars and is frequently associated with affected incisors as well" [10].

Clinically, MIH and HSPM affected teeth can be recognized by demarcated opacities with a white, yellow or brown aspect (Figure 1.1). Among oral healthcare professionals in the Netherlands, MIH and HSPM are often referred to as "cheese molars", because of their visual resemblance to cheese. The diagnosis of enamel

hypomineralization in most research is based on the European Academy of Pediatric Dentistry (EAPD)-criteria for MIH (Table 1.1) [10, 11]. Elfrink et al. made an adaptation to these criteria with regard to HSPM [12]. Furthermore, many researchers added a distinction between mild and severe hypomineralization [11]. The mild form only shows opacities and severe hypomineralization includes posteruptive enamel loss, atypical caries lesions, atypical restorations and/or atypical extractions [12].

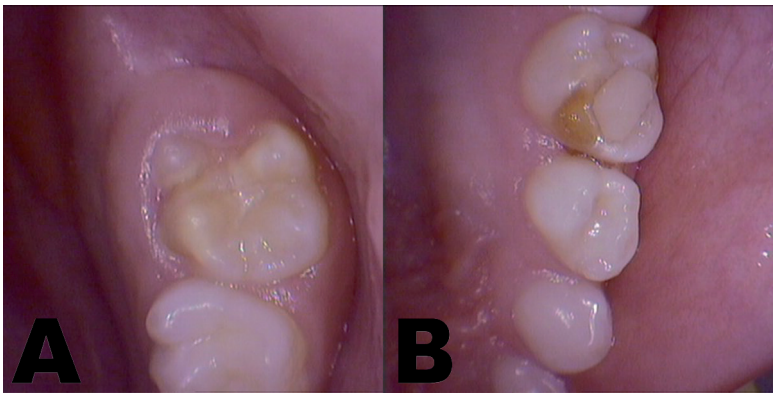


Figure 1.1. A: MIH of the first permanent molar in the right upper jaw showing yellow opacities over the entire occlusal surface. **B:** HSPM of the second primary molar in the right upper jaw showing a big occlusal restoration and a brown/yellow opacity on the mesiopalatal cusp.

Table 1.1. EAPD criteria for scoring HSPM and MIH on intra-oral photographs (Elfrink et al. 2009; Weerheijm et al. 2003)

Mild:	Opacity: A defect that changes the translucency of the enamel, variable in degree. The defective enamel is of normal thickness with a smooth surface and can be white, yellow or brown in color. The demarcated opacity is not caused by caries, ingestion of excess fluoride during tooth development or amelogenesis imperfect etc.
Severe:	Posteruptive enamel loss: A defect that indicates surface enamel loss after eruption of the tooth, e.g., hypomineralization related attrition. Enamel loss due to erosion was excluded, and/or
	Atypical caries: The size and form of the caries lesion do not match the present caries distribution in the child's mouth, and/or
	Atypical restoration: The size and form of the restoration do not match the present caries distribution in the child's mouth, and/or
	Atypical extraction: Absence of a molar that does not fit in the dental development and caries pattern of the child.

The burden of MIH is high with a calculated estimate of 878 million affected people worldwide, roughly 11% of the complete world population [13]. Within the Netherlands the reported prevalence of MIH is comparable and is somewhere around

10% [14-16]. The prevalence of HSPM in Dutch children is calculated between 5% and 10% [17, 18]. As a result of the weaker enamel, they are more susceptible to dental caries, dental pulp inflammation, pain and/or hypersensitivity [4, 8, 13, 19, 20]. This leads to extra usage of healthcare, probable extra healthcare costs and a decreased oral health-related quality of life [13, 21, 22]. Therefore, accurate management of MIH and HSPM in patients is important and prevention is desirable, but not possible yet.

Dental caries

Dental caries is defined as "localized destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates" [23]. Hence, caries is not caused by intrinsic factors, but the biofilm in which these bacteria fermentate carbohydrates.

The global burden of dental caries in both the primary and permanent dentition is tremendously high with almost three billion affected people [24]. Dental caries not only causes tooth pain, but also leads to significant disease burden in a population [25]. In children, caries decreases theirs and their parents' quality of life, and produces considerable health costs on the short and long term [26-28]. Therefore, prevention of this disease is desirable.

In the last decades, great steps have already been taken in caries prevention. Epidemiologic research showed a great global decline of the caries prevalence among 5- and 12-year-olds [29]. A similar trend had been observed in the Netherlands [30, 31]. One of the major causes for this decline was the introduction of fluoride-containing toothpastes in the seventies [32]. The preventive fraction was calculated to be between 23% and 36%, dependent of the fluoride concentration used [33]. Another contributing factor is the better oral health behavior of children in terms of more accurate biofilm control and less sugar intake [29, 34]. Still, there is potential for even more effective prevention. Especially in certain risk groups within the population.

Tooth development

Knowledge about the development of teeth gives clearer insight behind the etiological processes of dental enamel hypomineralization and dental caries. Therefore, the odontogenesis is presented within this paragraph before other research on the etiology of both enamel diseases is discussed.

Dental enamel formation is a highly complex process starting with the formation of the dental lamina at age 42–48 days of the embryo [1]. Within this lamina a dental

placode develops from which tooth development proceeds in three consecutive stages: the bud-, cap-, and bell stage [1]. In the bud stage the epithelial cells of the dental lamina invaginate into the ectomesenchyme of the jaw. During transformation to the cap stage, around this invagination, the cellular density of the ectomesenchyme increases [1]. The tooth bud grows larger around the condensed ectomesenchyme and forms a cap of epithelial cells [1]. The "cap" forms the enamel organ, the condensed ectomesenchyme the dental papilla, and around those two another structure develops; the dental follicle [1]. As histodifferentiation within the tooth germ proceeds, the bell stage is reached at the moment when the enamel organ morphology resembles a bell [1]. The deciduous teeth already reach the bell stage at the fetal age of fourteen weeks [1]. Eighteen weeks later, still in utero, also the permanent first molars reach the bell stage [1]. After this, the crown stage starts in which the amelogenesis starts from the ameloblasts within the enamel organ and dentinogenesis from the odontoblasts within the dental papilla [1]. All distinct stages of dentinogenesis are shown in Figure 1.2.

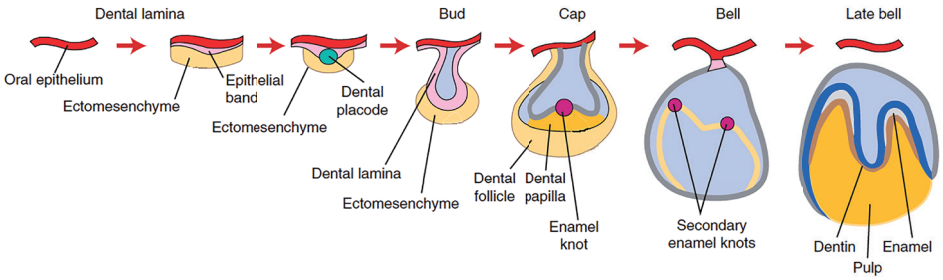


Figure 1.2. The distinct stages of dentinogenesis.
Adapted from Nanci, *ten Cate's Oral Histology 2012*, with permission from the publisher and author.

Shortly after the first dentin is formed by the odontoblasts, the ameloblasts of the inner enamel epithelium begin to secrete enamel proteins [1]. In this secretory phase, the ameloblasts develop a cytoplasmatic extension called the Tomes' process [1]. The Tomes' process plays a crucial role in the characteristic orientation of the enamel crystallites into enamel rods and interrod enamel [1]. When the enamel layer reaches its full thickness, the ameloblasts lose their Tomes' process and follow cyclical morphological changes of ruffle-ended cells or smooth-ended cells [1]. They either are able to excrete inorganic material or to take up protein fragments and water, respectively [1]. Both with the purpose to allow for crystal growth at the expense of enamel proteins and fluid that were secreted during the secretory phase [1]. This phase is called the maturation phase. Within this phase the dental enamel hardens to the hardest calcified

material of the body, with a mineral concentration of 96%, composed of hydroxyapatite crystals substituted with carbonate ions [1].

Complete crown formation of the deciduous teeth takes up to infancy. The central incisors are completed first at the age of two months and the second deciduous molars are finished last at the age of eleven months [2]. Crown formation of the permanent teeth takes even longer up to childhood and adolescence. In the permanent dentition the central incisors are finished first at the age of 5 years, up to the age of 14 for the third molars [2].

Etiology of dental enamel hypomineralization

Since enamel formation of the first permanent molars already starts at the embryo's age of 32 weeks and takes up to the age of 4 years, especially this time period could be interesting to study any potential risk factors for MIH. Pioneers who investigated the etiology of MIH, found various risk factors for MIH such as environmental conditions, respiratory tract problems early in life, perinatal complications, dioxins, calcium and phosphate metabolic disorders, drug use, and frequent childhood diseases [35-39]. However, the latest systematic review on the etiology of MIH only found substantial evidence for early childhood illness to be associated with the presence of MIH [40]. Clearly, the etiology of MIH has not been unraveled yet and seems to be caused by a complex interplay of multiple interruptive factors.

In the search for the etiology of HSPM, causative factors should be sought in the period between the eighteenth week in utero and the age of eleven months. The period in which the second primary molars are formed. However, literature about the etiology of HSPM is even scarcer than for MIH. MIH and HSPM seem to have some shared risk factors, i.e. a high experience of medical conditions around the perinatal period and any fever in the child's first year of life [15, 41]. Elfrink et al. also identified maternal alcohol consumption during pregnancy and low birth weight as risk factors for HSPM [15]. Interestingly, having HSPMs is found to be a risk factor for getting MIH which is indicative for shared risk factors [42]. This is important in the follow-up of children affected by HSPM. Recently, a prospective twin study was published about the etiology of HSPM [43]. They added infantile eczema, vitamin D at birth, in vitro fertilization (IVF), maternal smoking beyond the first trimester of pregnancy, and high socioeconomic position (SEP) to the list of risk factors for HSPM [43]. Genetic influence appeared to be limited in the development of HSPM [43].

Etiology of dental caries

As little is known about the etiology of dental enamel hypomineralization, as much is known about the etiology of dental caries. A review of Kidd and Fejerskov presented an informative overview of the histopathology of dental caries [44]. The key element that constitutes dental caries is the dental plaque or the dental biofilm [44]. A biofilm is defined as a 3D accumulation of interacting microorganisms attached to a surface, embedded in a matrix of extracellular polymers [45, 46]. The biofilm attaches to the dental pellicle and shows pH-fluctuations under influence of acidic by-products from bacterial fermentation of dietary carbohydrates [44]. If this fluctuation favors a low pH over a high pH, eventually demineralization may take the overhand resulting in a caries

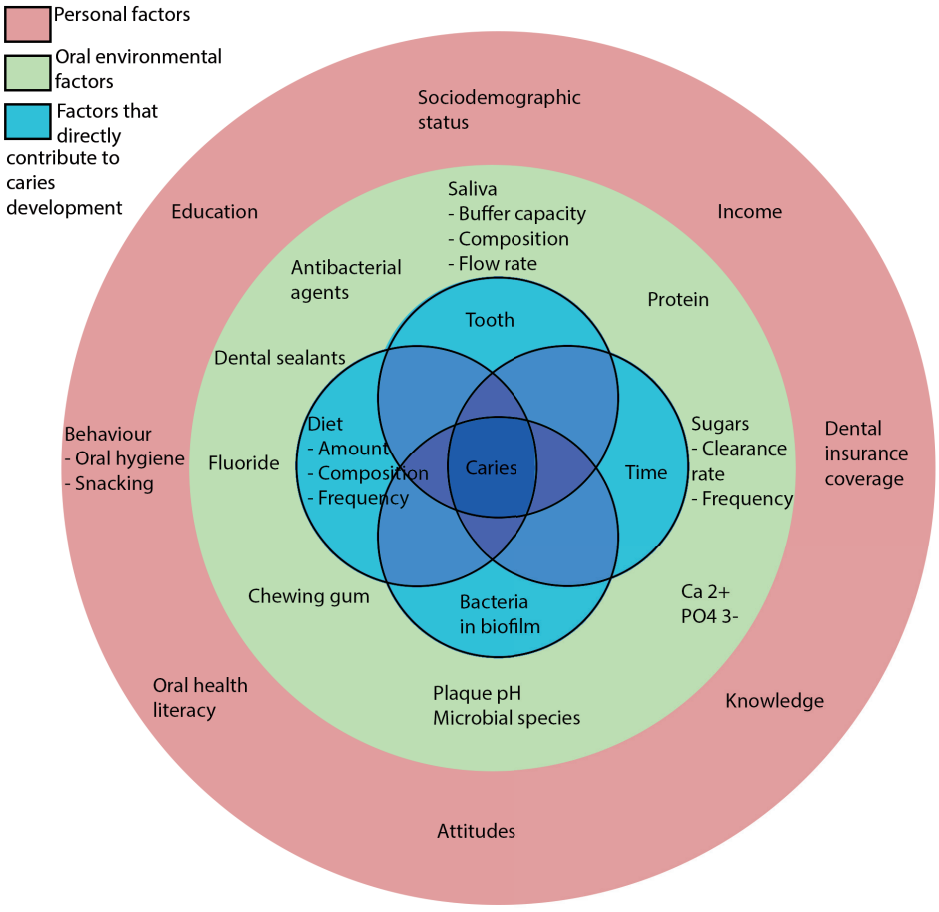


Figure 1.3. The multifactorial origin of dental caries.
Adapted from Fejerskov and Manji, 1990 with permission from the publisher and author.

lesion [44]. Hence, caries prevention and control are all about limiting the magnitude of the pH fluctuations within the biofilm [44, 47]. However, this is a multifactorial process and therefore difficult to control. Most important factors contributing to the caries process are diet, fluoride use, and salivary secretion rate, but many more factors play a role in keeping the equilibrium in favor of remineralization (Figure 1.3) [23].

Sociodemography of dental caries

One group of those factors are personal factors, i.e. ethnic background and SEP [23]. Differences in oral health among ethnic groups have been reported by various studies from the Netherlands and other parts of the world [30, 48-51]. These studies indicated a higher prevalence of caries among children from immigrant or ethnic minority groups. Furthermore, a low SEP was found to be significantly associated with a higher risk of having dental caries in both children and adults [52-57]. In the association between ethnicity and health, SEP might play an important mediating role. The equilibrium of re- and demineralization seems to tilt in favor of demineralization in children from an ethnic minority group and in children from a family with a low SEP.

Genetics and dental caries

Genetic susceptibility is a less common studied risk factor, but the first clue that genes play a role in developing caries was already suspected in an animal model 50 years ago and later twin studies confirmed this finding [58-60]. Heritability was found to play an important role in caries progression and severity in the primary and permanent dentition of children, with heritability estimates (H) ranging between H = 30.0 to H = 36.1 [60]. Only a few genetic loci, however, were associated with dental caries. Then, the first genome wide association studies (GWAS) for dental caries were performed in children and adults [61-64]. Before this thesis, the largest GWAS for dental caries in the permanent dentition was performed in a Hispanic and Latino population (n = 11,754) which reported an association to different genetic loci (NAMPT and BMP7), but without any evidence for replication across all the GWAS efforts, pleading for larger well-powered GWAS [65]. There is still great potential for GWAS in larger populations, because of the assumed modest effects of common genetic variants on caries susceptibility.

Early origin of dental caries

Another factor that may play a role in caries susceptibility is the pregnancy course. Sub-optimal intra-uterine circumstances might influence the process of tooth development in utero by insufficient enamel secretion and maturation negatively. Several studies

already assessed prenatal and early postnatal risk factors for dental caries, but literature has not been conclusive yet. Still, it seems that a trend towards a positive relationship between adverse pregnancy outcome and dental caries exists [66-71].

Oral health assessment in longitudinal studies

Population-based cohort studies offer great opportunities for identification of risk factors for both dental caries and MIH. Such a design is ideal for studying relatively rare diseases as dental enamel hypomineralization, because of the high numbers of included participants within the study but is also suitable for studying the effect of common diseases as dental caries on a population. However, these oral health parameters are rarely included in longitudinal cohort studies. The most prominent reason for the lack of dental research within large scale studies, is the effort, time and cost of the assessment of dental enamel hypomineralization and dental caries. Detection of both conditions is usually performed by oral examination of a dentist or comparable professional [12, 72]. However, in the context of a cohort study, in which multiple outcomes are studied, appointing a dental professional for clinical examination is logistically and financially difficult. Therefore, this thesis will try to provide a convenient solution for digital assessment of both dental enamel hypomineralization and dental caries.

Aims

The overall aim of this thesis is to contribute to the understanding of the etiology, sociodemography, and risk factors of dental enamel hypomineralization and caries by performing several epidemiologic studies. Therefore, the following research questions were formulated that will be answered in the upcoming chapters (Table 1.2):

1. How are bone health and vitamin D-status related to the presence of dental enamel hypomineralization in children?
2. Does vitamin D-status play a mediating role in a possible association between bone health and dental enamel hypomineralization?
3. Which six-year-old children are most prone to have dental caries and what are possible causes of those inequalities?
4. Are (fluorescent) digital photographs a reliable source for oral health assessment in the context of longitudinal cohort studies?

Table 1.2. Main characteristics of all included studies

Chapter	Study sample (N)	Population age	Study design	Main exposure	Main outcome
2.1	6,510	Six-year-olds	Cross-sectional analysis embedded within Generation R	Bone health	Dental enamel hypomineralization
2.2	4,750	Six-year-olds	Longitudinal analysis embedded within Generation R	Vitamin D	Dental enamel hypomineralization
3.1	4,306	Six-year-olds	Cross-sectional analysis embedded within Generation R	Ethnicity	Dental caries
3.2	17,037 13,353	2.5–12 years (primary dentition) 6.0–18 years (permanent dentititon)	Consortium based Genome Wide Association Study	Genotype	Dental caries
3.3	5,189	Six-year-olds	Cross-sectional analysis embedded within Generation R	Socioeconomic Position	Dental caries
3.4	5,323	Six-year-olds	Longitudinal analysis embedded within Generation R	Pregnancy complications	Dental caries
4	113	9.0–18 years	Cross-sectional analysis within a small dental practice	-	Dental caries and dental enamel hypomineralization

Setting

Most studies were conducted within the Generation R Study. This is a population-based prospective cohort study in which children are followed-up from fetal life until young adulthood [73]. The Generation R study includes a population with many different sociocultural and socioeconomic backgrounds, which enables to study the effect of those factors on a certain phenotype. Pregnant women living in Rotterdam, the Netherlands, were eligible for inclusion if the delivery date was expected to be between April 2002 and January 2006. After the inclusion period, 9,749 children were included in the study. More than 95% of those children (n = 9,278) and their parents were invited in early childhood (around the age of five years) to visit the research center for data collection, including intra-oral dental photography. Eventually, 6,690 children visited

the research center of whom intra-oral photographs were made to assess dental caries and dental enamel hypomineralization. Furthermore, two studies were situated in another setting. The genetic studies were carried out in the context of an international consortium including Generation R, which is needed to study the weak genetic effects typically identified by genome-wide association studies. Finally, we performed a study on a photography-based method to assess dental enamel hypomineralization and dental caries in a purely clinical setting.

Outline of the thesis

Chapter 2 focuses on dental enamel hypomineralization. In chapter 2.1 the association between bone mass and dental enamel hypomineralization is studied. In particular we investigate whether a smaller potential of bone mineralization may be associated with dental enamel hypomineralization. Chapter 2.2 elaborates on whether fetal, neonatal, and child vitamin D levels influence the susceptibility of dental enamel hypomineralization.

From Chapter 3 the focus is set on the other enamel disease studied in this thesis, dental caries. Chapter 3.1 sheds light on ethnic disparities in dental caries prevalence among Dutch children. The possible genetic caries risk is the main focus of Chapter 3.2. Chapter 3.3 tries to fill two knowledge gaps; one on the most important proxy of SEP in the association between SEP and dental caries and the other on the demographic distribution of caries prevalence within the study. In chapter 3.4 the possible influence of a complicated pregnancy course on later oral health is studied. Sociodemographic and prenatal factors influencing the risk of dental enamel hypomineralization were already studied by a colleague within the same population, and therefore not included in this thesis [15, 74].

Chapter 4 focuses on the possibility of fluorescent and white light photographs for the purpose of dental enamel hypomineralization and dental caries assessment in longitudinal population-based cohort studies, which is unstudied yet.

Finally, Chapter 5 summarizes our main findings, compares them with the existing literature, discusses limitations and strengths of our studies, and provides suggestions for future research.

References

[1] A. Nanci. Ten Cate's Oral Histology. 8th ed: Elsevier - Health Sciences Division 2012. p. 122-148.

[2] W.R. Profitt, H.W. Fields, D.M. Sarver. Contemporary Orthodontics. 5th ed: Elsevier - Health Sciences Division; 2012. p. 67.

[3] K. Weerheijm, B. Jälevik, S. Alaluusua, Molar-incisor hypomineralisation, Caries Res 35(5) (2001) 390-391.

[4] M.E. Elfrink , A.A. Schuller, J.S. Veerkamp, J.H. Poorterman, H.A. Moll, B.J. ten Cate, Factors increasing the caries risk of second primary molars in 5-year-old Dutch Children, Int J Paediatr Dent 20(2) (2010) 151-157.

[5] T. Walsh, H.V. Worthington, A.M. Glenny, V.C.C. Marinho, A. Jeroncic, Fluoride toothpastes of different concentrations for preventing dental caries, Cochrane Database of Systematic Reviews (3) (2019).

[6] J.H. Vermaire, Application of the Nexø method in a general dental practice in the Netherlands: 6-year results of a RCT, International Journal of Dental Hygiene 16(3) (2018) 419-425.

[7] A.A. Schuller, J.H. Vermaire, G.H.W. Verrips, Kies-voor-Tanden Study: the incidence of caries among 5-year-olds in the Netherlands, Ned Tijdschr Tandheelkd 126(7-8) (2019) 399-407.

[8] N.A. Lygidakis, F. Wong, B. Jälevik, A.M. Vierrou, S. Alaluusua, I. Espelid, Best Clinical Practice Guidance for clinicians dealing with children presenting with Molar-Incisor-Hypomineralisation (MIH), European Archives of Paediatric Dentistry 11(2) (2010) 75-81.

[9] M. Elfrink, A. Schuller, K. Weerheijm, J. Veerkamp, Hypomineralized second primary molars: prevalence data in Dutch 5-year-olds., Caries Res 42(4) (2008) 282-285.

[10] K.L. Weerheijm, M. Duggal, I. Mejàre, L. Papagiannoulis, G. Koch, L.C. Martens, et al., Judgement criteria for molar incisor hypomineralisation (MIH) in epidemiologic studies: a summary of the European meeting on MIH held in Athens, 2003, Eur J Paediatr Dent 4(3) (2003) 110-113.

[11] E. Allam, A. Ghoneima, K. Kula, Definition and scoring system of molar incisor hypomineralization: a review., Dent Oral Craniofac Res 3(2) (2017) 1-9.

[12] M.E. Elfrink, J.S. Veerkamp, I.H. Aartman, H.A. Moll, J.M. Ten Cate, Validity of scoring caries and primary molar hypomineralization (DMH) on intraoral photographs, JM Eur Arch Paediatr Dent 10(Suppl. 1) (2009) 5-10.

[13] F. Schwendicke, K. Elhennawy, S. Reda, K. Bekes, D.J. Manton, J. Krois, Global burden of molar incisor hypomineralization, Journal of Dentistry 68 (2018) 10-18.

[14] L. Jasulaityte, K. Weerheijm, J. Veerkamp, Prevalence of molarincisor-hypomineralisation among children participating in the Dutch National Epidemiological Survey (2003), Eur Arch Paediatr Dent 9 (2008) 218-223.

[15] M.E. Elfrink, H.A. Moll, J.C. Kiefte-de Jong, V.W. Jaddoe, A. Hofman, J.M. ten Cate, et al., Pre- and postnatal determinants of deciduous molar hypomineralisation in 6-year-old children. The generation R study, PLoS One 9(7) (2014) e91057.

[16] K.L. Weerheijm, H.J. Groen, V.E. Beentjes, J.H. Poorterman, Prevalence of cheese molars in eleven-year-old Dutch children, ASDC J Dent Child 68(4) (2001) 259-262.

[17] M.E. Elfrink, A.A. Schuller, K.L. Weerheijm, J.S. Veerkamp, Hypomineralized second primary molars: prevalence data in Dutch 5-year-olds., Caries Res 42(4) (2008) 282-285.

[18] M.E. Elfrink, J.M. ten Cate, V.W. Jaddoe, A. Hofman, H.A. Moll, J.S. Veerkamp, Deciduous molar hypomineralization and molar incisor hypomineralization., J Dent Res 91(6) (2012) 551-555.

[19] G.C. Americano, P.E. Jacobsen, V.M. Soviero, D. Haubek A systematic review on the association between molar incisor hypomineralization and dental caries, Int J Paediatr Dent 27(1) (2017) 11-21.

[20] K.L. Weerheijm, B. Jälevik, S. Alaluusua, Molar-incisor hypomineralisation, Caries Res 35(5) (2001) 390-391.
[21] N.B. Dantas-Neta, L.F. Moura, P.F. Cruz, M.S. Moura, S.M. Paiva, C.C. Martins, et al., Impact of molar-incisor hypomineralization on oral health-related quality of life in schoolchildren, Braz Oral Res 30(1) (2016) e117.
[22] L.M. Velandia, L.V. Álvarez, L.P. Mejía, M.J. Rodríguez, Oral health-related quality of life in Colombian children with Molar-Incisor Hypomineralization, Acta Odontol Latinoam 31(1) (2018) 38-44.
[23] R.H. Selwitz, A.I. Ismail, N.B. Pitts, Dental caries, Lancet 369(9555) (2007) 51-59.
[24] T. Vos, R. Lozano, M. Naghavi, A.D. Flaxman, C.J. Murray, and Collaborators, Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013, Lancet 386(9995) (2015) 743-800.
[25] W. Marcenes, N.J. Kassebaum, E. Bernabé, A. Flaxman, M. Naghavi, A. Lopez, et al., Global burden of oral conditions in 1990-2010: a systematic analysis, J Dent Res 92(7) (2013) 592-597.
[26] M.C. Gomes, T.C. Pinto-Sarmento, E.M. Costa, C.C. Martins, A.F. Granville-Garcia, S.M. Paiva, Impact of oral health conditions on the quality of life of preschool children and their families: a cross-sectional study, Health Qual Life Outcomes 12 (2014) 55.
[27] L. Kragt, J.T. van der Tas, H.A. Moll, M.E. Elfrink, V.W. Jaddoe, E.B. Wolvius, et al., Early Caries Predicts Low Oral Health-Related Quality of Life at a Later Age, Caries Res 50(5) (2016) 471-479.
[28] L.C.J. Slobbe, J.M. Smit, J. Groen, M.J.J.C. Poos, G.J. Kommer. Costs of illness in the Netherlands 2007; trends in Dutch health expenditures 1999-2010. 2011 [cited 31.10.2016]; Available from: www.rivm.nl/dsresource?objectid=rivmp:61294&type=org&disposition=inline&ns_nc=1
[29] J.E. Frencken, P. Sharma, L. Stenhouse, D. Green, D. Laverty, T. Dietrich, Global epidemiology of dental caries and severe periodontitis – a comprehensive review, Journal of Clinical Periodontology 44(S18) (2017) S94-S105.
[30] G.J. Truin, A.A. Schuller, J.H. Poorterman, J. Mulder, Secular trends of caries prevalence among 6 and 12 year-old youths in the Netherlands, Ned Tijdschr Tandheelkd 117(3) (2010) 143-147.
[31] A.A. Schuller, P. van Dommelen, J.H. Poorterman, Trends in oral health in young people in the Netherlands over the past 20 years: a study in a changing context, Community Dent Oral Epidemiol 42(2) (2014) 178-184.
[32] C. van Loveren, Preventive dentistry 1. Fluoride toothpaste, the cornerstone of caries prevention, Ned Tijdschr Tandheelkd 123(12) (2016) 601-608.
[33] T. Walsh, H.V. Worthington, A.M. Glenny, P. Appelbe, V.C. Marinho, X. Shi, Fluoride toothpastes of different concentrations for preventing dental caries in children and adolescents, Cochrane Database Syst Rev (1) (2010).
[34] J.J. Murray, C.R. Vernazza, R.D. Holmes, Forty years of national surveys: An overview of children's dental health from 1973-2013, Bdj 219 (2015) 281.
[35] F. Crombie, D. Manton, N. Kilpatrick, Aetiology of molar-incisor hypomineralization: a critical review, Int J Paediatr Dent 19(2) (2009) 73-83.
[36] K.L. Weerheijm, Molar incisor hypomineralization (MIH): clinical presentation, aetiology and management, Dent Update 31(1) (2004) 9-12.
[37] S.M. Allazzam, S.M. Alaki, O.A.S. El Meligy, Molar Incisor Hypomineralization, Prevalence, and Etiology, International Journal of Dentistry 2014 (2014) 234508.

[38] C. Serna, A. Vicente, C. Finke, A.J. Ortiz, Drugs related to the etiology of molar incisor hypomineralization: A systematic review, The Journal of the American Dental Association 147(2) (2016) 120-130.
[39] R. Whatling, J.M. Fearne, Molar incisor hypomineralization: a study of aetiological factors in a group of UK children, International Journal of Paediatric Dentistry 18(3) (2008) 155-162.
[40] M.J. Silva, K.J. Scurrah, J.M. Craig, D.J. Manton, N. Kilpatrick, Etiology of molar incisor hypomineralization - A systematic review, Community Dent Oral Epidemiol 44(4) (2016) 342-353.
[41] A.M. Ghanim, M.V. Morgan, R.J. Marino, D.L. Bailey, D.J. Manton, Risk factors of hypomineralised molars in a group of Iraqi schoolchildren, Eur Arch Paediatr Dent 13(3) (2012) 111–118.
[42] E. Garot, A. Denis, Y. Delbos, D. Manton, M. Silva, P. Rouas, Are hypomineralised lesions on second primary molars (HSPM) a predictive sign of molar incisor hypomineralisation (MIH)? A systematic review and a meta-analysis, Journal of Dentistry 72 (2018) 8-13.
[43] M.J. Silva, N.M. Kilpatrick, J.M. Craig, D.J. Manton, P. Leong, D. Burgner, et al., Etiology of Hypomineralized Second Primary Molars: A Prospective Twin Study, Journal of Dental Research (2018) 0022034518792870.
[44] E.A.M. Kidd, O. Fejerskov, What Constitutes Dental Caries? Histopathology of Carious Enamel and Dentin Related to the Action of Cariogenic Biofilms, Journal of Dental Research 83(1_suppl) (2004) 35-38.
[45] P.D. Marsh, Microbial Ecology of Dental Plaque and its Significance in Health and Disease, Advances in Dental Research 8(2) (1994) 263-271.
[46] H. Meyer-Lueckel, S. Paris, K.R. Ekstrand. Caries Management - Science and Clinical Practice. 1st ed: Thieme Publishing Group; 2013. p. 14.
[47] E.A.M. Kidd, O. Fejerskov, Changing concepts in cariology: forty years on, Dental Update 40(4) (2013) 277-286.
[48] A.A. Schuller, I.P.F. van Kempen, J.H.G. Poorterman, G.H.W. Verrips. Kies voor tanden: Een onderzoek naar mondgezondheid en preventief tandheelkundig gedrag van jeugdigen. 2013 [cited 05.09.2016]; Available from: www.tno.nl/media/1167/kiesvoortanden_tnols2013r10056.pdf
[49] C.C. Guarnizo-Herreño, G.L. Wehby, Children's Dental Health, School Performance and Psychosocial Well-Being, The Journal of pediatrics 161(6) (2012) 1153-1159.e1152.
[50] G. Matsuo, R.G. Rozier, A.M. Kranz, Dental Caries: Racial and Ethnic Disparities Among North Carolina Kindergarten Students, American Journal of Public Health 105(12) (2015) 2503-2509.
[51] T.I. Wigen, N.J. Wang, Caries and background factors in Norwegian and immigrant 5-year-old children, Community Dentistry and Oral Epidemiology 38(1) (2010) 19-28.
[52] C. Adam, A. Eid, P.J. Riordan, M. Wolikow, F. Cohen, Caries experience in the primary dentition among French 6-year-olds between 1991 and 2000, Community Dent Oral Epidemiol 33(5) (2005) 333-340.
[53] J.C. Carvalho, W. D'Hoore, J.P. van Nieuwenhuysen, Caries decline in the primary dentition of Belgian children over 15 years, Community Dent Oral Epidemiol 32(4) (2004) 277-282.
[54] L.B. Christensen, S. Twetman, A. Sundby, Oral health in children and adolescents with different socio-cultural and socio-economic backgrounds, Acta Odontol Scand 68(1) (2010) 34-42.
[55] M. Maliderou, S. Reeves, C. Noble, The effect of social demographic factors, snack consumption and vending machine use on oral health of children living in London, Br Dent J 201(7) (2006) 441-444.

[56] K. Pieper, S. Dressler, M. Heinzel-Gutenbrunner, A. Neuhäuser, M. Krecker, K. Wunderlich, et al., The influence of social status on pre-school children's eating habits, caries experience and caries prevention behavior, Int J Public Health 57(1) (2012) 207-215.
[57] F. Schwendicke, C. Dörfer, P. Schlattmann, L. Foster Page, W. Thomson, S. Paris, Socioeconomic inequality and caries: a systematic review and meta-analysis., J Dent Res 94(1) (2015) 10-18.
[58] J.C. Boraas, L.B. Messer, M.J. Till, A Genetic Contribution to Dental Caries, Occlusion, and Morphology as Demonstrated by Twins Reared Apart, Journal of Dental Research 67(9) (1988) 1150-1155.
[59] C.S. Chung, R.H. Larson, Factors and Inheritance of Dental Caries in the Rat, Journal of Dental Research 46(3) (1967) 559-564.
[60] W.A. Bretz, P.M. Corby, N.J. Schork, M.T. Robinson, M. Coelho, S. Costa, et al., Longitudinal Analysis of Heritability for Dental Caries Traits, Journal of Dental Research 84(11) (2005) 1047-1051.
[61] Z. Zeng, E. Feingold, X. Wang, D.E. Weeks, M. Lee, K.T. Cuenco, et al., Genome-wide association study of primary dentition pit-and-fissure and smooth surface caries, Caries Research 48(4) (2014) 330-338.
[62] J.R. Shaffer, X. Wang, E. Feingold, M. Lee, F. Begum, D.E. Weeks, et al., Genome-wide Association Scan for Childhood Caries Implicates Novel Genes, Journal of Dental Research 90(12) (2011) 1457-1462.
[63] X. Wang, J.R. Shaffer, Z. Zeng, F. Begum, A.R. Vieira, J. Noel, et al., Genome-wide association Scan of dental caries in the permanent dentition, BMC Oral Health 12 (2012) 57-57.
[64] Z. Zeng, J.R. Shaffer, X. Wang, E. Feingold, D.E. Weeks, M. Lee, et al., Genome-wide Association Studies of Pit-and-Fissure- and Smooth-surface Caries in Permanent Dentition, Journal of Dental Research 92(5) (2013) 432-437.
[65] J. Morrison, C.C. Laurie, M.L. Marazita, A.E. Sanders, S. Offenbacher, C.R. Salazar, et al., Genome-wide association study of dental caries in the Hispanic Communities Health Study/Study of Latinos (HCHS/SOL), Human Molecular Genetics 25(4) (2016) 807-816.
[66] H. Yokomichi, T. Tanaka, K. Suzuki, T. Akiyama, G. Okinawa Child Health Study, Z. Yamagata, Macrosomic Neonates Carry Increased Risk of Dental Caries in Early Childhood: Findings from a Cohort Study, the Okinawa Child Health Study, Japan, PLoS One 10(7) (2015).
[67] E. Bernabé, H. MacRitchie, C. Longbottom, N.B. Pitts, W. Sabbah, Birth Weight, Breastfeeding, Maternal Smoking and Caries Trajectories, J Dent Res 96(2) (2016) 171-178.
[68] A. Nirunsittirat, W. Pitiphat, C. McKinney, T.A. DeRouen, N. Chansamak, O. Angwaravong, et al., Adverse Birth Outcomes and Childhood Caries: A Cohort study, Community Dent Oral Epidemiol 44(3) (2016) 239-247.
[69] M.C. Saraiva, H. Bettiol, M.A. Barbieri, A.A. Silva, Are intrauterine growth restriction and preterm birth associated with dental caries?, Community Dent Oral Epidemiol 35(5) (2007) 364-376.
[70] J.D. Shulman, Is There an Association between Low Birth Weight and Caries in the Primary Dentition?, Caries Res 39(3) (2005) 161-167.
[71] K. Tanaka, Y. Miyake, Low birth weight, preterm birth or small-for-gestational-age are not associated with dental caries in young Japanese children, BMC Oral Health 14 (2014) 38-38.
[72] T. Gimenez, C. Piovesan, M.M. Braga, D.P. Raggio, C. Deery, D.N. Ricketts, et al., Visual Inspection for Caries Detection: A Systematic Review and Meta-analysis, J Dent Res 94(7) (2015) 895-904.
[73] M.N. Kooijman, C.J. Kruithof, C.M. van Duijn, O.H. Franco, M.H. van IJzendoorn, J.C. de Jongste, et al., The Generation R Study: design and cohort update 2017, Eur J Epidemiol 31(12) (2016) 1243-1264.
[74] M.E. Elfrink , B.J. ten Cate, H.A. Moll, J.S. Veerkamp. Deciduous molar hypomineralisation, its nature and nurture: Faculty of Dentistry (ACTA); 2012.

2

Dental enamel hypomineralization

Chapter 2.1

Association between bone mass and dental hypomineralization

Justin T. van der Tas | Marlies E. Elfrink | Strahinja Vucic | Denise H. Heppe | Jaap S. Veerkamp | Fernando Rivadeneira | Henriëtte A. Moll | Eppo B. Wolvius

Journal of Dental Research, 2016, 95(4), 395-401

Abstract

The aim of this study was to examine the association between the bone mass (Bone Mineral Content) and hypomineralized second primary molars (HSPM)/ molar incisor hypomineralization (MIH) in six-year-old children. This cross-sectional study was embedded in the Generation R Study, a population-based, prospective cohort study from fetal life until adulthood in Rotterdam, The Netherlands. The European Academy of Pediatric Dentistry (EAPD) criteria were used to score the intra-oral photographs on the presence or absence of HSPM and MIH. Bone mass was measured using a Dual-energy X-ray Absorptiometry scan (DXA-scan). Intra-oral photographs and DXA-scans were available in 6,510 six-year-old children. Binary logistic regression models were used to study the association between the bone mass and HSPM/MIH. In total, 5,586 children had their second primary molars assessed and a DXA-scan made, 507 children were diagnosed with HSPM. Of 2,370 children with data on their permanent first molars 203 were diagnosed with MIH. In the fully adjusted model, children with lower Bone Mineral Content (BMC) (corrected for bone area) were more likely to have HSPM (OR 1.13, 95%CI 1.02–1.26 per 1 SD decrease). A lower BMC (corrected for bone area) was not associated with MIH (OR 1.02, 95%CI 0.87–1.20 per 1 SD decrease). We observed a negative association between BMC (corrected for bone area) and HSPM. No association was found between BMC (corrected for bone area) and MIH. Future research should focus on investigating the mechanism underlying the negative association between the bone mass and HSPM. Our study, in a large population of six-year-old children, adds the finding that BMC (corrected for bone size) is associated with hypomineralized second primary molars, but not with molar incisor hypomineralization in childhood.

Introduction

Molar incisor hypomineralization (MIH) is defined as hypomineralization of systemic origin of 1 to 4 permanent first molars and it is frequently associated with affected incisors of the upper jaw and, more rarely, of the lower jaw [1]. This qualitative enamel defect, which can be seen as demarcated opacities, can also be found in second primary molars [2]. This defect has been defined as hypomineralized second primary molars (HSPM) [3]. Hypomineralization of teeth can be effectively measured using quantitative backscattered electron imaging (qBEI) of tooth biopsies, but is generally diagnosed by just the clinical view of a dentist [4]. Both MIH and HSPM are risk factors for caries [1, 5]. Moreover, a recent study in Generation R has shown that children with HSPM are at risk of developing MIH [6]. Two recent reviews on the risk factors of MIH identified several factors related to the occurrence of MIH such as exposure to polychlorinated biphenyls (PCBs)/dioxins, pre-, peri- and neonatal complications, childhood malnutrition, common childhood illnesses and/or their treatment, being part of a medically compromised population and genetic susceptibility [7, 8]. In addition, they have suggested similar risk factors for HSPM that occur earlier in life [9]. However, the exact etiology of MIH and HSPM remains unclear. As the enamel of molars with HSPM and MIH contains less mineral content compared with sound molars, enamel maturation should be taken into consideration for further identification of possible risk factors for HSPM and MIH [10, 11]. Also, the role of minerals such as calcium and bicarbonate needs to be elucidated. Evidently, the metabolism of minerals, such as calcium and phosphorus, plays a crucial role in enamel maturation [12]. The process of enamel maturation mimics bone remodeling, with calcium and phosphorus being the main bone-forming minerals [13]. Contrary to enamel formation bone tissue is remodeled continuously throughout life. Enamel formation of a first permanent molar starts at the gestational age of eight months and is completed by the age of four years [8]. Enamel of the second primary molar is developed earlier and begins at the 18th week of gestation until the age of one [14]. However, since the same minerals play an essential role in bone and enamel formation, the presence of HSPM or MIH have been suggested to be indirectly associated with the bone mass at the moment of development of the teeth [15]. Thus far, one cross-sectional study examined the association of Bone Mineral Density (BMD) with the occurrence of caries. They found that higher bone mineralization at a younger age (age ≤ 12 years) was associated with a lower risk of caries [15]. Further identification of prognostic factors for the early detection of HSPM and MIH in children may help to build preventive strategies.

Therefore, the purpose of this study was to examine the association between the bone mass and HSPM/MIH in six-year-old children in a cross-sectional analysis.

Materials & methods

Study design

The study was embedded in the Generation R Study, a population-based, prospective cohort study from fetal life until adulthood in Rotterdam, The Netherlands [16]. Mothers were eligible if their residence was in the study area and if they had a delivery date from April 2002 until January 2006. Enrollment in the study was aimed during the first trimester of pregnancy, but it was later extended until the birth of the child. Eventually, 9,778 mothers were enrolled in the study with a total of 9,745 children. The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam (MEC 198.782/2001/31). Written informed consent was obtained from all participants. From 6,510 (66.8%) children, both intra-oral photographs and Dual Energy X-ray Absorptiometry (DXA) scans were available (Figure 2.1.1). The children visited the Erasmus Medical Centre-Sophia between March 2008 and January 2012. Their mean age at assessment was 6.2 years (4.9–9.1 years, SD 0.52).

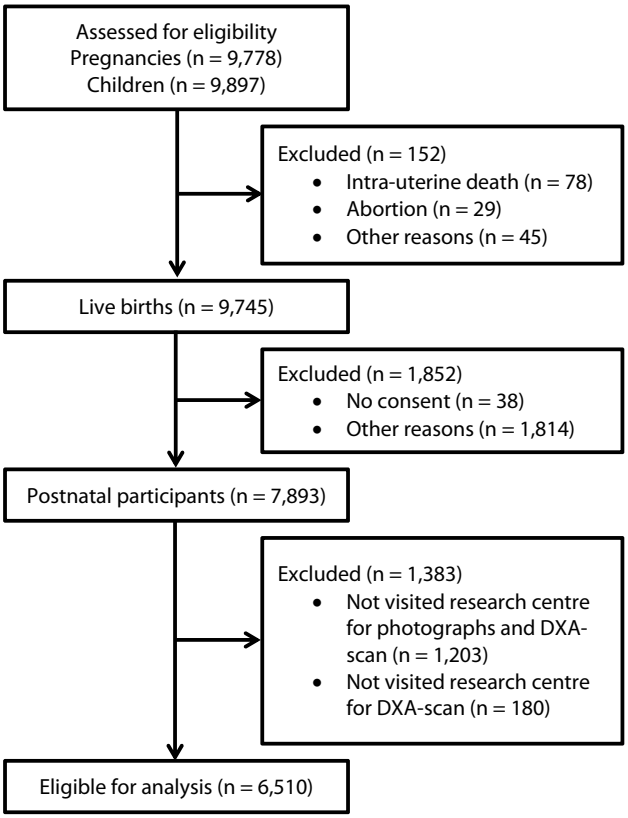


Figure 2.1.1. Flowchart of participants.

HSPM and MIH diagnoses

In order to visualize HSPM and MIH, an intra-oral camera was used [Poscam USB intra-oral (Digital Leader PointNix) or Sopro 717 (Acteon) autofocus camera, 640 X 480 pixels]. The minimal scene illumination of both cameras was f 1.4 and 30 lx. All children of Generation R were invited to participate in taking photographs. Before taking a photograph, participants were asked to brush their teeth. Thereafter, excessive saliva was removed with a cotton roll and trained nurses and dental students took photographs of the teeth. Approximately 10 photographs were taken per child, of all the teeth in 1–2 minutes. The validity of using an intraoral camera for detecting HSPM was tested and appeared to be high (sensitivity: 72.3%, specificity: 92.8%) [17]. The inter-observer agreement reliability was good (kappa coefficient 0.62) and the intra-observer agreement was excellent (kappa coefficient 0.95) [17]. Afterwards, a trained pediatric dentist scored the photographs, in full-screen mode, using the European Academy of Pediatric Dentistry (EAPD) criteria for HSPM and MIH (Table 2.1.1). If a first permanent molar or a second primary molar met one or more of the EAPD criteria, the child was diagnosed as having MIH or HSPM. The presence or absence of HSPM and MIH was considered 'unidentifiable' if the tooth, or the place where it should be, was not shown on the photographs. This value was given only if no photographs were made, if only one photograph was made or if there was a limitation in judging individual teeth. To test the intra-observer agreement, 649 children were scored for a second time after at least six weeks. The Cohen's kappa scores reached 0.82 for HSPM and 0.85 for MIH. A second trained pediatric dentist re-evaluated approximately 10% of the photographs (648 children) to estimate the inter-observer agreement. The Cohen's

Table 2.1.1. EAPD criteria for scoring HSPM and MIH on intra-oral photographs (Elfrink et al. 2009; Weerheijm et al. 2003)

Mild:	Opacity: A defect that changes the translucency of the enamel, variable in degree. The defective enamel is of normal thickness with a smooth surface and can be white, yellow or brown in color. The demarcated opacity is not caused by caries, ingestion of excess fluoride during tooth development or amelogenesis imperfect etc.
Severe:	Posteruptive enamel loss: A defect that indicates surface enamel loss after eruption of the tooth, e.g., hypomineralization related attrition. Enamel loss due to erosion was excluded, and/or
	Atypical caries: The size and form of the caries lesion do not match the present caries distribution in the child's mouth, and/or
	Atypical restoration: The size and form of the restoration do not match the present caries distribution in the child's mouth, and/or
	Atypical extraction: Absence of a molar that does not fit in the dental development and caries pattern of the child.

kappa scores were 0.60 for HSPM and 0.69 for MIH. When the observers disagreed on the diagnosis, the photographs were studied again and a joint consensus decision was made.

Bone mass measurements

The bone mass of the total body was measured by a DXA-scan (iDXA, General Electrics – Lunar, 2008, Madison, Wisconsin, USA). BMD is measured and averaged over the projected area in the scan, the Bone Area (BA). Multiplying BMD by the BA derives the bone mass or Bone Mineral Content (BMC) of the projected area. As recommended by the International Society for Clinical Densitometry (ISCD), bone parameters of the total body minus head were used [18]. For the procedure of measuring the bone parameters, we refer to the manuscript "Maternal first-trimester diet and childhood bone mass: the Generation R Study" of Heppe et al. [19]. Areal BMD is not useful in children as it is significantly influenced by bone size, which is dynamic during growth. Under this contention there is no proportional scaling between BMC and BA, which can lead to erroneous interpretation of the BMD value in children. Bone mineralization is best assessed in relation to height for age, BA for height, and BMC for BA which address different configurations leading to artifacts, reflected as decreased BMD: namely short bones, narrow bones, and light bones, respectively. Therefore, BMD should be corrected for size differences. The BMC regressed on bone area (BMC_{reggBA}) has been postulated as an adequate assessment of BMD which is corrected for skeletal size differences [20-22]. As also recommended by the ISCD, all statistical models are corrected for sex of the child in addition to age, weight, length of the child and any other confounding variables [18]. Table 2.1.2 depicts a brief explanation of all used DXA-scan-related variables.

Table 2.1.2. Explanation of the DXA-scan-related variables

Bone Mineral Density (BMD)	Bone mineral density in the projected area expressed in g/cm^2
Bone Area (BA)	The total area of the projected bone tissue expressed in cm^2
Bone mass or Bone Mineral content (BMC)	The total content of bone mineral in the projected area calculated by BMD*BA expressed in g
BMC corrected for bone area (BMC_{reggBA})	BMC regressed for BA in a statistical model

Covariates

Maternal age, pre-pregnancy length and weight, ethnicity, income, educational level and calcium intake were assessed using a questionnaire at recruitment. To examine maternal smoking and drinking habits, a questionnaire was sent to the mothers in each trimester of pregnancy asking about their cigarette and alcohol consumption. Furthermore, postnatal questionnaires were used to obtain information about breastfeeding initiation and continuation. Offspring sex, birth weight and birth length of the child were acquired from medical records and hospital registries. The frequency of fever in the first year of life was assessed by questionnaire at an age of 12 months. Ethnicity of the child was updated by consulting the Dutch Central Agency for Statistics – 'Centraal Bureau voor Statistiek' (CBS) at 6 years of age. Child's participation in sports was addressed by a questionnaire at the age of 6.

Statistical analysis

All bone parameters were corrected for sex, age, weight and length of the child in a regression model. A t-test was used to compare differences in the means of the bone parameters between affected children and non-affected children. We used a binary logistic regression model to investigate the association between the BMC and HSPM/MIH. To correct BMD for size effects, we initially adjusted BMC for the bone area in a separate regression model. In total we used three statistical models for our analyses. The first model was corrected for sex, age, weight, and length of the child. The second model was additionally adjusted for alcohol use during pregnancy, birth weight, child's ethnicity and fever in first year of life. These confounders were based on a previous publication of the Generation R Study of Elfrink et al. where a significant association of these variables with HSPM was found [23]. The third model was adjusted in addition for age of the mother, household income, smoking during pregnancy and child's participation in sports. The same models were used for testing the association of BMD, BMC and BA with HSPM and with MIH. We have used the Wald Chi-Square test to test the statistical significance of an individual regression coefficient for a variable. The Hosmer and Lemeshow test was used as a measure of goodness of model fit. In order to reduce potential bias associated with missing data, we performed multiple imputation of missing covariates by generating five independent datasets using the Markov Chain Monte Carlo (MCMC) method after which the pooled effect estimates were calculated [24]. Imputations were based on the relationships between covariates, determinants and outcomes under the assumption that data was missing at random. We opted for using all variables included in this study as predictors for the imputation of variables with missing values [24, 25]. Data were

imputed for the variables alcohol use during pregnancy (20.7% missing), ethnicity of the child (2.46%), fever in the first year of life (37.2%), household income (24.9%), smoking during pregnancy (13.3%) and child's participation in sports (15.9%). The

Table 2.1.3. Maternal and children characteristics in the total group of children with a DXA-scan[1, 2]

Maternal characteristics	Total group (n = 6,510)	Child characteristics	Total group (n = 6,510)
Age (y)	30.6 ± 5.16	Age (y)	6.18 ± 0.521
Length (cm)	168 ± 7.40	Male (%)	50.0
BMI (kg/cm^2)	24.8 ± 4.40	Birth weight (kg)	3.40 ± 0.570
Smoking during pregnancy (%)		Low birth weight (%)	5.84
Never	64.5	Weight (kg)	23.3 ± 4.26
Smoked until pregnancy was known	7.76	Birth length (cm)	50.2 ± 2.39
Continued	14.4	Length (cm)	120 ± 6.02
Missing	13.3	Fever in first year of life (%)	
Alcohol use during pregnancy (%)		Yes	51.1
Never	36.5	No	11.7
Alcohol use until pregnancy was known	10.9	Missing	37.2
Continued	31.9	Ethnicity (%)	
Missing	20.7	Dutch	55.6
Ethnicity (%)		Cape Verdian	3.01
Dutch	52.8	Moroccan	5.75
Cape Verdian	4.16	Surinamese	7.13
Moroccan	5.61	Turkish	7.51
Surinamese	7.77	European	7.44
Turkish	8.08	Other Non-European	11.1
European	7.43	Missing	2.46
Other Non-European	11.5	Participation in sports (%)	
Missing	2.65	Yes	37.9
Education level (%)		No	46.2
No education finished/primary education	8.49	Missing	15.9
Secondary education	39.8	BMD (g/cm^2)	0.554 ± 0.0516
Higher education	42.5	BMC (g)	528 ± 106
Missing	9.21	BA (cm^2)	945 ± 118
Calcium intake per day (mg)	1117 ± 451	Evaluable photographs (%)	
Breastfeeding (%)		HSPM	85.8
4 months exclusive	15.8	MIH	36.4
4 months partial	41.0	Prevalence HSPM (%)*	9.08
Never	5.99	Prevalence MIH (%)**	8.57
Missing	37.2		

[1] Values are means ± SDs for continuous variables and percentages for categorical variables.
[2] For the categorical variables the percentage of missing data is shown.
* Based on children with second primary molars (n = 5,586).
** Based on children with permanent first molars (n = 2,370).

Statistical Package of Social Sciences version 20.0 for Mac (SPSS Incorporated, Chicago, Illinois) was used for this study and a p-value < 0.05 was considered to be statistically significant.

The STROBE Guidelines were followed in the reporting of this observational study [26].

Results

In total 6,510 children had intra-oral photographs taken and underwent a DXA-scan. From the 6,510 children, 100% were scored on assessability of diagnosing HSPM and MIH. Maternal and child characteristics are depicted in Table 2.1.3. The mean BMD of the children was 0.554 g/cm^2, the mean BMC was 528 g and the mean BA was 945 cm^2. Furthermore, in the group of children with a DXA-scan, 85.8% had an evaluable intra-oral photograph for HSPM of which 507 (7.79%) children were diagnosed with HSPM. Mainly due to unerupted permanent first molars, judging individual teeth was not possible in all photographs. Therefore, the photographs of only 2,370 children could be used to evaluate MIH (36.4%). Some of the continuous variables had more than 10% missing data: Mother's length, mother's BMI, calcium intake and birth length.

Children with HSPM had on average 6.25 g ($p < 0.001$) lower BMC_{reggBA} and 0.15 cm^2 ($p = 0.001$) greater bone area than children without HSPM. No significant differences were observed in BMD or BMC, nor between any of the bone parameters and MIH status.

Table 2.1.4 shows the association of BMD, BA, BMC and BMC_{reggBA} with HSPM/MIH in standard deviations (SD) corrected for confounding variables. BMD levels are not significantly associated with HSPM (OR 1.06, 95%CI 0.92–1.22 per 1 SD decrease). While BMC is not significantly associated with HSPM (OR 0.81, 95%CI 0.64–1.03 per 1 SD decrease), the BA showed a significant association with HSPM in all three models (OR 0.68, 95%CI 0.55–0.84 per 1 SD decrease). Further, after correction for bone area, a lower BMC is seen significantly associated with HSPM (OR 1.13, 95%CI 1.02–1.26 per 1 SD decrease). This association remained significant across all consecutive models including different sets of potential confounders.

In the first model MIH and BMD were not associated (OR 1.07, 95%CI 0.86–1.33 per 1 SD decrease). This association remained non-significant in the consecutive models (OR 1.04, 95%CI 0.83–1.31 per 1 SD decrease). No other significant associations were found between the BMC, BA, BMC_{reggBA} and MIH (OR 1.18, 95%CI 0.82–1.70 per 1 SD decrease, OR 1.15, 95%CI 0.81–1.61 per 1 SD decrease and 1.02, 95%CI 0.87–1.20 per 1 SD decrease respectively). All models had a good fit based on Hosmer and Lemeshow statistics.

Table 2.1.4. Associations of childhood bone mineral content adjusted for bone area, bone mineral density, bone mineral content and bone area with the risk of HSPM and MIH, the Generation R Study, Rotterdam, the Netherlands[1]

	N	HSPM (Yes/No)[2] 507/5,079	Hosmer & Lemeshow test (df = 8)	MIH (Yes/No)[2] 203/2,167	Hosmer & Lemeshow test (df = 8)
BMD - SD					
Model I[a]		1.08 (0.93–1.22)	$X^2 = 13.5$ p = 0.09	1.07 (0.86–1.33)	$X^2 = 9.02$ p = 0.34
Model II[b]		1.05 (0.92–1.21)	$X^2 = 2.34$ p = 0.97	1.04 (0.83–1.30)	$X^2 = 2.37$ p = 0.97
Model III[c]		1.06 (0.92–1.22)	$X^2 = 4.14$ p = 0.84	1.04 (0.83–1.31)	$X^2 = 4.11$ p = 0.85
BMC - SD					
Model I[a]		0.85 (0.67–1.06)	$X^2 = 6.39$ p = 0.60	1.21 (0.84–1.73)	$X^2 = 10.7$ p = 0.22
Model II[b]		0.81 (0.64–1.03)	$X^2 = 5.55$ p = 0.70	1.17 (0.82–1.69)	$X^2 = 10.2$ p = 0.26
Model III[c]		0.81 (0.64–1.03)	$X^2 = 9.03$ p = 0.34	1.18 (0.82–1.70)	$X^2 = 9.22$ p = 0.32
BA – SD					
Model I[a]		0.70** (0.57–0.87)	$X^2 = 17.3$ p = 0.03*	1.13 (0.81–1.59)	$X^2 = 7.11$ p = 0.53
Model II[b]		0.68** (0.55–0.84)	$X^2 = 5.10$ p = 0.75	1.15 (0.81–1.61)	$X^2 = 13.9$ p = 0.08
Model III[c]		0.68** (0.55–0.84)	$X^2 = 6.39$ p = 0.60	1.15 (0.81–1.61)	$X^2 = 5.91$ p = 0.66
BMC_{reggBA} - SD					
Model I[a]		1.13* (1.02–1.25)	$X^2 = 8.21$ p = 0.41	1.03 (0.96–1.12)	$X^2 = 8.75$ p = 0.36
Model II[b]		1.13* (1.02–1.26)	$X^2 = 4.14$ p = 0.84	1.02 (0.87–1.20)	$X^2 = 5.95$ p = 0.65
Model III[c]		1.13* (1.02–1.26)	$X^2 = 4.19$ p = 0.84	1.02 (0.87–1.20)	$X^2 = 5.89$ p = 0.66

[1] Values are expressed as the Odds Ratio (per SD decrease) with 95%CI.
[2] P-values are based on the Wald Chi-Square test.
* $p < 0.05$. ** $p < 0.01$.
[a] Variables entered in the model: BMD, Sex Child, Age Child, Weight Child, Length Child.
[b] Variables entered in the model: BMD, Sex Child, Age Child, Weight Child, Length Child, Alcohol use during pregnancy, Birth weight, Ethnicity Child, Fever in first year of life.
[c] Variables entered in the model: BMD, Sex Child, Age Child, Weight Child, Length Child, Alcohol use during pregnancy, Birth weight, Ethnicity Child, Fever in first year of life, Age Mother, Household Income, Smoking during pregnancy, Child's participation in sports.

Discussion

The results from this study suggest an association between BMC corrected for bone area and HSPM in six-year-old children. As stated before, BMC corrected for bone area

has been considered a measure of bone mass unbiased to the artefact effects of body size and bone area. We do not observe a significant association between whole–body BMD and HSPM, nor between BMC and HSPM. No association was found between any of the bone parameters and MIH.

Differences in skeletal size are an important source of confounding in paediatric studies using areal BMD. For this reason, guidelines emphasize the need of correcting all association models for potential differences in skeletal frame size [18]. Greater bone size will overestimate BMD levels (as it reflects the thickness of mineral in the 2D region), while a larger scan area will correlate with a comparatively lower BMD (assuming a fixed bone mass). While we found no association with areal BMD corrected by size (length and weight), we do find an association with bone mass after correction for bone area. Children with HSPM have on average greater bone area than children without the condition. However, in absence of 3D evaluations (i.e. peripheral Quantitative Computed Tomography) where we can examine the relationship with volumetric density and bone dimensions, we can only conclude that there is an association of HSPM with bone mass only after correction for bone area.

This article is unique in the field of epidemiology and dentistry. It is the first that studied the association between the bone mass and HSPM/MIH in a large population-based, cohort study. The association between caries and BMD, was examined in a previous study, in which quantitative ultrasound (QUS) of 540 healthy adolescents was used to determine BMD [15]. The authors stated that the BMD of the adolescents was negatively associated with the prevalence of dental caries. Dental caries is a possible consequence of HSPM and MIH [1, 5, 7, 8]. Therefore, the result of Fabiani et al. seems to be partly in line with our findings for HSPM. However, QUS and DXA provide different information on bone tissue as they are differently influenced by factors such as bone size, bone geometry and soft tissue thickness [27]. Moreover, the study population of Fabiani et al. was much older (mean age 12.3 years) and consisted of fewer participants.

The development of second primary molars starts in the second trimester of pregnancy. The first permanent molars start developing in the third trimester of pregnancy [6]. Therefore, prenatal factors can influence this process of development and therewith increase the risk of both HSPM and MIH [28-31]. Early life factors can also influence bone health and the risk of developing osteoporosis throughout life [19, 32]. For example, maternal diet in the first trimester was associated with bone mass in childhood in a previous study within our study population [19]. In the current study bone mass measurements and intra-oral photographs were carried out at a mean age of 6.2 years. Second primary molars complete development by age of 10 months and first permanent molars by the age of four years [6]. Thus, we did not measure

bone mass at the time of the molar development. However, in general, a low bone mass found at a young age is correlated with a low bone mass in adulthood [33]. The complete development of the first permanent molar takes longer than the second primary molar. Possibly, during this time, compensative mechanisms might diminish the inferior mineralization of dental tissue in children with a low bone mass. However, the existence of such compensatory mechanisms should be further investigated.

A recent study of Kühnisch et al. revealed that elevated child serum 25-hydroxy-vitamin D (25(OH)D) concentrations were associated with a lower prevalence of MIH in ten-year-old children [34]. Interestingly, there is evidence that 25(OH)D concentration also plays a role in bone mineralization [35]. Therefore, it would be interesting to study in future work the association between serum levels of 25(OH)D and HSPM/MIH in the paediatric population of Generation R. Moreover, as suggested by Kühnisch et al. studying the genetic background of HSPM and MIH may also help to further unravel the underlying mechanisms of the development of HSPM and MIH [36]. A GWAS-study, embedded in the Generation R study, could be a promising opportunity for this.

Our study is not free of limitations. The cross-sectional design is a weakness to draw conclusions about causality. Multiple measurements over several years of both bone mass and teeth would have given a more accurate "risk" or "incidence rate" of developing HSPM or MIH given a certain bone mass. Assessing bone mass at infant age may have been more accurate since teeth are developed during that period. Moreover, due to the cross-sectional design, the causality and the direction of the association found cannot be established. However, in presence of a true association, mineralization of bone is more likely to influence the mineralization of the teeth than vice versa. Non-responding could not be completely avoided, despite stimulation and reminding patients. Because non-responding led to more than 10% missing data for some variables, we used multiple imputation to dissolve bias from missing data, a widely applied and accepted method [24]. Missing data was not disproportionally divided between the subgroups. Also, we could not include the children that did not have their first permanent molars yet. This resulted in a smaller population for analysis for MIH (n = 2,370) in comparison with the population for analysis for HSPM (n = 5,586). Thus, possible selection bias could not be completely avoided. We tried to correct for as many potential confounding variables as possible. However, residual confounding should still be considered as a result of unknown and unmeasured variables.

In conclusion, more research is needed to unravel the etiology of dental hypomineralization. Our study, in a large population of six-year-old children, adds the finding that BMC (corrected for bone size) is associated with HSPM, but not with MIH in childhood.

References

[1] K. Weerheijm, B. Jälevik, S. Alaluusua, Molar-incisor hypomineralisation, Caries Res 35(5) (2001) 390-391.

[2] K. Weerheijm, M. Duggal, I. Mejàre, L. Papagiannoulis, G. Koch, L. Martens, A. Hollonsten, Judgement criteria for molar incisor hypomineralisation (MIH) in epidemiologic studies: a summary of the European meeting on MIH held in Athens, 2003, Eur J Paediatr Dent 4(3) (2003) 110-113.

[3] M. Elfrink, A. Schuller, K. Weerheijm, J. Veerkamp, Hypomineralized second primary molars: prevalence data in Dutch 5-year-olds., Caries Res 42(4) (2008) 282-285.

[4] T. Koehne, R. Marshall, A. Jeschke, B. Kahl-Nieke, T. Schinke, M. Amling, Osteopetrosis, osteopetrorickets and hypophosphatemic rickets differentially affect dentin and enamel mineralization, Bone 53(1) (2013) 25-33.

[5] M. Elfrink , A. Schuller, J. Veerkamp, J. Poorterman, H. Moll, B. ten Cate, Factors increasing the caries risk of second primary molars in 5-year-old Dutch Children, Int J Paediatr. Dent 20(2) (2010) 151-157.

[6] M. Elfrink, J. ten Cate, V. Jaddoe, A. Hofman, H. Moll, J. Veerkamp, Deciduous molar hypomineralization and molar incisor hypomineralization., J Dent Res 91(6) (2012) 551-555.

[7] F. Crombie, D. Manton, N. Kilpatrick, Aetiology of molar-incisor hypomineralization: a critical review, Int J Paediatr Dent 19(2) (2009) 73-83.

[8] S. Alaluusua, Aetiology of Molar-Incisor Hypomineralisation: A Systematic Review, Eur Arch Paediat Dent 11(4) (2010) 53-58.

[9] A. Ghanim, M. Morgan, R. Marino, D. Bailey, D. Manton, Risk factors of hypomineralised molars in a group of Iraqi schoolchildren, Eur Arch Paediatr Dent 13(3) (2012) 111–118.

[10] M. Elfrink, J. ten Cate, L. van Ruijven, J. Veerkamp, Mineral content in teeth with deciduous molar hypomineralisation (DMH), J Dent Res 41(11) (2013) 974-978.

[11] R. Farah, M. Swain, B. Drummond, R. Cook, M. Atieh, Mineral density of hypomineralised enamel, J Dent 38(1) (2010) 50-58.

[12] C. Smith, Cellular and chemical events during enamel maturation, Crit Rev Oral Biol Med 9(2) (1998) 128-161.

[13] K. Zhu, R. Prince, Calcium and bone, Clin Biochem 45(12) (2012) 936-942.

[14] R. Lunt, D. Law, A review of the chronology of calcification of deciduous teeth, JADA 89(3) (1974) 599-606.

[15] L. Fabiani, G. Mosca, D. Giannini, A. Giuliani, G. Farello, M. Marci, E. Ballatori, Dental caries and bone mineral density: a cross sectional study, Eur J Paediatr Dent 7(2) (2006) 67-72.

[16] V. Jaddoe, C. van Duijn, O. Franco, A. van der Heijden, M. van Iizendoorn, J. de Jongste, A. van der Lugt, J. Mackenbach, H. Moll, H. Raat, e. al., The Generation R Study: design and cohort update 2012, Eur J Epidemiol 27(9) (2012) 739–756.

[17] M. Elfrink, J. Veerkamp, I. Aartman, H. Moll, J. Ten Cate, Validity of scoring caries and primary molar hypomineralization (DMH) on intraoral photographs, JM Eur Arch Paediatr Dent 10(1 Suppl) (2009) 5-10.

[18] E. Lewiecki, M. Gordon, S. Baim, N. Binkley, J. Bilezikian, D. Kendler, D. Hans, S. Silverman, N. Bishop, M. Leonard, M. Bianchi, H. Kalkwarf, C. Langman, H. Plotkin, F. Rauch, B. Zemel, Special report on the 2007 adult and pediatric Position Development Conferences of the International Society for Clinical Densitometry, Osteoporos Int 19(10) (2008) 1369-1378.

[19] D. Heppe, C. Medina-Gomez, A. Hofman, O. Franco, F. Rivadeneira, V. Jaddoe, Maternal first-trimester diet and childhood bone mass: the Generation R Study, Am J Clin Nutr 98(1) (2013) 224-232.

[20] R. Heaney, Bone mineral content, not bone mineral density, is the correct bone measure for growth studies, Am J Clin Nutr 78(2) (2003) 350–351, author reply 351–352.

[21] A. Prentice, T. Parsons, T. Cole, Uncritical use of bone mineral density in absorptiometry may lead to size-related artifacts in the identification of bone mineral determinants, Am J Clin Nutr 60(6) (1994) 837–842.
[22] C. Mølgaard, B. Thomsen, A. Prentice, T. Cole, K. Fleischer Michaelsen, Whole body bone mineral content in healthy children and adolescents, Arch Dis Child 76(1) (1997) 9-15.
[23] M. Elfrink, H. Moll, J. Kiefte-de Jong, V. Jaddoe, A. Hofman, J. ten Cate, J. Veerkamp, Pre- and postnatal determinants of deciduous molar hypomineralisation in 6-year-old children. The generation R study, PLos One, 2014, p. e91057.
[24] J. Sterne, I. White, J. Carlin, M. Spratt, P. Royston, M. Kenward, A. Wood, J. Carpenter, Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls, BMJ, 2009, p. b2393.
[25] L. Collins, J. Schafer, C. Kam, A comparison of inclusive and restrictive strategies in modern missing data procedures, Psychol Methods 6(4) (2001) 330-351.
[26] E. von Elm, D. Altman, M. Egger, S. Pocock, P. Gøtzsche, J. Vandenbroucke, The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)statement: guidelines for reporting observational studies, J Clin Epidemiol 61(4) (2008) 344-349.
[27] G. Baroncelli, Quantitative ultrasound methods to assess bone mineral status in children: technical characteristics, performance, and clinical application, Pediatr Res 63(3) (2008) 220-228.
[28] V. Beentjes, K. Weerheijm, H. Groen, Factors Involved in the aetiology of molar-incisor hypomineralisation (MIH), Eur J Paediatr Dent 3(1) (2002) 9-13.
[29] H. Sönmez, G. Yıldırım, T. Bezgin, Putative factors associated with molar incisor hypomineralisation: an epidemiological study, Eur Arch Paediatr Dent 14(6) (2013) 375-380.
[30] N. Lygidakis, G. Dimou, D. Marinou, Molar-incisor-hypomineralisation (MIH). A retrospective clinical study in Greek children. II. Possible medical aetiological factors, Eur Arch Paediatr Dent 9(4) (2008) 207-217.
[31] M. Elfrink, H. Moll, J. Kiefte-de Jong, H. El Marroun, V. Jaddoe, A. Hofman, B. Stricker, J. ten Cate, J. Veerkamp, Is maternal use of medicines during pregnancy associated with deciduous molar hypomineralisation in the offspring? A prospective, population-based study, Drug Saf 36(8) (2013) 627-633
[32] C. Cooper, S. Westlake, N. Harvey, K. Javaid, E. Dennison, M. Hanson, Review: developmental origins of osteoporotic fracture, Osteoporos Int 17(3) (2006) 337-347.
[33] I. van der Sluis, M. de Ridder, A. Boot, E. Krenning, S. de Muinck Keizer-Schrama, Reference data for bone density and body composition measured with dual energy x ray absorptiometry in white children and young adults, Arch Dis Child 87(4) (2002) 341–347.
[34] J. Kühnisch, E. Thiering, J. Kratzsch, R. Heinrich-Weltzien, R. Hickel, J. Heinrich, Elevated Serum 25(OH)-Vitamin D Levels Are Negatively Correlated with Molar-Incisor Hypomineralization, J Dent Res 94(2) (2015) 381-387.
[35] T. Winzenberg, S. Powell, K. Shaw, G. Jones, Vitamin D supplementation for improving bone mineral density in children, Cochrane Database Syst Rev, 2010.
[36] J. Kühnisch, E. Thiering, D. Heitmüller, C. Tiesler, H. Grallert, R. Heinrich-Weltzien, R. Hickel, J. Heinrich, Genome-wide association study (GWAS) for molar-incisor hypomineralization (MIH), Clin Oral Invest 18(2) (2014) 677-682.

Chapter 2.2

Foetal, neonatal and child vitamin D status and enamel hypomineralization

Justin T. van der Tas | Marlies E. Elfrink | Annemieke C Heijboer | Fernando Rivadeneira | Vincent W. Jaddoe | Henning Tiemeier | Josje D. Schoufour | Henriëtte A. Moll | Edwin M. Ongkosuwito | Eppo B. Wolvius | Trudy Voortman

Community Dentistry and Oral Epidemiology, 2018, 46(4), 343-251

Abstract

Recent literature suggested that higher vitamin D concentrations in childhood are associated with a lower prevalence of Molar Incisor Hypomineralization (MIH). Since tooth development already starts in utero, we aimed to study whether vitamin D status during fetal, postnatal, and childhood periods is associated with the presence of Hypomineralized Second Primary Molars (HSPMs) and/or MIH at the age of six. Our study was embedded in the Generation R Study, a population-based, prospective cohort from fetal life onwards in Rotterdam, the Netherlands. HSPMs and MIH were scored from intra-oral photographs of the children at their age of six. Serum 25(OH)D concentrations were measured at three points in time, which resulted in three different samples; mid-gestational in mothers' blood (n = 4,750), in umbilical cord blood (n = 3,406), and in children's blood at the age of six years (n = 3,983). The children had a mean (± SD) age of 6.2 (± 0.5) years at the moment of taking the intra-oral photographs. After adjustment for confounders, no association was found between fetal 25(OH)D concentrations and the presence of HSPMs (OR 1.02 per 10 nmol/L higher 25(OH)D, 95% CI: 0.98–1.07) or MIH (OR 1.05 per 10 nmol/L increase, 95% CI: 0.98–1.12) in six-year-olds. A higher 25(OH)D concentration in umbilical cord blood resulted in neither lower odds of having HSPM (OR 1.05, 95% CI: 0.98–1.13) nor lower odds of having MIH (OR 0.95, 95% CI: 0.84–1.07) by the age of six. Finally, we did not find higher 25(OH)D concentrations at the age of six to be associated with a significant change in the odds of having HSPM (OR 0.97, 95% CI: 0.92–1.02) or MIH (OR 1.07, 95% CI: 0.98–1.16).

25(OH)D concentrations in prenatal, early postnatal and later postnatal life are not associated with the presence of HPSMs or with MIH at the age of six. Future observational research is required to replicate our findings. Furthermore, it is encouraged to focus on identifying other modifiable risk factors, because prevention of hypomineralization is possible only if the causes are known.

Introduction

Dental enamel hypomineralization is an anomaly of dental enamel in which the affected enamel contains less mineral than sound enamel and is more susceptible to caries [1-3]. This anomaly can be divided into hypomineralization of second primary molars, called hypomineralized second primary molars (HSPMs), and hypomineralization of permanent first molars, called molar incisor hypomineralization (MIH) [3-5]. In patients with MIH incisors of the upper jaw can also be involved and in rare cases incisors of the lower jaw [3]. Although hypomineralization is not restricted to those few index teeth and can be diagnosed in any tooth of both dentitions, a patient can only be diagnosed with HSPM/MIH if he or she has at least one affected second primary molar or first permanent molar respectively [6]. The prevalence of HSPMs is about 4.9% in six-year-old Dutch children [7]. For MIH the prevalence ranges between 8% and 19% among Dutch and Scandinavian children, aged six to thirteen years [3, 5, 7, 8]. Children with HSPM have a higher chance of developing MIH [9, 10]. Identifying modifiable risk factors is important to prevent development of dental enamel hypomineralization in children.

Several early-life risk factors for HSPM and MIH have been identified. For HSPM, maternal alcohol consumption during pregnancy, low birth weight, and fever during the first year of life are mentioned [11]. Other illnesses in early life and the use of antibiotics were proposed as risk factors for MIH [12, 13]. The exact etiology of dental enamel hypomineralization, however, remains unclear [4, 11-14]. In the search to unravel the etiology of dental hypomineralization, a recent study of Kühnisch et al showed that higher serum 25-hydroxy-vitamin D (25(OH)D) concentrations were correlated with less MIH and dental caries in 1,048 German children at age ten [15]. To our knowledge, this is the only study to have examined 25(OH)D and dental enamel hypomineralization. Several other studies examined vitamin D in relation to caries and generally observed that vitamin D supplementation in early life may be preventative for dental caries, as reviewed by Hujoel et al. [16].

The main function of vitamin D is to maintain plasma calcium concentrations at a constant level, which is important for healthy bone development and increasing evidence suggests also for healthy tooth development. [17, 18]. Vitamin D stimulates mineralization of dental enamel and bone by binding to receptors that are expressed in both dental cells and bone cells [19, 20]. Because vitamin D is important in the mineralization of these tissues, it is noteworthy that we recently discovered that lower bone mass is associated with the presence of HSPM but not with MIH in six-year-old children [21]. Our hypothesis is that this association could be explained by differences of 25(OH)D status between children, affecting mineralization of dental enamel and bone.

A limitation of the previous study of Kühnisch et al. was that information on vitamin D status of the children was only available at 10 years of age, whereas tooth development and enamel mineralization already start earlier in life [12, 15, 22]. Accordingly, we aimed to replicate and extend these previous analyses by examining whether 25(OH)D concentrations during fetal life, early postnatal life, and childhood are associated with HSPMs and/or MIH in six-year-old children. Based on the previous literature, our hypothesis was that children, affected by HSPM or MIH, have significant lower 25(OH)D concentrations during earlier phases in their life than children with unaffected teeth.

Methods

Study design and population

The analysis was embedded in the Generation R Study, a population-based, prospective cohort from fetal life onwards in Rotterdam, the Netherlands [23]. Pregnant women living in the study area with a due date between April 2002 and January 2006 were eligible for enrolment. We enrolled 9,778 mothers, who gave birth to a total of 9,745 live-born children. The study has been approved by the Medical Ethics Committee of Erasmus Medical Center, Rotterdam (MEC 198.782/2001/31). Written informed consent was obtained from parents of all participants. Concentrations of 25(OH)D were measured at three points in time; at mid-gestation (18–25 weeks of pregnancy) in 7,179 mothers, after birth from a blood sample of the umbilical cord in 5,023 children, and at six years of age (mean 6.2 [range 4.9–9.1]) in 4,167 children. Intra-oral photographs were made of 6,325 children during the same visit at the age of six. The number of children with data on both 25(OH)D and intra-oral photographs ranged from 3,406 to 4,750 for the different analyses (Figure 2.2.1).

HSPM and MIH diagnoses

In order to visualize HSPM and MIH, an intra-oral camera was used (Poscam USB intra-oral [Digital Leader PointNix] or Sopro 717 [Acteon] autofocus camera, 640 X 480 pixels). The minimal scene illumination of both cameras was 3.0 lx (F1.4). During the data collection period, pictures of the teeth were taken by six trained nurses, twelve dental students, and six PhD students. A pediatric dentist (ME) gave them a presentation about the how and why of taking the dental photographs and repeated that each half a year. Before the employees/students were allowed to make photographs themselves, they had to accompany an experienced employee/student for a day and learned how

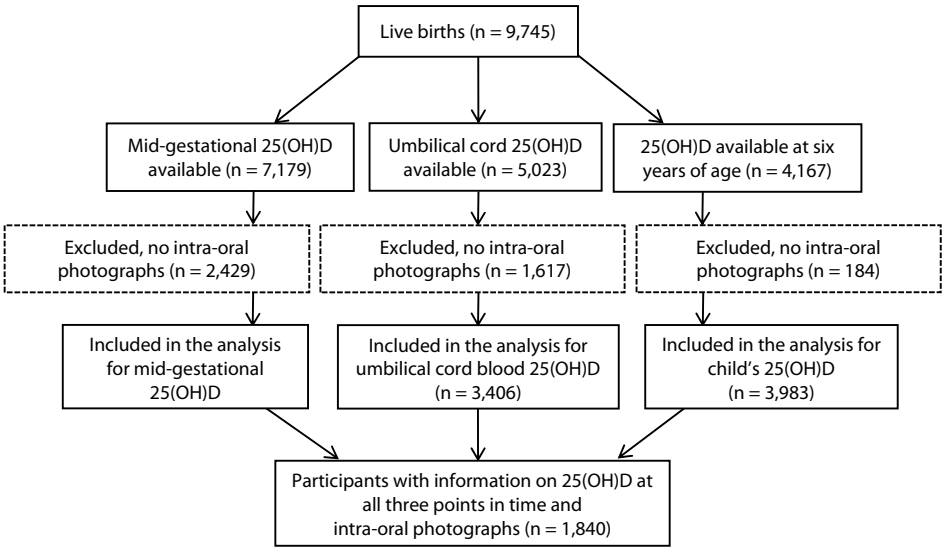


Figure 2.2.1. Flowchart of participants.

to make high-quality photographs. Afterwards, a pediatric dentist (ME) evaluated all photographs within two or four weeks If she found the quality to be too low, she further instructed the respective employee/student on how to improve their quality or she trained them individually. Before taking the photographs, the children had to brush their teeth and excess saliva was removed with a cotton roll. Photographs were scored by a pediatric dentist (ME) on the presence of HSPM and MIH by using the European Academy of Pediatric Dentistry (EAPD)criteria [24]. After completion of the data collection period, the same pediatric dentist (ME) re-evaluated the photographs of 649 children (10%) with a minimal time gap of six weeks. This resulted in a kappa for the intraobserver agreement of 0.82 for HSPM and 0.85 for MIH [21]. A second pediatric dentist (JV) re-evaluated the photographs of 648 children (10%). The kappa's for the interobserver agreement for ME and JV were 0.60 for HSPM and 0.69 for MIH [10, 21]. JV evaluated the photographs only once. Hence, we were not able to calculate a kappa value for the intraobserver agreement of this examiner. ME and JV had a calibration session each two or three months. Before this session, ME randomly chose a couple photographs and discussed them together with JV. Since photographs were taken at the age of six, not all children had their permanent first molars yet, resulting in a smaller number of children with data on MIH than HSPM. Children without data on MIH were on average younger (mean age 6.00 vs. 6.41 years), were more often male (52.7% vs. 44.7%), and more often had a Dutch or other Western background (68.3% vs. 59.8%) than children with data on MIH (Supplementary Table S2.2.1).

25(OH)D measurement

Maternal venous blood samples were collected during mid-pregnancy at a median gestational age of 20.4 weeks (95% range 18.5–23.4). After delivery, midwives or obstetricians collected cord blood from the umbilical vein at a median gestational age of 40.1 weeks (95% range 36.7–42.3). Blood samples of the children were collected at the research center at the six-year visit. Concentrations of 25(OH)D in these samples were analyzed in two different labs.

25(OH)D concentrations in maternal blood samples and in umbilical cord blood were measured at the Eyles Laboratory of the Queensland Brain Institute, University of Queensland, Australia. Samples were quantified using isotope dilution liquid chromatography/tandem mass spectrometry (LC-MS/MS). The method limit of quantification was 6 nmol/L and inter-assay imprecision was < 11% [25].

Vitamin D status of children's blood samples was measured at the Endocrine Laboratory of the VU University Medical Center, Amsterdam, the Netherlands as described in detail previously [26]. Briefly, 25(OH)D was measured using isotope dilution online solid phase extraction LC-MS/MS, a similar method as used for the fetal sample. The limit of quantitation was 4.0 nmol/L; intra-assay coefficient of variation was < 6%, and inter-assay coefficient of variation was < 8% for concentrations between 25 and 180 nmol/L [26]. This method was perfectly aligned with the reference methods [27].

A cross-validation in 31 umbilical cord and pregnancy blood samples that were analyzed in both laboratories showed an excellent correlation between both methods (r = 0.99). The Passing & Bablok regression analysis resulted in $25(OH)D_{Eyles} = 0.93 * 25(OH)D_{VUmc} + 0.3$ nmol/L. This means that a small calibration difference of about 7% exists between the two LC-MS/MS methods. As both assays show some inter-assay variation, we decided not to correct for this small difference.

We categorized 25(OH)D concentrations: ≥ 75 nmol/L (optimal), 50 to < 75 nmol/L (sufficient), 25 to < 50 nmol/L (deficient), and < 25 nmol/L (severely deficient) on the basis of recommendations and cut-offs used in previous studies [26, 28].

Measuring the 25(OH)D concentrations at three different time points and assessing hypomineralization at one time point resulted in four different subsets of the population; three subsets with a 25(OH)D measurement at one point in time and dental data, and one subset with measurements at all three points in time and dental data. These subsets were highly comparable in terms of population characteristics (Supplementary Table S2.2.2).

Covariates

Maternal age, educational level (low, mid-low, mid-high, or high), parity, folic acid supplement use before/during pregnancy (start 1st ten weeks, start periconceptional, or never), and household income (< 2,000, 2,000–3,300, > 3,300 Euros/month) were assessed at enrolment in the study (i.e. during pregnancy) using questionnaires. Maternal smoking and alcohol consumption during pregnancy were assessed in each trimester of pregnancy and categorized into never, until pregnancy was known, or continued. Information on child's birth weight was acquired from medical records and hospital registries. Low birth weight was defined as a birth weight below 2,500 grams. Children's ethnicity was defined based on birth country of both parents and categorized into Western (Dutch, other European, American, and Oceanian), Moroccan and Turkish, African (Surinamese-Creole, Antillean, Cape Verdean, and other African), or Asian (Indonesian, other Asian, and Surinamese-Hindustani) on the basis of expected similarities in skin color [26, 29]. Frequency of fever in the first year of life was assessed at age 12 months with questionnaires. During the research center visit at the child's age of six years, we measured length and weight of the child. At the six-year follow-up, we assessed duration of television watching (< 2 / ≥ 2 hours/day), and playing outside during daytime (< 2 / ≥ 2 hours/day) with questionnaires. At the six-year follow-up, we re-assessed household income (< 2,000, 2,000–3,200, or > 3,200 Euros/month) and maternal educational level [30]. For all blood sample analyses, we kept a record of the month and season of the year in which blood was drawn.

Statistical analyses

First, we constructed three binary logistic regression models in which having HSPMs at the age of six (yes/no) was defined as the outcome (dependent variable) and the fetal serum 25(OH)D concentrations were included as a predictor (independent variable). Fetal serum 25(OH)D concentrations were included as both a categorical variable and as a continuous variable per 10 nmol/L. The categories were compared to an optimal serum concentration of ≥ 75 nmol/L (reference category). Model 1 adjusted only for the child's sex, gestational age at blood withdrawal, mother's age and BMI before pregnancy. Model 2 additionally adjusted for variables that were associated with HSPMs in the Generation R Study population [11]. In model 3, we added variables that were associated with serum 25(OH)D concentrations in our study population [26]. We followed the same approach for studying the association between MIH (outcome) and fetal 25(OH)D serum concentrations (predictor). Moreover, we made use of the same models to study the association between HSPM and MIH as outcomes and cord blood serum 25(OH)D concentrations as predictor. For the approach in which the child's serum

25(OH)D concentrations at the age of six was used as a predictor, minor modifications in the model were made: Model 1 was adjusted for child's sex, age, weight, and length, model 2 did not change, and model 3 was adjusted for household income and maternal educational level at the child's age of six instead of at enrolment, and child's watching television and playing outside were added because these factors have been shown to be important for children's vitamin D status [27].

In order to be able to compare results of fetal, birth and childhood 25(OH)D, we repeated the analyses in a subgroup with data available on 25(OH)D at all three time points (n = 1,840, Figure 2.2.1). We tested for statistical interaction between vitamin D status and children's age, sex, and ethnicity separately in model 3. Multicollinearity was evaluated but was found not to be a problem in our models, because the tolerance statistic exceeded 0.20 for all variables. Moreover, we examined whether we could assume 25(OH)D levels to be linear to the logit by using natural cubic splines (degrees of freedom = 3). Missing data of covariates were handled by applying multiple imputation (n = 10 imputations) [31]. The pooled Odds Ratios (ORs) and 95% confidence intervals (95% CIs) were derived from pooling the results of the ten imputed datasets. Effect estimates were similar to the results of analyses of the original data, therefore we only report pooled results after the imputation procedure. SPSS version 22.0 for Mac (IBM Corp, Armonk, NY, USA) was used for all analyses and a two-sided p-value of < 0.05 was considered to be statistically significant. The STROBE Guidelines were used to ensure adequate reporting of this observational study [32].

Results

Children in our sample had a mean (± SD) age of 6.2 (± 0.5) years at assessment (Table 2.2.1). Half of all participants had optimal or sufficient 25(OH)D serum concentrations above 50 nmol/L (50.1%) at the mid-gestational period; 26.5% were deficient, and 23.4% were severely deficient in 25(OH)D. 10% of the children had incomplete image sets or too low quality photographs to score HSPMs. To score MIH, 62.5% of the children had incomplete image sets (i.e., no erupted first permanent molars) or too low quality photographs. The prevalence of HSPM in this population was 8.9% (381 out of 4,278), and it was 8.1% (146 out of 1,780) for MIH.

The results of the logistic regression analyses with mid-gestational serum 25(OH) D concentration as a predictor and dental enamel hypomineralization as the outcome are shown in Table 2.2.2. In model 1, children from mothers with severely deficient mid-gestational 25(OH)D concentrations had significantly lower odds of having HSPMs (OR, 0.67; 95% CI 0.50–0.91) than those from mothers with sufficient or optimal 25(OH)

Table 2.2.1. Maternal and child characteristics in the total group of children with fetal 25(OH)D concentration measurements[1, 2]

Maternal characteristics	Total group (n = 4,750)	Child characteristics	Total group (n = 4,750)
Age (y)	30.4 ± 5.0	Age (y)	6.2 ± 0.5
Length (cm)	168 ± 7.4	Male (%)	49.7 (2,359)
BMI (kg/cm^2)	24.7 ± 4.4	Birth weight (kg)	3.4 ± 0.6
Parity (% (n))		Low birth weight (% (n))	5.0 (236)
Nulliparous	57.4 (2,727)	Weight (kg)	23.2 ± 4.2
Primi- or multiparous	42.6 (1,991)	Length (cm)	119 ± 5.9
Missing	0.7 (32)	Fever in first year of life (% (n))	
Educational level (% (n))		Yes	82.0 (2,573)
High	28.9 (1,165)	No	18.0 (566)
Mid-high	27.6 (1,115)	Missing	33.9 (1,611)
Mid-low	31.4 (1,268)	Ethnicity (% (n))	
Low	12.1 (488)	Dutch and other Western	65.0 (3,035)
Missing	15.0 (714)	Moroccan and Turkish	14.0 (653)
Household Income / month (% (n))		African	14.8 (691)
> 3,200 euro	49.8 (1,912)	Asian	6.2 (288)
2,000–3,200 euro	26.1 (1,000)	Missing	1.8 (83)
< 2,000 euro	24.1 (925)	Watching television (% (n))*	
Missing	19.2 (913)	< 2 hours/day	80.5 (2,621)
Alcohol use during pregnancy (% (n))		≥ 2 hours/day	19.5 (636)
Never	45.1 (1,887)	Missing	21.8 (910)
Alcohol use until pregnancy was known	14.3 (598)	Playing outside during daytime (% (n))*	
Continued	40.6 (1,697)	≥ 2 hours/day	23.0 (704)
Missing	12.0 (568)	< 2 hours/day	77.0 (2,358)
Folic acid use during pregnancy (% (n))		Missing	26.5 (1,105)
Start 1st 10 weeks	32.2 (1,170)	Season of blood withdrawal (% (n))	
Start periconceptional	43.5 (1,580)	Winter	24.0 (1,140)
Never	24.2 (879)	Spring	28.3 (1,343)
Missing	23.6 (1,121)	Summer	22.3 (1,060)
		Fall	25.4 (1,207)
		25(OH)D Concentration (% (n))	
		Optimal + sufficient (≥ 50 nmol/L)	50.1 (2,381)
		Deficient (25 to 50 nmol/L)	26.5 (1,258)
		Severely deficient (< 25 nmol/)	23.4 (1,111)
		Evaluable photographs (% (n))	
		HSPM	90.0 (4,278)
		MIH	37.5 (1,780)
		Prevalence HSPM (% (n))**	8.9 (381)
		Prevalence MIH (% (n))***	8.2 (146)

[1] Values are means ± SDs for continuous variables and percentages for categorical variables based on the number of valid cases.
[2] For the categorical variables the percentage of missing data is shown.
* Based on group of children with childhood 25(OH)D concentration measurements (n = 4,167).
** Based on group of children with evaluable photographs for HSPM (n = 4,278).
*** Based on group of children with evaluable photographs for MIH (n = 1,780).

Table 2.2.2. Associations of mid-gestational serum 25(OH)D concentrations with HSPM and MIH

		Mid-gestational Serum 25(OH)D concentrations			
		≥ 50 nmol/L (sufficient to optimal)	25–50 nmol/L (deficient)	< 25 nmol/L (severely deficient)	Per 10 nmol/L
HSPM	(n = 4,278)	n = 2,184	n = 1,126	n = 968	n = 4,728
	(Yes vs. No)	(222 vs. 2,184)	(96 vs. 1,030)	(63 vs. 905)	(381 vs. 3,897)
OR (95% CI)	Model 1[1]	Reference	0.85 (0.66–1.10)	**0.67 (0.50–0.91)**	**1.04 (1.01–1.08)**
	Model 2[2]	Reference	0.93 (0.72–1.21)	0.87 (0.62–1.23)	1.01 (0.97–1.05)
	Model 3[3]	Reference	0.89 (0.68–1.16)	0.82 (0.57–1.18)	1.02 (0.98–1.07)
MIH	(n = 1,780)	n = 650	n = 498	n = 507	n = 1,780
	(Yes vs. No)	(66 vs. 709)	(38 vs. 498)	(42 vs. 465)	(146 vs. 1,634)
(Yes vs. No) OR (95% CI)	Model 1[1]	Reference	0.87 (0.57–1.32)	0.92 (0.60–1.41)	1.04 (0.98–1.10)
	Model 2[2]	Reference	0.92 (0.60–1.42)	1.15 (0.71–1.87)	1.02 (0.96–1.08)
	Model 3[3]	Reference	0.85 (0.55–1.33)	0.99 (0.58–1.69)	1.05 (0.98–1.12)

Values are odds ratios (OR) with 95% confidence interval (CI).
[1] Model 1 = adjusted for child's sex, gestational age (mid gestational), age of mother, BMI before pregnancy;
[2] Model 2 = adjusted for all factors in model 1 and additionally adjusted for factors related to enamel hypomineralization (Alcohol use during pregnancy, child's ethnicity, low birth weight and fever in first year of life);
[3] Model 3 = adjusted for all factors in model 2 and additionally adjusted for factors related to 25(OH)D levels (Household income at intake, educational level mother at intake, folic acid use during pregnancy, parity, and season of blood draw);
Significant associations are **bold**.
HSPM = Hypomineralized Second Primary Molar; MIH = Molar Incisor Hypomineralization.

D concentrations. Similar associations were observed for 25(OH)D as a continuous variable. However, no association with HSPMs remained statistically significant in model 2 and 3. The fetal 25(OH)D concentration was not associated with the presence of MIH in children.

Children with severely deficient 25(OH)D concentrations in umbilical cord blood serum had significantly lower odds of having HSPMs than children with sufficient to optimal levels (Table 2.2.3; OR, 0.63; 95% CI 0.45–0.88). Further adjustment for other confounders in model 2 and 3, however, showed no associations. The level of the cord blood serum 25(OH)D concentrations was not associated with the presence of MIH in the six-year-old children.

Table 2.2.4 shows the associations between 25(OH)D concentrations, measured at the age of six, with HSPM and MIH. Model 1 showed that children with a deficient

Table 2.2.3. Associations of cord blood serum 25(OH)D concentrations with HSPM and MIH

		Cord blood serum 25(OH)D concentrations			
		≥ 50 nmol/L (sufficient to optimal)	25–50 nmol/L (deficient)	< 25 nmol/L (severely deficient)	Per 10 nmol/L
HSPM	(n = 3,092)	n = 650	n = 1,106	n = 1,336	n = 3,092
	(Yes vs. No)	(72 vs. 578)	(109 vs. 997)	(88 vs. 1,248)	(269 vs. 2,823)
OR (95% CI)	Model 1[1]	Reference	0.90 (0.66–1.23)	**0.63 (0.45–0.88)**	**1.09 (1.03–1.15)**
	Model 2[2]	Reference	0.95 (0.69–1.31)	0.80 (0.56–1.14)	1.05 (0.99–1.12)
	Model 3[3]	Reference	0.94 (0.67–1.31)	0.79 (0.53–1.18)	1.05 (0.98–1.13)
MIH	(n = 1,315)	n = 233	n = 438	n = 644	n = 1,315
	(Yes vs. No)	(21 vs 212)	(37 vs. 401)	(57 vs. 587)	(115 vs. 1200)
(Yes vs. No) OR (95% CI)	Model 1[1]	Reference	0.92 (0.53–1.62)	0.95 (0.55–1.65)	0.98 (0.89–1.08)
	Model 2[2]	Reference	0.97 (0.55–1.70)	1.11 (0.62–1.98)	0.94 (0.84–1.05)
	Model 3[3]	Reference	0.94 (0.52–1.70)	1.06 (0.55–2.02)	0.95 (0.84–1.07)

Values are odds ratios (OR) with 95% confidence interval (CI).
[1] Model 1 = adjusted for child's sex, gestational age (mid gestational), age of mother, BMI before pregnancy;
[2] Model 2 = adjusted for all factors in model 1 and additionally adjusted for factors related to enamel hypomineralization (Alcohol use during pregnancy, child's ethnicity, low birth weight and fever in first year of life);
[3] Model 3 = adjusted for all factors in model 2 and additionally adjusted for factors related to 25(OH)D levels (Household income at intake, educational level mother at intake, folic acid use during pregnancy, parity, and season of blood draw);
Significant associations are **bold**.
HSPM = Hypomineralized Second Primary Molar; MIH = Molar Incisor Hypomineralization.

25(OH)D status had significantly lower odds for having HSPM (OR, 0.73; 95% CI 0.55–0.98) than those with optimal levels. After more extensive adjustment in models 2 and 3, this association was no longer apparent. Results for MIH were similar. In model 1, children with a deficient 25(OH)D status had significantly lower odds of having MIH (OR 0.68, 95% CI 0.53–0.88) than those with an optimal serum concentration. However, after further adjustment all ORs were non-significant.

In sensitivity analyses, in which we restricted our analyses to a subgroup of children with 25(OH)D data available at all three time points, similar effect estimates were observed as those obtained in the full populations (Supplementary Table S2.2.3). We found no significant interaction between 25(OH)D and child's age, sex, or ethnicity for the association with HSPMs or MIH. No better fit of any model was found after applying natural cubic splines (all $p > 0.05$), indicating linearity to the logit.

Table 2.2.4. Associations of childhood serum 25(OH)D concentrations with HSPM and MIH

		Serum 25(OH)D concentrations				
		≥ 75 nmol/L (optimal)	50–75 nmol/L (sufficient)	25–50 nmol/L (deficient)	< 25 nmol/L (severely deficient)	Per 10 nmol/L
HSPM	(n = 3,642)	n = 1,254	n = 1,327	n = 843	n = 218	n = 3,642
	(Yes vs. No)	(124 vs. 1130)	(134 vs. 1,193)	(59 vs. 784)	(12 vs. 206)	(329 vs. 3,313)
OR (95% CI)	Model 1[1]	Reference	1.05 (0.92–1.20)	0.73 (0.54–1.00)	0.59 (0.32–1.09)	1.03 (0.99–1.07)
	Model 2[2]	Reference	1.12 (0.86–1.46)	0.88 (0.63–1.24)	0.84 (0.44–1.60)	1.00 (0.95–1.04)
	Model 3[3]	Reference	1.20 (0.91–1.57)	1.02 (0.70–1.48)	1.03 (0.52–2.04)	0.97 (0.92–1.02)
MIH	(n = 1,556)	n = 459	n = 548	n = 431	n = 118	n = 1,556
	(Yes vs. No)	(45 vs. 414)	(42 vs. 506)	(27 vs. 404)	(9 vs. 109)	(123 vs. 1,433)
(Yes vs. No) OR (95% CI)	Model 1[1]	Reference	**0.79 (0.63–0.99)**	**0.68 (0.53–0.88)**	0.96 (0.65–1.43)	1.06 (1.03–1.10)
	Model 2[2]	Reference	0.81 (0.52–1.26)	0.75 (0.44–1.27)	1.14 (0.51–2.57)	1.05 (0.98–1.13)
	Model 3[3]	Reference	0.81 (0.51–1.29)	0.72 (0.40–1.31)	1.05 (0.42–2.61)	1.07 (0.98–1.16)

Values are odds ratios (OR) with 95% confidence interval (CI).
[1] Model 1 = adjusted for child's sex, age, weight and length;
[2] Model 2 = additionally adjusted for factors related to enamel hypomineralization (Alcohol use during pregnancy, child's ethnicity, low birth weight and fever in first year of life);
[3] Model 3 = additionally adjusted for factors related to 25(OH)D levels (Household income, educational level mother, folic acid use during pregnancy, parity, watching television, playing outside, season of blood draw);
Significant associations are **bold**.
HSPM = Hypomineralized Second Primary Molar; MIH = Molar Incisor Hypomineralization.

Discussion

Our findings provide no evidence for an association between 25(OH)D status during fetal life, at birth, or at age six with the presence of HSPMs or MIH in six-year-olds. Despite a tendency towards lower odds for both HSPMs and MIH in children with lower 25(OH)D concentrations in the basic models, all apparent associations disappeared after adjusting for possible confounders. Furthermore, associations did not differ by child age, sex or ethnicity.

To put the above findings in perspective, some limitations of our study have to be addressed. Children who did not have their first permanent molar yet could not be included, which resulted in smaller sample sizes for MIH in all analyses compared to HSPM. This may have introduced possible selection bias. Children with complete data on MIH were older on average, more often female, and more often had a non-Dutch

or other non-Western background. This may have resulted in underestimation of the MIH-prevalence, but it is not likely to have biased our results. Ideally, diagnoses of HPSM and MIH were based on clinical examinations, but due to the study setting we had to make use of digital intra-oral photographs [33]. This may have led to an underestimation and/or non-differential misclassification of pathological findings, resulting in possible information bias. We tried to minimalize the chance of having this bias by using a reliable method, but some bias may still be present [34]. Furthermore, not all children with 25(OH)D measurements had photographs of sufficiently high quality for diagnosing HSPM and/or MIH due to blurriness of the pictures (e.g. movements) which have led to excluded children. Sampling bias may therefore have occurred, reducing generalizability to the population, but may not have biased our effect estimates. It is expected that the association between 25(OH)D status and dental enamel hypomineralization is the same for included and excluded participants. Another limitation of our study is that we did not consider different distribution patterns of HSPMs and MIH. This limited the possibility to associate vitamin D status with numerical data. Still, the number of children with HSPMs and MIH would have been the same, since the diagnosis was based on the index teeth as stated in the EAPD-criteria [24]. Furthermore, in order to prevent possible attrition bias, we implemented a multiple imputation method for missing data of covariates. Sensitivity analyses of the imputed data, however, did not result in significant differences in outcome compared to analyses of the original data. The major strength of our study was that we were able to include not only 25(OH)D status when the teeth were already developed, but we have also measured 25(OH)D concentrations at two time points in time during tooth mineralization, which is unique [22, 35]. Another strength of our study was the large and diverse study population of children that could be included and analyzed. Furthermore, we were able to adjust for many important factors related to 25(OH)D concentrations, which were not always considered in previous studies.

We were the first to study the association between serum 25(OH)D status and HSPM prevalence in children. Enamel of HSPMs are thought to be less resistant to dental caries and recent studies concluded high 25(OH)D concentrations in children, high prenatal maternal 25(OH)D concentrations, and even higher vitamin D intake during pregnancy, to be associated with lower risk of dental caries in primary dentition [2, 36-38]. Given these results, we hypothesized that 25(OH)D deficiency during tooth development would result in weaker enamel or even hypomineralized enamel. Moreover, the odds of having HSPMs is higher in children with a lower bone mass, which is influenced by vitamin D [21, 39]. It was therefore unexpected to observe no association between 25(OH)D and HSPM prevalence in six-year-old children. In line

with our findings for enamel hypomineralization, however, we also recently observed no association of fetal vitamin D status with children's bone mass [28].

Kühnisch et al. were the first to have studied the association between a child's serum 25(OH)D status and MIH [15]. Contradictory to their results, we did not find elevated serum 25(OH)D concentrations in children to be negatively correlated with MIH. Compared to their study, we included children that were on average four years younger, but it is unlikely that this could explain the discrepancy in results. Despite the inclusion of younger children, we had the same number of MIH-cases as Kühnisch et al. [15]. Furthermore, they reported the lack of earlier 25(OH)D concentration measurements during the period of tooth development as a limiting factor of their study [15], because the development of teeth already starts in utero [22]. We were also able to examine 25(OH)D in a prenatal and early postnatal period. This was a major strength of our study. However, neither the prenatal nor the postnatal 25(OH)D status showed a significant association with HSPMs or MIH.

In conclusion, in this large population-based cohort, 25(OH)D concentrations in prenatal, early postnatal and later postnatal life are not associated with HPSMs or with MIH at the age of six. To our knowledge, we are the only research group, together with Kühnisch et al, to have studied the association between 25(OH)D status and dental enamel hypomineralization with contradictory results [15]. Therefore, we encourage other cohorts to replicate our findings. Replication in observational studies is needed to confirm whether or not vitamin D supplementation, as a preventive agent against enamel hypomineralization, is worth to be investigated in clinical trials. This could be done by setting up a cohort or by embedding a study within an existing cohort with repeated and early measurements of serum 25(OH)D in children. Ideally, during the developmental period of teeth. Furthermore, it is important to keep on searching for different preventive possibilities and etiological factors for dental hypomineralization in children, which still are unknown [14]. Moreover, despite null findings with hypomineralization, it would be interesting to study the association between 25(OH)D status and dental caries in our population. The pathway in which vitamin D affects the risk of developing dental caries may involve pathways other than enamel mineralization [40].

References

[1] G.C. Americano, P.E. Jacobsen, V.M. Soviero, D. Haubek A systematic review on the association between molar incisor hypomineralization and dental caries, Int. J. Paediatr. Dent. 27(1) (2017) 11-21.

[2] M.E. Elfrink , A.A. Schuller, J.S. Veerkamp, J.H. Poorterman, H.A. Moll, B.J. ten Cate, Factors increasing the caries risk of second primary molars in 5-year-old Dutch Children, Int. J. Paediatr. Dent. 20(2) (2010) 151-157.

[3] K.L. Weerheijm, B. Jälevik, S. Alaluusua, Molar-incisor hypomineralisation, Caries Res. 35(5) (2001) 390-391.

[4] A.M. Ghanim, M.V. Morgan, R.J. Marino, D.L. Bailey, D.J. Manton, Risk factors of hypomineralised molars in a group of Iraqi schoolchildren, Eur. Arch. Paediatr. Dent. 13(3) (2012) 111–118.

[5] M.E. Elfrink, A.A. Schuller, K.L. Weerheijm, J.S. Veerkamp, Hypomineralized second primary molars: prevalence data in Dutch 5-year-olds., Caries Res. 42(4) (2008) 282-285.

[6] A.M. Ghanim, M.E. Elfrink, K.L. Weerheijm, R.J. Mariño, D.J. Manton, A practical method for use in epidemiological studies on enamel hypomineralisation, Eur. Arch. Paediatr. Dent. 16(3) (2015) 235-246.

[7] M.E. Elfrink, A. Ghanim, D.J. Manton, K.L. Weerheijm, Standardised studies on Molar Incisor Hypomineralisation (MIH) and Hypomineralised Second Primary Molars (HSPM): a need, Eur. Arch. Paediatr. Dent. 16(3) (2015) 247-255.

[8] L. Jasulaityte, K.L. Weerheijm, J.S. Veerkamp, Prevalence of molarincisor-hypomineralisation among children participating in the Dutch National Epidemiological Survey (2003), Eur. Arch. Paediatr. Dent. 9(4) (2008) 218-223.

[9] A. Negre-Barber, J.M. Montiel-Company, M. Boronat-Catalá, M. Catalá-Pizarro, J.M. Almerich-Silla, Hypomineralized Second Primary Molars as Predictor of Molar Incisor Hypomineralization, Sci. Rep. 6 (2016) 31929.

[10] M.E. Elfrink, J.M. ten Cate, V.W. Jaddoe, A. Hofman, H.A. Moll, J.S. Veerkamp, Deciduous molar hypomineralization and molar incisor hypomineralization., J. Dent. Res. 91(6) (2012) 551-555.

[11] M.E. Elfrink, H.A. Moll, J.C. Kiefte-de Jong, V.W. Jaddoe, A. Hofman, J.M. ten Cate, J.S. Veerkamp, Pre- and postnatal determinants of deciduous molar hypomineralisation in 6-year-old children. The generation R study, PLoS ONE 9(7) (2014) e91057.

[12] S. Alaluusua, Aetiology of Molar-Incisor Hypomineralisation: A Systematic Review, Eur. Arch. Paediat. Dent. 11(4) (2010) 53-58.

[13] F. Crombie, D. Manton, N. Kilpatrick, Aetiology of molar-incisor hypomineralization: a critical review, Int. J. Paediatr. Dent. 19(2) (2009) 73-83.

[14] M.J. Silva, K.J. Scurrah, J.M. Craig, D.J. Manton, N. Kilpatrick, Etiology of molar incisor hypomineralization - A systematic review, Community Dent. Oral Epidemiol. 44(4) (2016) 342-353.

[15] J. Kühnisch, E. Thiering, J. Kratzsch, R. Heinrich-Weltzien, R. Hickel, J. Heinrich, Elevated Serum 25(OH)-Vitamin D Levels Are Negatively Correlated with Molar-Incisor Hypomineralization, J. Dent. Res. 94(2) (2015) 381-387.

[16] P.P. Hujoel, Vitamin D and dental caries in controlled clinical trials: systematic review and meta-analysis, Nutr. Rev. 71(2) (2013) 88-97.

[17] H.F. DeLuca, Overview of general physiologic features and functions of vitamin D, Am. J. Clin. Nutr. 80(Suppl. 6) (2004) 1689S-1696S.

[18] A. Berdal, P. Papagerakis, D. Hotton, I. Bailleul-Forestier, J.L. Davideau, Ameloblasts and odontoblasts, target-cells for 1,25-dihydroxyvitamin D3: a review, Int. J. Dev. Biol. 39(1) (1995) 257-262.

[19] F. Lézot, V. Descroix, D. Hotton, N. Mauro, S. Kato, A. Berdal, Vitamin D and tissue non-specific alkaline phosphatase in dental cells, Eur. J. Oral Sci. 114(Suppl. 1) (2006) 178-182.
[20] M. Mesbah, I. Nemere, P. Papagerakis, J.R. Nefussi, S. Orestes-Cardoso, C. Nessmann, A. Berdal, Expression of a 1,25-dihydroxyvitamin D3 membrane-associated rapid-response steroid binding protein during human tooth and bone development and biomineralization, J. Bone Miner. Res. 17(9) (2002) 1588-1596.
[21] J.T. van der Tas, M.E. Elfrink, S. Vucic, D.H. Heppe, J.S. Veerkamp, V.W. Jaddoe, F. Rivadeneira, A. Hofman, H.A. Moll, E.B. Wolvius, Association between Bone Mass and Dental Hypomineralization., J. Dent. Res. 95(4) (2016) 395-401.
[22] R.C. Lunt, D.B. Law, A review of the chronology of calcification of deciduous teeth, JADA 89(3) (1974) 599-606.
[23] M.N. Kooijman, C.J. Kruithof, C.M. van Duijn, O.H. Franco, M.H. van IJzendoorn, J.C. de Jongste, C.C. Klaver, A. van der Lugt, J.P. Mackenbach, H.A. Moll, R.P. Peeters, H. Raat, E.H. Rings, F. Rivadeneira, M.P. van der Schroeff, E.A. Steegers, H. Tiemeier, A.G. Uitterlinden, F.C. Verhulst, E. Wolvius, J.F. Felix, V.W. Jaddoe, E.J.E. 1243., The Generation R Study: design and cohort update 2017, Eur. J. Epidemiol. 31(12) (2016) 1243-1264.
[24] K.L. Weerheijm, M. Duggal, I. Mejàre, L. Papagiannoulis, G. Koch, L.C. Martens, A.L. Hallonsten, Judgement criteria for molar incisor hypomineralisation (MIH) in epidemiologic studies: a summary of the European meeting on MIH held in Athens, 2003, Eur. J. Paediatr. Dent. 4(3) (2003) 110-113.
[25] A.A. Vinkhuyzen, D.W. Eyles, T.H. Burne, L.M. Blanken, C.J. Kruithof, F. Verhulst, V.W. Jaddoe, H. Tiemeier, J.J. McGrath, Prevalence and predictors of vitamin D deficiency based on maternal mid-gestation and neonatal cord bloods: The Generation R Study, J. Steroid. Biochem. Mol. Biol. 164 (2016) 161-167.
[26] T. Voortman, E.H. van den Hooven, A.C. Heijboer, A. Hofman, V.W. Jaddoe, O.H. Franco, Vitamin D deficiency in school-age children is associated with sociodemographic and lifestyle factors, J. Nutr. 145(4) (2015) 791-798.
[27] N.F. Dirks, H.W. Vesper, A.E. van Herwaarden, J.M. van den Ouweland, I.P. Kema, J.G. Krabbe, A.C. Heijboer, Various calibration procedures result in optimal standardization of routinely used 25(OH)D ID-LC-MS/MS methods, Clin. Chim. Acta 462 (2016) 49-54.
[28] A.H. Garcia, N.S. Erler, V.W.V. Jaddoe, H. Tiemeier, E.H. van den Hooven, O.H. Franco, F. Rivadeneira, T. Voortman, 25-hydroxyvitamin D concentrations during fetal life and bone health in children aged 6 years: a population-based prospective cohort study, Lancet Diabetes Endocrinol. 5(5) (2017) 367-376.
[29] CBS, Centraal Bureau voor de Statistiek Jaarrapport Integratie, 2014. http://www.cbs.nl/NR/rdonlyres/4735C2F5-C2C0-49C0-96CB-0010920EE4A4/0/jaarrapportintegratie2014pub.pdf. (Accessed December 10, 2016 CBS).
[30] R. Schaart, S. Westerman, M.B. Moens, The Dutch standard classification of education SOI 2006, Statistics Netherlands, 2008. www.cbs.nl/en-gb/background/2008/24/the-dutch-standard-classification-of-education-soi-2006. (Accessed November 24, 2016 Schaart R, Westerman S, Moens MB).
[31] J.A.C. Sterne, I.R. White, J.B. Carlin, M. Spratt, P. Royston, M.G. Kenward, A.M. Wood, J.R. Carpenter, Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls, BMJ 338 (2009) b2393.
[32] E. von Elm, D.G. Altman, M. Egger, S.J. Pocock, P.C. Gøtzsche, J.P. Vandenbroucke, The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)statement: guidelines for reporting observational studies, J. Clin. Epidemiol. 61(4) (2008) 344-349.
[33] A. Ghanim, M. Elfrink, K. Weerheijm, R. Mariño, D. Manton, A practical method for use in epidemiological studies on enamel hypomineralisation, Eur. Arch. Paediatr. Dent. 16(3) (2015) 235-246.

[34] M. Elfrink, J. Veerkamp, I. Aartman, H. Moll, J. Ten Cate, Validity of scoring caries and primary molar hypomineralization (DMH) on intraoral photographs, Eur Arch Paediatr Dent 10(1 Suppl) (2009) 5-10.
[35] W. Profitt, H. Fields, Contemporary orthodontics, third ed., St. Louis, MO: Mosby, 2000.
[36] R.J. Schroth, J.A. Levi, E.A. Sellers, J. Friel, E. Kliewer, M.E. Moffatt, Vitamin D status of children with severe early childhood caries: a case-control study, BMC Pediatr. 13(1) (2013) 174.
[37] R.J. Schroth, N.S. Jeal, E. Kliewer, E.A. Sellers, The relationship between vitamin D and severe early childhood caries: a pilot study, Int. J. Vitam. Nutr. Res. 82(1) (2012) 53-62.
[38] K. Tanaka, S. Hitsumoto, Y. Miyake, H. Okubo, S. Sasaki, N. Miyatake, M. Arakawa, Higher vitamin D intake during pregnancy is associated with reduced risk of dental caries in young Japanese children, Ann. Epidemiol. 25(8) (2015) 620-625.
[39] T. Winzenberg, G. Jones, Vitamin D and bone health in childhood and adolescence, Calcif. Tissue Int. 92(2) (2013) 140-150.
[40] R. Schroth, C. Lavelle, R. Tate, S. Bruce, R.J. Billings, M.E. Moffatt, Prenatal vitamin D and dental caries in infants, Pediatrics 133(5) (2014) 1277-1284.

Supplemental material

Table S2.2.1. Comparison between participants with complete dental data on MIH and participants with dental photographs, but without information on MIH (n = 4,750)[1]

		Complete data (%)	No complete data (%)
Age child	(n = 4,750)	n = 1,780 (37.5)	n = 2,970 (62.5)
	Mean (95% CI)	6.41 (6.38 to 6.44)	6.00 (5.99 to 6.01)
Sex child	(n = 4,750)	n = 1,780 (37.5)	n = 2,970 (62.5)
	Boy	796 (44.7)	1,565 (52.7)
	Girl	984 (55.3)	1,405 (47.3)
Child's ethnicity	(n = 4,651)	n = 1,738 (37.3)	n = 2,913 (62.7)
	Dutch & other Western	1,039 (59.8)	1,991 (68.3)
	Moroccan & Turkish	313 (18.0)	339 (11.6)
	African	279 (16.1)	403 (13.8)
	Asian	107 (6.16)	180 (6.18)
Alcohol use during pregnancy	(n = 4,168)	n = 1,542 (37.0)	n = 2,626 (63.0)
	Never	767 (49.7)	1,115 (42.5)
	Until pregnancy known	184 (11.9)	412 (15.7)
	Continued	591 (38.3)	1,099 (41.9)
Low birth weight	(n = 4,725)	n = 1,773 (37.5)	n = 2,952 (62.5)
	No	1,700 (95.9)	2,789 (94,5)
	Yes	73 (4.12)	163 (5.52)
Fever first year of life	(n = 3,134)	n = 999 (31.8)	n = 2,135 (68.2)
	No	186 (18.6)	378 (17.7)
	Yes	813 (81.4)	1,757 (82.3)
Maternal educational level	(n = 4,462)	n = 1,648 (36.9)	n = 2,814 (63.1)
	High	351 (21.3)	763 (27.1)
	Mid-high	316 (19.2)	644 (22.9)
	Mid-low	531 (32.2)	882 (31.3)
	Low	450 (27.3)	525 (18.7)
Household Income	(n = 1,648)	n = 679 (41.2)	n = 969 (58.8)
	> €3,300	300 (44.2)	483 (49.8)
	€2,000–€3,300	252 (37.1)	339 (35.0)
	< €2,000	127 (18.7)	147 (15.2)
Folic acid use	(n = 3,616)	n = 1,317 (36.4)	n = 2,299 (63.6)
	Never	401 (30.4)	472 (20.5)
	Start first ten weeks	408 (31.0)	758 (33.0)
	Start periconceptional	508 (38.6)	1,068 (46.5)
Parity	(n = 4,702)	n = 1,764 (37.5)	n = 2,938 (62.5)
	Mean (95% CI)	0.61 (0.57 to 0.65)	0.57 (0.54 to 0.60)

[1] Percentages of categorical variables are based on the number of valid cases.

Table S2.2.2. Comparison between participants with complete dental data and fetal serum 25(OH)D measurements (n = 4,750), complete dental data and serum 25(OH)D measurements at birth (n = 3,406), complete dental data and serum 25(OH)D measurements at age six (n = 3,983), complete dental data and serum 25(OH)D measurements at all three points in time

	Dental data and fetal 25(OH)D	Dental data and 25(OH)D at birth	Dental data and 25(OH)D at age 6	Dental data and 25(OH)D at all 3
Age child	n = 4,750	n = 3,406	n = 3,983	n = 1,840
Mean (± SD)	6.16 (± 0.51)	6.18 (± 0.52)	6.20 (± 0.54)	6.16 (± 0.50)
Missing	-	-	-	-
Sex child				
Boy	2,359 (49.7)	1,726 (50.7)	2,054 (51.6)	940 (51.1)
Girl	2,391 (50.3)	1,680 (49.3)	1,929 (48.4)	900 (48.9)
Missing	-	-	-	-
Child's ethnicity				
Dutch & other Western	3,035 (65.0)	2,227 (66.4)	2,562 (66.1)	1,229 (67.5)
Moroccan & Turkish	653 (14.0)	474 (14.1)	520 (13.4)	245 (13.5)
African	691 (14.8)	483 (14.4)	566 (14.6)	266 (14.5)
Asian	288 (6.17)	168 (5.01)	229 (5.91)	81 (4.40)
Missing	83 (1.75)	54 (1.59)	106 (2.66)	19 (1.03)
Alcohol use during pregnancy				
Never	1,887 (45.1)	1,344 (44.9)	1,398 (44.6)	698 (42.3)
Until pregnancy known	598 (14.3)	413 (13.8)	435 (13.9)	243 (14.9)
Continued	1,697 (40.6)	1,239 (41.4)	1,305 (41.6)	698 (42.8)
Missing	568 (12.0)	410 (12.0)	845 (21.2)	210 (11.4)
Low birth weight				
No	4,505 (95.0)	3,331 (97.8)	3,753 (94.4)	1,804 (98.0)
Yes	236 (5.00)	75 (2.20)	222 (5.58)	36 (2.00)
Missing	9 (0.19)	-	8 (0.20)	-
Fever first year of life				
No	566 (18.0)	400 (17.5)	492 (19.4)	223 (17.1)
Yes	2,573 (82.0)	1,891 (82.5)	2,044 (80.6)	1,082 (82.9)
Missing	1,611 (33.9)	1,115 (32.7)	1,447 (36.3)	535 (29.1)
Maternal educational level				
High	1,115 (24.9)	820 (25.4)	933 (25.8)	450 (25.5)
Mid-high	960 (21.4)	704 (21.8)	803 (22.2)	403 (22.9)
Mid-low	1,422 (31.8)	982 (30.5)	1,099 (30.4)	530 (30.1)
Low	979 (21.9)	717 (22.2)	778 (21.5)	379 (21.5)
Missing	274 (5.77)	183 (5.37)	370 (9.29)	78 (4.24)
Household income				
> €3,300	785 (47.3)	576 (47.7)	717 (47.7)	314 (49.6)
€2,000–€,3300	596 (35.9)	452 (37.4)	515 (34.3)	224 (35.4)
< €2,000	277 (16.7)	179 14.8)	271 (18.0)	95 (15.0)
Missing	3,092 (65.1)	2,199 (64.6)	2,480 (62.3)	1,207 (65.6)
Folic acid use				
Never	879 (24.2)	635 (24.4)	668 (24.5)	332 (23.4)
Start first ten weeks	1,170 (32.2)	831 (31.9)	872 (32.0)	459 (32.4)
Start periconceptional	1,580 (43.5)	1,141 (43.8)	1,185 (43.5)	626 (44.2)
Missing	1,121 (23.6)	799 (23.5)	1,258 (31.6)	423 (23.0)
Parity				
Mean (± SD)	0.58 (± 0.81)	0.60 (± 0.82)	0.63 (± 0.85)	0.59 (± 0.82)
Missing	32 (0.67)	18 (0.53)	39 (0.98)	6 (0.33)

[1] Percentages of categorical variables are based on the number of valid cases.

Table S2.2.3. Associations of 25(OH)D concentrations with HSPM and MIH among children with 25(OH)D data available at all three time points

		Mid-gestational Serum 25(OH)D concentration			
		≥ 50 nmol/L (sufficient to optimal)	25–50 nmol/L (deficient)	< 25 nmol/L (severely deficient)	Per 10 nmol/L
HSPM	**(n = 1,698)**	n = 879	n = 461	n = 358	n = 1,698
	(Yes vs No)	(89 vs. 790)	(38 vs. 423)	(21 vs. 337)	(148 vs. 1,550)
OR (95% CI)	Model 1[1]	Reference	0.83 (0.56–1.25)	0.63 (0.37–1.05)	1.03 (0.98–1.09)
	Model 2[2]	Reference	0.92 (0.62–1.39)	0.98 (0.55–1.73)	0.99 (0.93–1.05)
	Model 3[3]	Reference	0.88 (0.57–1.34)	0.89 (0.48–1.67)	1.00 (0.93–1.07)
MIH	**(n = 702)**	n = 309	n = 221	n = 172	n = 702
	(Yes vs No)	(24 vs. 285)	(16 vs. 205)	(14 vs. 158)	(54 vs. 648)
OR (95% CI)	Model 1[1]	Reference	0.91 (0.47–1.78)	1.01 (0.49–2.10)	1.05 (0.96–1.16)
	Model 2[2]	Reference	0.95 (0.48–1.87)	1.29 (0.56–2.97)	1.04 (0.93–1.16)
	Model 3[3]	Reference	0.89 (0.43–1.82)	1.34 (0.52–3.46)	1.05 (0.93–1.18)
		Cord Blood Serum 25(OH)D concentration			
		≥ 50 nmol/L (sufficient to optimal)	25–50 nmol/L (deficient)	< 25 nmol/L (severely deficient)	Per 10 nmol/L increase
HSPM	**(n = 1,698)**	n = 365	n = 616	n = 717	n = 1,698
	(Yes vs No)	(40 vs. 325)	(63 vs. 553)	(45 vs. 672)	(148 vs. 1.550)
OR (95% CI)	Model 1[1]	Reference	0.94 (0.62–1.43)	**0.60 (0.38–0.96)**	1.06 (0.98–1.14)
	Model 2[2]	Reference	0.99 (0.65–1.52)	0.80 (0.49–1.29)	1.00 (0.92–1.09)
	Model 3[3]	Reference	0.97 (0.62–1.53)	0.76 (0.44–1.33)	1.00 (0.90–1.10)
MIH	**(n = 702)**	n = 117	n = 237	n = 348	n = 702
	(Yes vs No)	(10 vs. 107	(18 vs. 219)	(26 vs. 322)	(54 vs. 648)
OR (95% CI)	Model 1[1]	Reference	0.84 (0.37–1.90)	0.80 (0.36–1.79)	0.98 (0.84–1.15)
	Model 2[2]	Reference	0.90 (0.39–2.04)	0.96 (0.41–2.22)	0.94 (0.79–1.11)
	Model 3[3]	Reference	0.81 (0.34–1.95)	0.77 (0.29–2.03)	0.94 (0.77–1.15)

Values are odds ratios with 95% confidence interval (CI).

[1] Model 1 = adjusted for child's sex, gestational age of birth, age of mother, BMI before pregnancy; [2] Model 2 = adjusted for all factors in model 1 and additionaly adjusted for factors related to enamel hypomineralization (Alcohol use during pregnancy, child's ethnicity, low birth weight and fever in first year of life); [3] Model 3 = adjusted for all factors in model 2 and additionally adjusted for factors related to 25(OH) D levels (Household income at intake, educational level mother at intake, folic acid use during pregnancy, parity, season of blood draw, and serum 25(OH)D levels at 6 years of age);

Signifcant associations are **bold**. HSPM = Hypomineralized Second Primary Molar; MIH = Molar Incisor Hypomineralization.

3

Dental caries

Chapter 3.1

Ethnic disparities in dental caries among six-year-old children in the Netherlands

Justin T. van der Tas | Lea Kragt | Jaap S. Veerkamp | Vincent W. Jaddoe | Edwin M. Ongkosuwito | Marlies E. Elfrink | Eppo B. Wolvius

Caries Research, 2016, 50(5), 489-497

Abstract

The aim of this study was to investigate potential differences in caries prevalence of children from ethnic minority groups compared to native Dutch children and the influence of socioeconomic status (SES) and parent-reported oral health behaviour on this association. The study had a cross-sectional design, embedded in a population based prospective multi-ethnic cohort study. 4,306 children with information on caries experience, belonging to seven different ethnic groups, participated in this study. The decayed, missing, and filled teeth (dmft) index was assessed at the age of six and categorized in two ways for analysis: children without caries (dmft = 0) versus any caries experience (dmft > 0) and children without caries (dmft = 0) versus children with mild caries (dmft = 1–3) or severe caries (dmft > 3). Compared to native Dutch children, children with a Surinamese-Hindustani, Surinamese-Creoles, Turkish, Moroccan, and Cape Verdean background had significantly higher odds for dental caries. Especially the Surinamese-Hindustani, Turkish, and Moroccan group had significantly higher odds for severe dental caries. Household income and educational level of the mother explained up to 43% of the association between ethnicity and dental caries, whereas parent-reported oral health behaviour did not mediate the association. Alarming disparities in caries prevalence between different ethnic (minority) groups exist, which cannot be fully explained by social inequalities. Public health strategies can apply this new knowledge and specifically focus on the reduction of ethnic disparities in oral health. More research is needed to explain high caries prevalence among different ethnic minority groups.

Introduction

Dental caries is a major public health problem with a prevalence between 60 and 90 percent among all school children worldwide 1]. This problem leads not only to impaired oral health-related quality of life in (pre-) school children and their parents, but also affects children's development [2]. For this reason, a reduction in the prevalence of dental caries, known as a preventable disease, is desirable [3].

Children with an ethnic minority background form a vulnerable population group. They have a higher risk of being born preterm or postterm, having respiratory allergies, or having an adverse cardiovascular risk profile [4-6]. Moreover, differences in oral health among ethnic groups have been reported by various studies from the Netherlands and other parts of the world [7-11]. These studies indicate a higher prevalence of caries among children from immigrant or ethnic minority groups. In the association between ethnicity and health, socioeconomic status (SES) might play an important mediating role. The relationship between lower SES and various non-communicable diseases is widely accepted [12]. Also in dental research, the relationship between SES and caries prevalence is visible [13]. Although declines in caries prevalence occur in all SES groups, lower SES remains associated with worse oral health in the Netherlands [10, 14]. In addition, oral health behaviour, such as toothbrushing frequency and sugar consumption, might play a mediating role in the association between ethnicity and dental caries. Obviously, oral health behaviour is the main determinant for oral health, but it also appears to be associated with immigrant status [15]. Our hypothesis is that ethnic disparities in caries prevalence can be mostly explained by SES and differences in oral health behaviour between ethnic groups.

Thus far, European studies did not distinguish between major, country-dependent, ethnic minority groups [10, 11, 15-18]. It is important to fill this knowledge gap, to be able to focus prevention programs at the most vulnerable groups. The purpose of our study was to investigate potential differences in caries prevalence of children from ethnic minority groups compared to native Dutch children. We further explored whether the associations between ethnicity and dental caries were explained by differences in SES or oral health behaviour.

Materials & methods

Study design

Our study had a cross-sectional design and was embedded in the Generation R Study. The Generation R study is a prospective multi-ethnic cohort study that follows children

from fetal life until adulthood in Rotterdam, The Netherlands [19]. The Generation R Study aims to identify early environmental and genetic determinants of growth, development and health, described in detail elsewhere [19]. The Medical Ethics Committee of Erasmus Medical Centre, Rotterdam, the Netherlands, approved this study (MEC-2007-413). All participants gave written informed consent.

Study population

All pregnant women living in the study area between April 2002 and January 2006, that were in their first trimester of pregnancy until birth of their child were eligible to enroll in the Generation R Study. Primarily, 9,778 mothers were enrolled in the study, with a total of 9,745 children. Of these, 8,305 mothers (84.9%) gave consent to participate in the school aged period (5 years onwards) of the Generation R Study and 6,690 (68.7%) children visited the research centre where information on oral health was collected. Finally, we excluded participants with missing information on dental caries (n = 1,367) and children with missing information on ethnicity (n = 127). We also excluded 890 children because of the small country-specific sample sizes (< 100 per group), leaving a total study group of 4,306 children belonging to one of the major ethnic groups.

Ethnic groups

All children were born in Rotterdam, the Netherlands, however the ethnic background of a child was defined as non-Dutch if one of the parents was born in another country than the Netherlands CBS [20]. If both parents were born in another country than the Netherlands, the birth country of the mother was conclusive for the ethnicity of the child. Information about ethnic background of the parents was obtained at enrolment by questionnaires [19]. Ethnic background of all mothers and children was updated by consulting the Dutch Central Agency for Statistics (Centraal Bureau voor de Statistiek) at the age of six. Different ethnic groups for this study were defined. We have distinguished between the following non-Dutch groups: Surinamese-Hindustani (n = 152), Surinamese-Creole (n = 154), Turkish (n = 402), Dutch Antillean (n = 161), Moroccan (n = 308), and Cape Verdean (n = 172).

Assessment of dmft index

The decayed, missing, filled teeth index (dmft index) was obtained from intraoral photographs, taken by trained nurses and dental students. For this, we have used two intraoral cameras, the Poscam USB intra-oral (Digital Leader PointNix) or Sopro 717 (Acteon) autofocus camera. Both cameras had a resolution of 640x480 pixels and

a minimal scene illumination of f 1.4 and 30 lx. Scoring dental caries per tooth on intraoral photographs has been described elsewhere and was shown to have high sensitivity and specificity (85.5% and 83.6% respectively), in children with a mean age of 4.96 years, when compared to ordinary oral examination [21]. On average, all teeth were captured on in total 10 photographs showing the occlusal, buccal, lingual and palatal sides of the teeth.

After at least six weeks, 10% of the photographs were scored for a second time to test the intra-observer agreement. The Cohen's (quadratic weighted) kappa score reached a perfect agreement of 0.98 [22]. A re-evaluation of approximately 10% of the photographs (460) was done by a second trained pediatric dentist to test the inter observer agreement. The two dentists reached almost a perfect agreement of 0.89 [22].

For the analysis, the dmft index was categorized into two ways. First, we categorized the children into caries free experience (dmft = 0) versus any caries experience (dmft > 0). Second, we categorized the children into three groups: children without caries experience (dmft = 0), children with mild caries (dmft = 1–3), and children with severe caries (dmft > 3). The cut-off for the latter categorization was based on the mean dmft index ± standard deviation dmft index (1.6 ± 2.5) of five-year-old Dutch children and was also used in other literature [10, 23].

Covariates

The following variables were considered as potential mediators: Mother's age, educational level of mother, household income, marital status, child's sex, child's age, child's BMI, age at first dental visit, dental visit in the past year and toothbrushing frequency. At enrolment in the Generation R Study mothers were asked about their age. At the children's age of six, the mothers had to fill in questionnaires that asked about their highest followed educational level, household income, marital status, and children's oral health related behaviour. Maternal educational level was subdivided into two groups; higher (higher vocational training, university or PhD degree) versus lower. Three categories were constructed for household income; < 2,000 euro/month, 2,000–3,200 euro/month, and > 3,200 euro/month. Marital status was divided in married/registered partnership or unmarried/no registered partnership. Information about oral health related behaviour was assessed by questions about age at first dental visit (0–3 years, > 3 years, or never), dental visit in the past year (yes/no), and toothbrushing frequency (once a day, twice a day, or more than twice a day). BMI was calculated based on anthropometric measurements, conducted during the child's visit at the research centre.

Statistical analysis

Descriptive statistics are used to characterize the study population and children from different ethnic backgrounds. Differences in sample characteristics among the ethnic groups were evaluated using one-way ANOVAs, Mann-Whitney-U and X^2 -tests.

Associations between ethnic background and caries prevalence were evaluated using logistic regression models for the dichotomous outcome (dmft = 0 vs. dmft > 0) and multinomial logistic regression models for the outcome categorized into three groups (dmft = 0 vs. dmft = 1–3 or vs. dmft > 3). Two different models were built. The first model was a crude model adjusted for child's age and sex only. The second model was built to investigate whether the association between ethnic background and dmft index is either explained by SES-related indicators or by oral health behaviour related indicators. For this we first included household income and educational level of the mother, as indicators for SES, into the model. Second, we added children's age at first dental visit, dental visit in the past year, and toothbrushing frequency, as indicators for oral health behaviour to the crude model. In both the second as the third model we have added the product term of ethnicity with the other predictor variables to test for statistical interaction. No interaction terms were significant and therefore we did not present stratified analysis. In the second model, which adjusted for mother's education and household income, we had also tested maternal life style factors. However, we excluded them from the model, because they did not have a significant influence on the association. Collinearity analysis was performed by using the tolerance statistics. A tolerance statistic > 0.20 was considered to exclude multi-collinearity. In our models the tolerance statistic exceeded 0.50 for all variables, thus excluding multi-collinearity. The natural logarithm of age was computed to perform a Box-Tidwell test. This test showed linearity of age with any of the caries outcomes ($p > 0.05$) Independence of errors was estimated by calculating the dispersion parameter, which is the ratio of the chi-square goodness-of-fit statistic to its degrees of freedom. Because the dispersion parameter approximated 1.0 and was < 2.0, we assumed independence of errors.

A multiple imputation of missing covariates was performed by generating ten independent datasets using the Markov Chain Monte Carlo (MCMC) method after which the pooled effect estimates were calculated [24]. Multiple imputation models were based on relationships between all variables included in this study. A sensitivity analyses was performed to compare results of the non-imputed dataset with the imputed dataset. We have used the Statistical Package of Social Sciences version 22.0 for Mac (IBM Corp, Armonk, NY, USA) for our statistical analyses. A p-value < 0.05 was considered to be statistically significant. The STROBE guidelines were followed in the reporting of this observational study [25].

Results

Population characteristics

In Table 3.1.1 the maternal and child characteristics of our study population by ethnic background are shown (n = 4,360). Educational level of the mother and household income, indicators for the SES, were significantly higher in the Dutch group (n = 2,957) than in the ethnic minority groups. Dutch children went earlier and more often to the dentist than children from the ethnic minority groups, but did not brush their teeth more often. The group of children with a Dutch background had the highest percentage of children without caries (77.1%). By contrast, in the group of Turkish (n = 402) and Moroccan (n = 308) children, only 41.5% and 40.9% of the children respectively had a caries-free dentition. At last, the distribution of the dmft index was significantly different in all ethnic groups compared to the distribution of the dmft index in Dutch children. Figure 3.1.1 shows the cumulative distribution of caries across all ethnic groups.

Ethnicity and dental caries

The associations between children's ethnicity and the dmft index are shown in Table 3.1.2. Compared to native Dutch children, children from other ethnic backgrounds were more likely to have higher dmft indices. The logistic regression model showed that children with a Surinamese-Hindustani, Surinamese-Creole, Turkish, Moroccan, and Cape Verdean background had significantly higher odds of having dental caries than Dutch children. These associations remained significant after correction for SES-related variables for the Surinamese-Hindustani (odds ratio [OR], 1.54; 95% confidence interval [95% CI], 1.08 to 2.19), Turkish (OR, 3.18; 95% CI, 2.50 to 4.04), and Moroccan group (OR, 3.25; 95% CI, 2.47 to 4.27). Correcting for oral health behaviour related variables did not change the OR significantly compared to the OR of the crude model.

The multinomial logistic regression showed a comparable association between ethnicity and the dmft index. Turkish and Moroccan children had significantly higher odds of having mild caries (dmft 1–3: OR, 2.73; 95% CI, 2.09 to 3.57; OR, 2.85; 95% CI, 2.12 to 3.83, respectively) and even higher odds of having severe dental caries (dmft > 3: OR, 8.90; 95% CI, 6.76 to 11.7; OR, 8.43; 95% CI, 6.21 to 11.4, respectively) than Dutch children. The association between the Surinamese-Creole group and the dmft index (OR 1.58; 95% CI, 1.07 to 2.33) was only significant for mild caries, whereas the association between the Surinamese-Hindustani (OR 3.77; 95% CI, 2.43 to 5.84) and the Cape Verdean group with the dmft index (OR 2.04; 95% CI, 1.25 to 3.32) was only significant for the severe caries group. The influence of mother's education and household income on the relationship between ethnicity and the dmft index was similar to its influence

Table 3.1.1. Sample characteristics of the study population (n = 4,306)

	Dutch n = 2,957	Surinamese-Hindustani n = 152	Surinamese-Creole n = 154	Turkish n = 402	Dutch Antillean n = 161	Moroccan n = 308	Cape Verdean n = 172
Parental characteristics							
Age mother, median (95% range), years	31.9 (21.3–39.8)	28.8 (17.8–39.1)*	30.2 (18.3–40.9)*	27.5 (19.3–38.7)*	26.3 (18.0–39.9)*	28.6 (20.2–40.7)*	30.8 (19.7–39.8)*
Missing, %	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Educational level mother, Higher, %							
Higher, %	66.3	23.3*	40.3*	18.5*	34.6*	27.5*	22.1*
Missing, %	7.9	15.1**	19.5**	22.1**	19.3**	33.5**	29.1**
Household income, %							
< 2,000 euro	11.1	40.4	42.5	55.6	63.2	64.1	67.5
2,000–3,200 euro	25.0	34.2	34.5	31.1	18.4	25.7	19.7
> 3,200 euro	63.9	25.4*	23.0*	13.2*	18.4*	10.2*	12.8*
Missing, %	13.1	25.0**	26.6**	24.9**	22.4**	33.1**	32.0**
Marital status, %							
Married/registered partnership	69.5	57.8*	36.6*	85.4*	28.9*	80.2*	29.1*
Missing, %	8.69	15.8**	20.1**	19.9**	20.5**	27.9**	26.2**
Child characteristics							
Boys, %	50.3	44.7*	53.9*	53.0	44.1	52.9	51.7
Missing, %	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Age, median (95% range), years	6.00 (5.68–7.44)	6.07 (5.63–8.09)*	6.10 (5.67–8.22)*	6.08 (5.63–7.88)*	6.11 (5.62–8.47)*	6.11 (5.69–8.04)*	6.12 (5.75–8.08)*
Missing,%	0.0	0.0	0.0	0.0	0.0	0.0	0.0

BMI, median (95% range), kg/m^2	15.7 (13.7–19.6)	15.0 (12.8–22.2)*	16.1 (13.4–23.6)*	16.5 (13.9–23.0)*	16.5 (14.0–24.1)*	16.5 (14.0–22.4)*	16.6 (13.8–22.5)
Missing, %	0.2	0.0	0.0	0.0	0.6	0.0	0.0
Age at first dental visit							
0–3 years	62.3	39.1	38.2	42.7	47.3	36.1	34.6
> 3 years	35.9	57.0	55.3	53.2	41.9	56.6	53.5
Never	1.80	3.99*	6.50*	4.11*	10.9*	7.31*	11.8*
Missing, %	8.05	15.8**	20.1**	21.4**	19.9**	28.9**	26.2**
Dental visits in the past year							
Yes	94.3	90.4	92.2	89.0*	89.8*	88.0*	87.9*
Missing, %	9.30	17.8**	24.7**	22.9**	26.7**	32.5**	32.6**
Brushing teeth per day, %							
Once	19.1	9.68	13.7	43.5	15.5	24.5	5.17
Twice or more	80.9	90.3*	86.3	56.5*	84.5	75.5*	94.8*
Missing, %	9.67	18.4**	24.0**	23.9**	28.0**	33.8**	32.6**
Index dmft, %							
0	77.1	60.5	66.2	41.5	67.7	40.9	64.5
1–3	13.6	19.1	26.0	25.4	21.7	26.9	22.7
> 3	9.2	20.4*	7.79*	33.1*	10.6*	32.1*	12.8*

[1] Values are presented as median(90%-range) for continuous variables and percentages for categorical variables. Percentages are based on the number of valid cases. P-values were estimated by using one-way ANOVA, Mann-Whitney-U and Chi-square tests. For all comparisons, children with a Dutch background were used as a reference group. dmft = decayed, missing, and filled teeth.

* P-value < 0.05 for comparison of non-missing variables.

** P-value < 0.05 for comparison of missing variables.

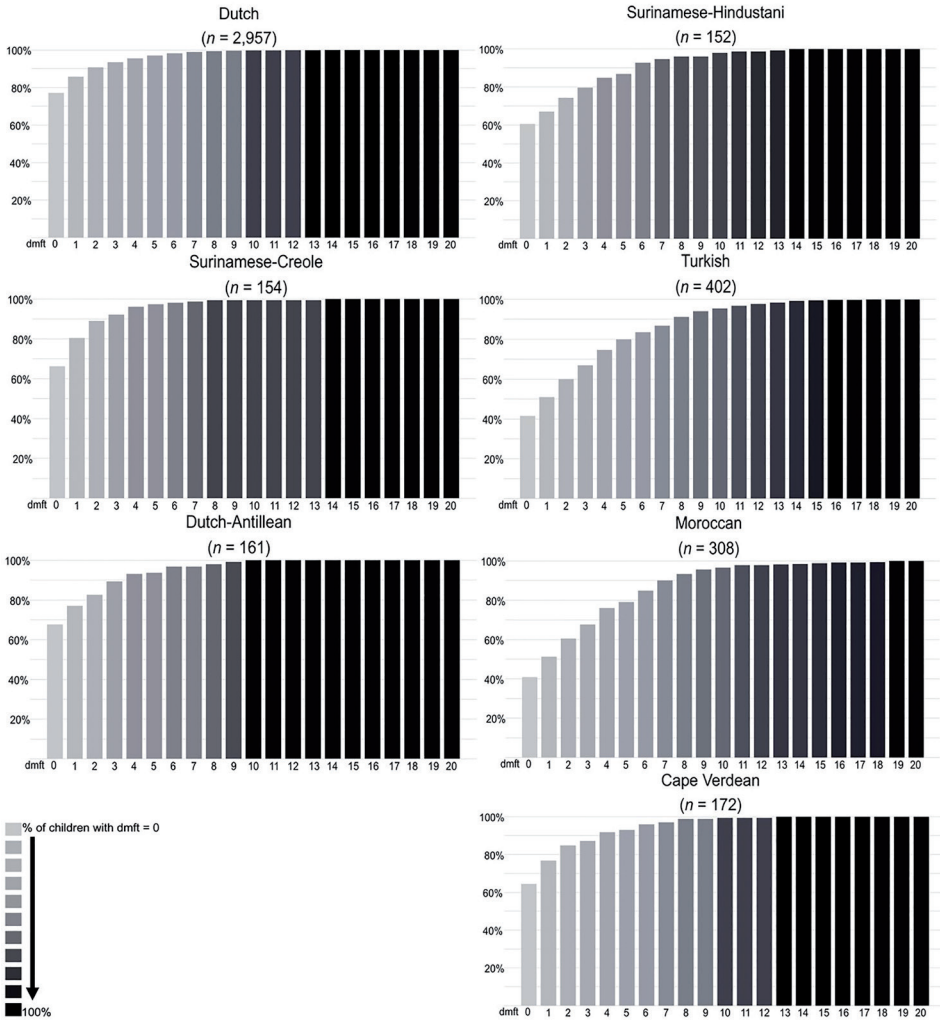


Figure 3.1.1. Cumulative caries distribution per ethnic group.

in the binary logistic regression model. After correction for SES-related indicators the association between Surinamese-Creoles and mild caries as well as Cape Verdean and severe caries lost significance. The influence of SES factors was more profound in the relation between ethnicity and severe caries groups than for the relation with mild caries and explained up to 43% of the association in the Turkish group. However, the associations between Surinamese-Hindustani (OR, 2.36; 95% CI, 1.49 to 3.73), Turkish (OR, 5.12; 95% CI, 3.73 to 7.04), or Moroccan background and severe caries (OR, 5.12; 95% CI, 3.54 to 7.41) remained significant after correcting for SES factors. Correcting

Table 3.1.2. Associations of ethnicity with dmft indices, after adjustment for SES factors and oral health behaviour (n = 4,306)

			Ethnicity groups					
Caries measures		Dutch	Surinamese-Hindustani	Surinamese-Creole	Turkish	Dutch Antillean	Moroccan	Cape Verdean
dmft		n = 2,957	n = 152	n = 154	n = 402	n = 161	n = 308	n = 172
(0 vs. > 0)	Crude model[1] OR (95% CI)	Reference	**2.06 (1.46–2.89)**	**1.45 (1.02–2.07)**	**4.48 (3.60–5.58)**	1.28 (0.90–1.83)	**4.43 (3.46–5.67)**	**1.63 (1.17–2.26)**
	+ SES factors[2] OR (95% CI)	Reference	**1.54 (1.08–2.19)**	1.18 (0.81–1.70)	**3.18 (2.50–4.04)**	0.96 (0.66–1.40)	**3.25 (2.47–4.27)**	1.16 (0.81–1.65)
	+ Oral health behaviour[3] OR (95% CI)	Reference	**2.02 (1.42–2.86)**	1.42 (0.98–2.04)	**4.34 (3.42–5.52)**	1.27 (0.88–1.84)	**4.27 (3.29–5.54)**	**1.56 (1.11–2.20)**
dmft	n_1 / n_2	484 / 2281	29 / 92	40 / 102	102 / 167	35 / 109	83 / 126	39 / 111
(0 vs. 1-3)	Crude model[1] OR (95% CI)	Reference	1.39 (0.90–2.14)	**1.58 (1.07–2.33)**	**2.73 (2.09–3.57)**	1.22 (0.81–1.82)	**2.85 (2.12–3.83)**	1.47 (1.00–2.15)
	+ SES factors[2] OR (95% CI)	Reference	1.15 (0.74–1.79)	1.38 (0.93–2.05)	**2.18 (1.63–2.90)**	1.00 (0.66–1.53)	**2.32 (1.69–3.21)**	1.16 (0.78–1.74)
	+ Oral health behaviour[3] OR (95% CI)	Reference	1.36 (0.88–2.11)	**1.54 (1.04–2.28)**	**2.66 (2.00–3.52)**	1.19 (0.79–1.80)	**2.74 (2.02–3.72)**	1.40 (0.94–2.07)
	n_1 / n_2	192 / 2281	31 / 92	12 / 102	133 / 167	17 / 109	99 / 126	22 / 111
(0 vs. > 3)	Crude model[1] OR (95% CI)	Reference	**3.77 (2.43–5.84)**	1.14 (0.61–2.13)	**8.90 (6.76–11.7)**	1.44 (0.84–2.50)	**8.43 (6.21–11.4)**	**2.04 (1.25–3.32)**
	+ SES factors[2] OR (95% CI)	Reference	**2.36 (1.49–3.73)**	0.79 (0.41–1.53)	**5.12 (3.73–7.04)**	0.90 (0.50–1.61)	**5.12 (3.54–7.41)**	1.18 (0.69–2.01)
	+ Oral health behaviour[3] OR (95% CI)	Reference	**3.69 (2.35–5.81)**	1.11 (0.58–2.13)	**8.63 (6.28–11.9)**	1.48 (0.83–2.62)	**8.16 (5.80–11.5)**	**1.97 (1.18–3.28)**

[1] Values are odds ratios with 95% confidence interval (CI) for the crude model adjusted for child's gender and age at dmft assessment; [2] for the crude model additionally adjusted for SES factors (educational level mother and household income); [3] for the crude model additionally adjusted for oral health behaviour related factors (children's age at first dental visit, dental visit in the past year and toothbrushing frequency); Significant associations are **bold**. SES = Social Economic Status; dmft = decayed, missing, and filled teeth; n_1 / n_2 = odds for having caries versus no caries if same ethnic background.

for oral health behaviour related indicators did not have any influence on the OR in the multinomial logistic regression analysis. The sensitivity analyses showed no difference in outcomes between the imputed and non-imputed dataset. Results for the non-imputed dataset are presented in a supplemental table (Table S3.1.1).

Discussion

Our results showed great disparities in caries prevalence between different ethnic groups living Rotterdam, the Netherlands. We confirmed a significantly higher prevalence of dental caries among non-Dutch ethnic children and the highest prevalence was observed among Surinamese-Hindustani, Turkish, and Moroccan children. These ethnic disparities were only partly explained by maternal educational level and household income, both indicators for SES. Parent-reported oral health behaviour related indicators did not explain any of the ethnic disparities.

Ethnic disparities in dental caries risk have been reported from all over the world [9, 26]. Most studies were conducted in subjects having a permanent dentition, but we showed that these already exist during childhood. Previous studies in the Netherlands indicated that caries prevalence might be higher in Turkish and Moroccan children. However, these were not conclusive, most likely because of their small sample size, especially among the ethnic minority groups [10, 18, 27]. To our knowledge, this study is the first in Europe to address ethnic disparities in dental caries prevalence among six-year-old children in a large population based multi-ethnic cohort.

SES plays an important role in the association between ethnicity and oral health [13]. In addition, we showed that the importance of SES, indicated by mother's education and household income, in the relationship between ethnicity and dmft indices increases with the severity of the caries experience. However, the current study also shows that ethnic disparities in dental caries are not fully explained by mother's education and household income. Generally, the dental literature proposes three hypotheses that might explain the association; bad oral hygiene, unhealthy dietary habits with high and frequent sugar consumption, and genetic factors [28-30]. In our results, in disagreement with Vanobbergen et al. [28], oral health behaviour did not attribute to the differences in caries prevalence between the ethnic groups. Within the scope of our study we were not able to investigate sugar consumption. Still, we strongly suggest it is a possible mechanism behind the association [30-32]. The literature is not conclusive, but ethnic minority groups tend to have higher sugar consumption compared to native Dutch [27, 33]. A new approach is the genome wide association studies that may shed some light on the susceptibility to dental caries. While the specific genes influencing the risk of

dental caries still remain largely unknown, genes may play a relevant role in explaining ethnic disparities in dental caries [29, 34, 35].

Definitely ethnic minorities need to be specifically targeted in oral disease prevention strategies, because they appeared to be more vulnerable for dental disease. To date, attempting to enhance oral health behaviour by awareness raising programs in ethnic minority groups has only a limited positive effect on oral health [36]. The way ethnicity influences dental caries is not well known yet. Therefore, Duijster et al. [37] tried to tackle modifiable risk factors that could be responsible for ethnic inequalities in the prevalence of childhood dental caries. They investigated parental and family factors as possible mediators and found that children without caries had parents with a more internal locus of control regarding dental health and that they were more likely to show positive parenting practices compared to parents of children with dental caries [37]. Interestingly, the locus of control regarding oral health was more external in parents with a Turkish or Moroccan background, compared to parents with a Dutch background. Thus, parental factors need to be further examined when prevention strategies are being developed. Research on behavioural, biological, psychological, or family factors explaining the relation between ethnicity and oral health is important, because health promotion strategies are only successful when they target these modifiable risk factors, instead of ethnicity as such.

The major strength of our study was the large population of children, so that different ethnic minority groups could be compared to each other. Furthermore, the prospective design of the Generation R study was well suited to investigate caries risk by embedding our cross-sectional analysis within the cohort, as caries is still a common population disease. Some limitations of our study need to be addressed. Since oral examination is the gold standard for clinically diagnosing dental caries, one limitation might be the assessment of dental caries from intraoral photographs [38]. The use of intraoral photographs has been validated by Elfrink et al. [21], but the performance was only moderate. Thus, the assessment of dmft indices in this study might be affected by a non-differential measurement error leading to an underestimation of the association between ethnicity and dental caries. Still, the present caries data are much more reliable than data obtained from self-report. In our study we have only found a minimal influence of oral health behaviour on the odds for having caries, although they are known risk factors for dental caries in young children [39]. Possible explanations for this could be a tendency of parents towards reporting socially desirable answers and the cross-sectional setting of our study. The effect of preventive behaviour is only visible over time and should therefore be evaluated in longitudinal data. Moreover, the effect of toothbrushing frequency on dental caries is proven, but might be influenced by

unhealthier diets [40]. This reinforces our suggestion that the diet of children explains some of the ethnic disparities in oral health. However, we were not able to evaluate the diet of children, which poses another limitation of our study. Power limitations are another point of attention for Surinamese-Creole, Dutch Antillean, and Cape Verdean children in the dmft > 3 category. Results of the multinomial regression for these categories should therefore be interpreted with caution. Furthermore, slightly less participants from ethnic minority groups and lower SES were included in the Generation R Study than expected from the population figures in Rotterdam, the Netherlands [19]. This may have led to a reduced generalizability of our study results, but exploring the mean caries prevalence in children from the Netherlands is beyond the scope of our paper. Missing information on dental caries was equally distributed between the ethnic groups. As some SES and oral behaviour variables had more than 10% missing data due to non-response, we used a multiple imputation method to dissolve possible bias from missing data. No significant differences in the outcome were observed in the sensitivity analysis that compared results from the original dataset with the results from the imputed dataset. In this study a wide array of important measures could be controlled for. However, residual confounding due to unmeasured SES and oral health indicators should still be considered.

In conclusion, the odds of having dental caries were much higher among children from all ethnic minority groups compared to the odds in native Dutch children. It is alarming to observe these differences in caries prevalence in a developed country as the Netherlands, in which oral health care and education is available for everyone. The magnitude of this problem in specific ethnic minority groups was not yet known. Our results emphasize to critically appraise health policies concerning prevention of dental caries. Public health strategies should apply this new knowledge and specifically focus at parents and children from these groups. More research is needed to find an explanation for the high caries prevalence among different ethnic (minority) groups.

References

[1] World Health Organization: fact sheet N°318: Oral Health, 2012. http://www.who.int/mediacentre/factsheets/fs318/en/#. (Accessed December 8 2015).

[2] P. Martins-Júnior, R. Vieira-Andrade, P. Corrêa-Faria, F. Oliveira-Ferreira, L. Marques, M. Ramos-Jorge, Impact of early childhood caries on the oral health-related quality of life of preschool children and their parents., Caries Res 47(3) (2013) 211-218.

[3] A. Rugg-Gunn, Dental caries: strategies to control this preventable disease., Acta Med Acad 42(2) (2013) 117-130.

[4] O. Gishti, R. Gaillard, J. Felix, S. Bouthoorn, E. Steegers, H. Raat, A. Hofman, L. Duijts, O. Franco, V. Jaddoe, Early origins of ethnic disparities in cardiovascular risk factors, Prev Med 76 (2015) 84-91.

[5] A. Khanolkar, S. Wedrén, B. Essén, P. Sparén, I. Koupil, Preterm and postterm birth in immigrant- and Swedish-born parents: a population register-based study, Eur J Epidemiol 30(5) (2015) 435-447.

[6] N. Mehta, H. Lee, K. Ylitalo, Child health in the United States: recent trends in racial/ethnic disparities., Soc Sci Med 95 (2013) 6-15.

[7] G. Truin, A. Schuller, J. Poorterman, J. Mulder, Trends in de prevalentie van cariës bij de 6- en 12-jarige jeugd in Nederland, Ned Tijdschr Tandheelkd 117 (2010) 143-147.

[8] C. Guarnizo-Herreño, G. Wehby, Explaining racial/ethnic disparities in children's dental health: a decomposition analysis, Am J Public Health 102(5) (2012) 859-66.

[9] G. Matsuo, R. Rozier, A. Kranz, Dental Caries: Racial and Ethnic Disparities Among North Carolina Kindergarten Students, Am J Public Health. 105(12) (2015) 2503–2509.

[10] A. Schuller, I. van Kempen, J. Poorterman, G. Verrips, Kies voor tanden: Een onderzoek naar mondgezondheid en preventief tandheelkundig gedrag van jeugdigen., 2013. www.tno.nl/media/1167/kiesvoortanden_tnols2013r10056.pdf. (Accessed December 10 2015).

[11] T. Wigen, N. Wang, Caries and background factors in Norwegian and immigrant 5-year-old children., Community Dent Oral Epidemiol 38(1) (2010) 19–28.

[12] M. Marmot, J. Allen, R. Bell, E. Bloomer, P. Goldblatt, Consortium for the European Review of Social Determinants of Health and the Health Divide. Divide, WHO European review of social determinants of health and the health divide, Lancet 380(9846) (2012) 1011-1029.

[13] F. Schwendicke, C. Dörfer, P. Schlattmann, L. Foster Page, W. Thomson, S. Paris, Socioeconomic inequality and caries: a systematic review and meta-analysis., J Dent Res 94(1) (2015) 10-18.

[14] A. Schuller, P. van Dommelen, J. Poorterman, Trends in oral health in young people in the Netherlands over the past 20 years: a study in a changing context., Community Dent Oral Epidemiol 42(2) (2014) 178-184.

[15] C. Stecksén-Blicks, P. Hasslöf, C. Kieri, K. Widman, Caries and background factors in Swedish 4-year-old children with special reference to immigrant status, Acta Odontol Scand 72(8) (2014) 852-858.

[16] M. Fontana, E. Santiago, G. Eckert, A. Ferreira-Zandona, Risk factors of caries progression in a Hispanic school-aged population, J Dent Res 90(10) (2011) 1189-1196.

[17] T. Marthaler, Changes in dental caries 1953-2003., Caries Res 38(3) (2004) 173-181.

[18] G. Truin, J. Frencken, J. Mulder, A. Kootwijk, E. de Jong, Prevalentie van tandcariës en tanderosie bij Haagse schoolkinderen in de periode 1996-2005, Ned Tijdschr Tandheelkd 114 (2007) 335-342.

[19] V. Jaddoe, C. van Duijn, O. Franco, A. van der Heijden, M. van IJzendoorn, J. de Jongste, A. van der Lugt, J. Mackenbach, H. Moll, H. Raat, F. Rivadeneira, E. Steegers, H. Tiemeier, A. Uitterlinden, F. Verhulst, A. Hofman, The Generation R Study: design and cohort update 2012, Eur J Epidemiol 27(9) (2012) 739-756.

[20] CBS, Centraal Bureau voor de Statistiek: Jaarrapport Integratie 2014, 2014. http://www.cbs.nl/NR/rdonlyres/4735C2F5-C2C0-49C0-96CB-0010920EE4A4/0/jaarrapportintegratie2014pub.pdf. (Accessed December 15 2015).
[21] M. Elfrink, J. Veerkamp, I. Aartman, H. Moll, J. Ten Cate, Validity of scoring caries and primary molar hypomineralization (DMH) on intraoral photographs, JM Eur Arch Paediatr Dent 10(1 Suppl) (2009) 5-10.
[22] J. Landis, G. Koch, An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers, Biometrics 33(2) (1977) 363-374.
[23] J. Pulache, J. Abanto, L. Oliveira, M. Bönecker, J. Porras, Exploring the association between oral health problems and oral health-related quality of life in Peruvian 11- to 14-year-old children, Int J Paediatr Dent 26(2) (2016) 81-90.
[24] J. Sterne, I. White, J. Carlin, M. Spratt, P. Royston, M. Kenward, A. Wood, J. Carpenter, Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls, BMJ 338 (2009) b2393.
[25] E. von Elm, D. Altman, M. Egger, S. Pocock, P. Gøtzsche, J. Vandenbroucke, Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies., BMJ 335(7624) (2007) 806-8.
[26] E. Delgado-Angulo, E. Bernabé, W. Marcenes, Ethnic inequalities in dental caries among adults in East London, J Public Health (Oxf) (2015) [Epub ahead of print].
[27] G. Verrips, H. Kalsbeek, M. Eijkman, Ethnicity and maternal education as risk indicators for dental caries, and the role of dental behavior, Community Dent Oral Epidemiol 21(4) (1993) 209-214.
[28] J. Vanobbergen, L. Martens, E. Lesaffre, K. Bogaerts, D. Declerck, Assessing risk indicators for dental caries in the primary dentition, Community Dent Oral Epidemiol 29(6) (2001) 424-434.
[29] J. Morrison, C. Laurie, M. Marazita, A. Sanders, S. Offenbacher, C. Salazar, M. Conomos, T. Thornton, D. Jain, C. Laurie, K. Kerr, G. Papanicolaou, K. Taylor, L. Kaste, J. Beck, J. Shaffer, Genome-wide association study of dental caries in the Hispanic Communities Health Study/Study of Latinos (HCHS/SOL), Hum Mol Genet 25(4) (2016) 807-816.
[30] M. Peres, A. Sheiham, P. Liu, F. Demarco, A. Silva, M. Assunção, A. Menezes, F. Barros, K. Peres, Sugar Consumption and Changes in Dental Caries from Childhood to Adolescence, J Dent Res 95(4) (2016) 388-394.
[31] I. Johansson, P. Holgerson, N. Kressin, M. Nunn, A. Tanner, Snacking habits and caries in young children, Caries Res 44 (2010) 421-430.
[32] W. Sohn, B. Burt, M. Sowers, Carbonated soft drinks and dental caries in the primary dentition, J Dent Res 85(3) (2006) 262-266.
[33] E. de Boer, H. Brants, M. Beukers, M. Ocké, L. Dekker, M. Nicolaou, M. Snijder, Voeding van Marokkaanse, Turkse, Surinaamse en autochtone Nederlanders in Amsterdam, RIVM rapport 2015-0099 (2015).
[34] X. Wang, M. Willing, M. Marazita, S. Wendell, J. Warren, B. Broffitt, B. Smith, T. Busch, A. Lidral, S. Levy, Genetic and environmental factors associated with dental caries in children: the Iowa Fluoride Study, Caries Res 46(3) (2012) 177-184.
[35] R. Werneck, F. Lázaro, A. Cobat, A. Grant, M. Xavier, L. Abel, A. Alcaïs, P. Trevilatto, M. Mira, A major gene effect controls resistance to caries, J Dent Res 90(6) (2011) 735-739.
[36] L. Schou, C. Wight, Does dental health education affect inequalities in dental health?, Community Dent Health 11(2) (1994) 97-100.
[37] D. Duijster, M. de Jong-Lenters, C. de Ruiter, J. Thijssen, C. van Loveren, E. Verrips, Parental and family-related influences on dental caries in children of Dutch, Moroccan and Turkish origin, Community Dent Oral Epidemiol 43(2) (2015) 152-162.

[38] T. Gimenez, C. Piovesan, M. Braga, D. Raggio, C. Deery, D. Ricketts, K. Ekstrand, F. Mendes, Visual Inspection for Caries Detection: A Systematic Review and Meta-analysis, J Dent Res 94(7) (2015) 895-904.
[39] R. Harris, A. Nicoll, P. Adair, C. Pine, Risk factors in young children: a systematic review of the literature, Community Dent Health 21 (2014) 71-85.
[40] S. Kumar, J. Tadakamadla, N. Johnson, Effect of Toothbrushing Frequency on Incidence and Increment of Dental Caries: A Systematic Review and Meta-Analysis, J Dent Res 95(11) (2016) 1230-1236.

Table S3.1.1. Associations of ethnicity with dmft indices, after adjustment for SES factors and oral health behaviour, original dataset (n = 4,306)

Caries measures		Ethnicity groups						
		Dutch	Surinamese-Hindustani	Surinamese-Creole	Turkish	Dutch Antillean	Moroccan	Cape Verdean
dmft		n = 2,957	n = 152	n = 154	n = 402	n = 161	n = 308	n = 172
(0 vs. > 0)	Crude model[1] OR (95% CI)	Reference	**2.06 (1.46–2.89)**	**1.45 (1.02–2.07)**	**4.48 (3.60–5.58)**	1.28 (0.90–1.83)	**4.43 (3.46–5.67)**	**1.63 (1.17–2.26)**
		n = 2,564	n = 114	n = 112	n = 291	n = 124	n = 195	n = 110
	+ SES factors[2] OR (95% CI)	Reference	**1.65 (1.10–2.47)**	1.36 (0.89–2.07)	**3.19 (2.42–4.20)**	0.91 (0.59–1.40)	**2.74 (1.98–3.78)**	0.99 (0.63–1.54)
		n = 2,662	n = 123	n = 113	n = 295	n = 114	n = 203	n = 113
	+ Oral health behaviour[3] OR (95% CI)	Reference	**1.99 (1.36–2.93)**	**1.63 (1.08–2.45)**	**4.74 (3.67–6.14)**	1.17 (0.76–1.81)	**3.90 (2.89–5.25)**	**1.68 (1.11–2.53)**
dmft	n_1 / n_2	484 / 2,281	29 / 92	40 / 102	102 / 167	35 / 109	83 / 126	39 / 111
(0 vs. 1–3)	Crude model[1] OR (95% CI)	Reference	1.39 (0.90–2.14)	**1.58 (1.07–2.33)**	**2.73 (2.09–3.57)**	1.22 (0.81–1.82)	**2.85 (2.12–3.83)**	1.47 (1.00–2.15)
	n_1 / n_2	407 / 2,016	21 / 70	29 / 74	73 / 127	26 / 88	50 / 92	18 / 77
	+ SES factors[2] OR (95% CI)	Reference	1.17 (0.70–1.95)	1.52 (0.96–2.40)	**2.15 (1.54–3.00)**	0.97 (0.60–1.57)	**2.00 (1.35–2.95)**	0.81 (0.46–1.39)
	n_1 / n_2	422 / 2,088	21 / 77	29 / 75	74/124	22 / 81	54 / 93	22 / 74
	+ Oral health behaviour[3] OR (95% CI)	Reference	1.24 (0.75–2.04)	**1.71 (1.09–2.69)**	**2.81 (2.05–3.86)**	1.05 (0.64–1.73)	**2.59 (1.81–3.71)**	1.28 (0.78–2.11)
	n_1 / n_2	192 / 2,281	31 / 92	12 / 102	133 / 167	17 / 109	99 / 126	22 / 111

(0 vs. > 3)	Crude model[1] OR (95% CI)	Reference	**3.77 (2.43–5.84)**	1.14 (0.61–2.13)	**8.90 (6.76–11.7)**	1.44 (0.84–2.50)	**8.43 (6.21–11.4)**	**2.04 (1.25–3.32)**
	n_1 / n_2	141 / 2,016	23 / 70	9 / 74	91 / 127	10 / 88	53 / 92	15 / 77
	+ SES factors[2] OR (95% CI)	Reference	**2.75 (1.63–4.64)**	1.03 (0.49–2.15)	**5.43 (3.82–7.73)**	0.80 (0.39–1.63)	**4.38 (2.89–6.63)**	1.39 (0.75–2.57)
	n_1 / n_2	152 / 2,088	25 / 77	9 / 75	97 / 124	11 / 81	56 / 93	17 / 74
	+ Oral health behaviour[3] OR (95% CI)	Reference	**4.10 (2.51–6.70)**	1.42 (0.69–2.91)	**10.1 (7.29–14.0)**	1.52 (0.78–2.96)	**7.54 (5.15–11.0)**	**2.75 (1.56–4.85)**

[1] Values are odds ratios with 95% confidence interval (CI) for the crude model adjusted for child's gender and age at dmft assessment;
[2] for the crude model additionally adjusted for SES factors (education level mother and household income);
[3] for the crude model additionally adjusted for oral health behaviour related factors (children's age at first dental visit, dental visit in the past year and toothbrushing frequency);
Significant associations are bold. SES = Social Economic Status; dmft = decayed, missing, and filled teeth; n_1 / n_2 = odds for having caries versus no caries if same ethnic background.

Chapter 3.2

Consortium-based genome-wide meta-analysis for childhood dental caries traits

Simon Haworth* | Dmitry Shungin* | Justin T. van der Tas | Strahinja Vucic | Carolina Medina-Gomez | Victor Yakimov | Bjarke Feenstra | John R. Shaffer | Myoung Keun Lee | Marie Standl | Elisabeth Thiering | Carol Wang | Klaus Bønnelykke | Johannes Waage | Leon Eyrich Jessen | Pia Elisabeth | Nørrisgaard | Raimo Joro | Ikka Seppälä | Olli Raitakari | Tom Dudding | Olja Grgic | Edwin Ongkosuwito | Anu Vierola | Aino-Maija Eloranta | Nicola X. West | Steven J. Thomas | Daniel W. McNeil | Steven M. Levy | Rebecca Slayton | Ellen A. Nohr | Terho Lehtimäki | Timo Lakka | Hans Bisgaard | Craig Pennell | Jan Kühnisch | Mary L. Marazita | Mads Melbye | Frank Geller | Fernando Rivadeneira | Eppo B. Wolvius | Paul W. Franks | Ingegerd Johansson | Nicholas J. Timpson

*Shared first authors

Human Molecular Genetics, 2018, 27(17), 3113-3127
Supplemental material can be accessed via https://academic.oup.com/hmg/article/27/17/3113/5040780#supplementary-data

Abstract

Prior studies suggest dental caries traits in children and adolescents are partially heritable, but there has been no large-scale consortium genome-wide association study (GWAS) to date. We therefore performed GWAS for caries in participants aged 2.5–18.0 years from 9 contributing centres. Phenotype definitions were created for the presence or absence of treated or untreated caries, stratified by primary and permanent dentition. All studies tested for association between caries and genotype dosage and results were combined using fixed–effects meta-analysis. Analysis included up to 19,003 individuals (7,530 affected) for primary teeth and 13,353 individuals (5,875 affected) for permanent teeth. Evidence for association with caries status was observed at rs1594318-C for primary teeth (intronic within *ALLC*, Odds Ratio (OR) 0.85, Effect Allele Frequency (EAF) 0.60, p 4.13e-8) and rs7738851-A (intronic within *NEDD9*, OR 1.28, EAF 0.85, p 1.63e-8) for permanent teeth. Consortium-wide estimated heritability of caries was low (h^2 of 1% [95% CI: 0%:7%] and 6% [95% CI 0%:13%] for primary and permanent dentitions, respectively) compared to corresponding within-study estimates (h^2 of 28%, [95% CI: 9%:48%] and 17% [95% CI: 2%:31%]) or previously published estimates. This study was designed to identify common genetic variants with modest effects which are consistent across different populations. We found few single variants associated with caries status under these assumptions. Phenotypic heterogeneity between cohorts and limited statistical power will have contributed; these findings could also reflect complexity not captured by our study design, such as genetic effects which are conditional on environmental exposure.

Introduction

Dental caries remains a prevalent public health problem in both children and adults. Untreated dental caries was estimated to affect 621 million children worldwide in 2010, with little change in prevalence or incidence between 1990 and 2010 [1]. This problem is not unique to lower income countries; around 50% of children have evidence of caries by age 5 in industrialized nations [2-4]. Dental caries results from reduced mineral saturation of fluids surrounding teeth, driven by ecological shifts in the oral microbiome [5]. Many different factors predispose towards dental caries, of which high sugar consumption, poor oral hygiene and low socio-economic status are the most notorious [6-8]. Over the last decades there has been increasing appreciation for the role of genetic influences in dental caries. The importance of genetic susceptibility for dental caries experience was demonstrated in an animal model over 50 years ago, a finding since substantiated in twin studies in humans [9-11]. Of particular relevance to caries traits in children and adolescents, Bretz et al. analyzed longitudinal rates of change in caries status in children and found that caries progression and severity were highly heritable in the primary and permanent dentition [10]. It has also been suggested that heritability for dental caries does not depend entirely on genetic predisposition to sweet food consumption [12]. Despite evidence of a genetic contribution to caries susceptibility, few specific genetic loci have been identified.

Shaffer et al. performed the first GWAS for dental caries in 2011 [13], studying the primary dentition of 1,305 children. They found evidence for association at novel and previously studied candidate genes (*ACTN2*, *MTR*, *EDARADD*, *MPPED2* and *LPO*), but no individual single nucleotide polymorphisms (SNPs) exceeded the genome wide significance threshold ($p \leq 5.0e-08$), possibly as a consequence of the modest sample size [13]. The first GWAS for dental caries in the permanent dentition in adults was performed at a similar time by Wang et al. [14]. They included 7,443 adults from five different cohorts and identified several suggestive loci ($p \leq 10E-05$) for dental caries (*RPS6KA2*, *PTK2B*, *RHOU*, *FZD1*, *ADMTS3* and *ISL1*), different loci from those mentioned above for the primary dentition and again with no single variants reaching genome-wide significance.

The next wave of GWAS of caries suggested association at a range of different loci. Two GWAS used separate phenotype definitions for pit-and-fissure and smooth tooth surfaces and identified different loci associated with dental caries susceptibility in both primary and permanent dentition [15, 16]. The GWAS in primary dentition used a sample of approximately 1,000 children and found evidence for association at loci reported in previous studies, including *MPPED2*, *RPS6KA2*, and *AJAP1* [13-16]. The largest GWAS for dental caries in permanent dentition was performed in a Hispanic and

Latino sample of 11,754 adults [17]. This study identified unique genetic loci (*NAMPT* and *BMP7*) compared to previous GWAS in individuals of European ancestry. To date, it is unclear whether the variability in nominated loci reflects true variability in the genetic architecture of dental caries across different populations, age periods and sub-phenotypic definitions, or merely represent chance differences between studies given the modest power in the studies performed to date.

Dental caries is a complex and multifactorial disease, caused by a complex interplay between environmental, behavioral and genetic factors. Until now there has been a lack of large-scale studies of dental caries traits in children and the genetic basis of these traits remains poorly characterized. This investigation set out to examine the hypothesis that common genetic variants influence dental caries with modest effects on susceptibility. We anticipated that a) caries in both primary and permanent teeth would be heritable in children and adolescents aged 2.5 to 18 years and b) common genetic variants are likely to only have small effects on the susceptibility of a complex disease such as dental caries. Therefore, the aim of this large-scale, consortium-based GWAS is to examine novel genetic loci associated with dental caries in primary and permanent dentition in children and adolescents.

Methods

Study samples

We performed genome-wide association (GWA) analysis for dental caries case/control status in a consortium including 9 coordinating centres. Study procedures differed between these centres. We use the term 'clinical dental assessment' to mean that a child was examined in person, whether this was in a dental clinic or a study centre. We use the term 'examiner' to refer to a dental professional and use the term 'assessor' to refer to an individual with training who is not a dental professional, for example a trained research nurse.

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a longitudinal birth cohort which recruited pregnant women living near Bristol, UK with an estimated delivery date between 1991 and 1992. Follow up has included clinical assessment and questionnaires and is ongoing [43]. A subset of children attended clinics including clinical dental assessment by a trained assessor at age 31, 43 and 61 months of age. Parents were asked to complete questionnaires about their children's health regularly, including comprehensive questions at a mean age of 5.4 and 6.4 years. Parents and children were asked to complete questionnaires about oral health at a mean age of age 7.5, 10.7 and 17.8 years. Please note that the study website contains details of all

the data that are available through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/). Both clinical and questionnaire derived data were included in this analysis, with priority given to clinical data where available (Table S3.2.3).

The Copenhagen Prospective Studies on Asthma in Childhood includes two population based longitudinal birth cohorts in Eastern Denmark. COPSAC2000 recruited pregnant women with a history of asthma between 1998 and 2001 [44]. Children who developed wheeze in early life were considered for enrollment in a nested randomized trial for asthma prevention. COPSAC2010 recruited pregnant women between 2008 and 2010 and was not selected on asthma status. Both COPSAC2000 and COPSAC2010 studies included regular clinical follow up. Within Denmark clinical dental assessment is routinely offered to children and adolescents until the age of 18 years and summary data from these examinations are stored in a national register. These data were obtained via index linkage for participants of COPSAC2000 and COPSAC2010 and used to perform joint analysis across both cohorts.

The Danish National Birth Cohort (DNBC) is a longitudinal birth cohort which recruited women in mid-pregnancy from 1996 onwards [45]. For this analysis, index linkage was performed to obtain childhood dental records for mothers participating in DNBC. As with the COPSAC studies, these data were originally obtained by a qualified dentist and included surface level dental charting.

The Generation R study (GENR) recruited women in early pregnancy with expected delivery dates between 2002 and 2006 living in the city of Rotterdam, the Netherlands. The cohort is multi-ethnic with representation from several non-European ethnic groups. Follow-up has included clinical assessment visits and questionnaires and is ongoing [46]. Intra-oral photography was performed as a part of their study protocol, with surface level charting produced by a dental examiner (a specialist in paediatric dentistry) [47]. Analysis in GENR included a) a multi-ethnic association study including all individuals with genetic and phenotypic data [48] and b) analysis including only individuals of European ancestry.

The GENEVA consortium is a group of studies which undertake coordinated analysis across several phenotypes. [49] Within GENEVA, the Center for Oral Health Research in rural Appalachia, West Virginia and Pennsylvania, USA (COHRA), the Iowa Fluoride Study in Iowa, USA (IFS) and the Iowa Head Start (IHS) study participated in analysis of dental traits in children [50]. COHRA recruited families with at least one child aged between 1 and 18 years of age, with dental examination performed at baseline [51]. IFS recruited mothers and newborn infants in Iowa between 1992 and 1995 with a focus on longitudinal fluoride exposures and dental and bone health outcomes. Clinical

dental examination in IFS was performed by trained assessors age 5, 9 13 and 17 years [52]. IHS recruited children participating in an early childhood education program which included a one-time clinical dental examination [13].

The "German Infant study on the influence of Nutrition Intervention plus air pollution and genetics on allergy development" (GINIplus) is a multi-centre prospective birth cohort study which has an observational and interventional arm which conducted a nutritional intervention during the first four months of life. The study recruited new born infants with and without family history of allergy in the Munich and Wesel areas, Germany between 1995 and 1998 [53, 54].The "Lifestyle-related factors, Immune System and the development of Allergies in East and West Germany" study (LISA) is a longitudinal birth cohort which recruited between 1997 and 1999 across four sites in Germany [53, 55]. For participants living in the Munich area, follow up used similar protocols in both GINIplus and LISA, with questionnaire and clinic data including clinical dental examination by trained examiners at age 10 and 15 years. Analysis for caries in GINIplus and LISA was therefore performed across both studies for participants at the Munich study centre.

The Physical Activity and Nutrition in Children (PANIC) Study is an ongoing controlled physical activity and dietary intervention study in a population of children followed retrospectively since pregnancy and prospectively until adolescence. Altogether 512 children 6–8 years of age were recruited in 2008–2009 [56]. The main aims of the study are to investigate risk factors and pathophysiological mechanisms for overweight, type 2 diabetes, atherosclerotic cardiovascular diseases, musculoskeletal diseases, psychiatric disorders, dementia and oral health problems and the effects of a long-term physical activity and dietary intervention on these risk factors and pathophysiological mechanisms. Clinical dental examinations were performed by a qualified dentist with tooth level charting.

The Cardiovascular Risk in Young Finns Study (YFS) is a multi-centre investigation which aimed to understand the determinants of cardiovascular risk factors in young people in Finland. The study recruited participants who were aged 3, 6, 9, 12 ,15 and 18 years old in 1980. Eligible participants living in specific regions of Finland were identified at random from a national population register and were invited to participate. Regular follow-up has been performed through physical examination and questionnaires [57]. Clinical dental examination was performed by a qualified dentist with tooth level charting.

The Western Australian Pregnancy Cohort (RAINE) study is a birth cohort which recruited women between 16th and 20th week of pregnancy living in the Perth area, Western Australia. Recruitment occurred between 1989 and 1991 with regular follow

up of mothers and their children through research clinics and questionnaires [58]. The presence or absence of dental caries was recorded by a trained assessor following clinical dental examination at the year 3 clinic follow up.

Further details of study samples are provided in Table S3.2.1.

Medical ethics

Within each participating study written informed consent was obtained from the parents of participating children after receiving a full explanation of the study. Children were invited to give assent where appropriate. All studies were conducted in accordance with the Declaration of Helsinki.

Ethical approval for the ALSPAC study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committee. Full details of ethical approval policies and supporting documentation are available online (http://www.bristol.ac.uk/alspac/researchers/research-ethics/.) Approval to undertake analysis of caries traits was granted by the ALSPAC executive committee (B2356).

The COPSAC2000 cohort was approved by the Regional Scientific Ethical Committee for Copenhagen and Frederiksberg (KF 01-289/96) and the Danish Data Protection Agency (2008-41-1574). The 2010 cohort (COPSAC2010) was approved by the Danish Ethics Committee (H-B-2008-093) and the Danish Data Protection Agency (2008-41-2599).

The DNBC study of caries was approved by the Scientific Ethics Committee for the Capital City Region (Copenhagen), the Danish Data Protection Agency, and the DNBC steering committee.

Each participating site in the GENEVA consortium caries analysis received approval from the local university institutional review board (federal wide assurance number for GENEVA caries project: FWA00006790). Within the COHRA arm local approval was provided by the University of Pittsburgh (020703/0506048) and West Virginia University (15620B), whilst the IFS and IHS arms received local approval from the University of Iowa's Institutional Review Board.

The GENR study design and specific data acquisition were approved by the Medical Ethical Committee of the Erasmus University Medical Center, Rotterdam, the Netherlands (MEC-2007-413).

The GINIplus and LISA studies were approved by the ethics committee of the Bavarian Board of Physicians (10 year follow up: 05100 for GINIplus and 07098 for LISA, 15 year follow up 10090 for GINIplus, 12067 for LISA).

The PANIC study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo. All participating children and their parents gave informed written consent.

The YFS study protocol was approved by local ethics committees for contributing sites.

The RAINE study was approved by the University of Western Australia Human Research Ethics Committee.

Phenotypes

Primary teeth exfoliate and are replaced by permanent teeth between 6 and 12 years of age. We aimed to separate caries status in primary and permanent teeth wherever possible using clinical information or age criteria, in line with our expectation that the genetic risk factors for dental caries might differ between primary and permanent dentition. For children in the mixed dentition we created two parallel case definitions, whilst in younger or older children a single case definition was sufficient.

All study samples included a mixture of children with dental caries and children who were caries-free, with varying degrees of within-mouth or within-tooth resolution. To facilitate comparison across these differing degrees of resolution all analysis compared children who were caries-free (unaffected) or had dental caries (affected). Missing teeth could represent exfoliation or delayed eruption rather than the endpoint of dental caries and therefore missing teeth were not included in classifying children as caries-free or caries affected.

In children aged 2.50 years to 5.99 years any individual with 1 or more decayed or filled tooth was classified as caries affected, with all remaining individuals classified as unaffected. In children aged 6.00 years to 11.99 years of age parallel definitions were determined for the primary dentition and permanent dentition respectively. Any individual with at least 1 decayed or filled primary tooth was classified as caries affected for primary teeth, while all remaining participants were classified as unaffected. In parallel, any individual with at least 1 decayed or filled permanent tooth was classified as caries affected for permanent teeth, while all remaining individuals were classified as unaffected. In children and adolescents aged 12.00 to 17.99 years of age any individual with 1 or more decayed or filled tooth or tooth surface (excluding third molar teeth) was classified as caries affected, with remaining individuals classified as unaffected.

Analysis was conducted in cross-section, meaning a single participant could only be represented in a single phenotype definition once. Where multiple sources of dental data were available for a single participant within a single phenotype definition window, the first source of data was selected (reflecting the youngest age at participation), in line with our expectation that caries status would be most heritable in the near-eruption period.

The sources of data used to create these phenotypic definitions are given in Table S3.2.3. Within ALSPAC only, questionnaire responses were used to supplement data

from clinical examination. The questions asked did not distinguish between primary and permanent teeth. Based on the age at questionnaire response we derived variables which prioritized responses from questionnaires before 6.00 years of age (thought to predominantly represent caries in primary teeth), and responses after 10.00 years of age (which might predominantly represent caries in permanent teeth). The final data sweep considered in this analysis targeted adolescents at age 17.50 years. Some participants responded to this after their eighteenth birthday. Data derived from this final questionnaire sweep were not included in the principal meta-analyses but were included in the GCTA heritability analysis.

Genotypes and imputation

All participating studies used genetic data imputed to a comprehensive imputation panel. The 1,000 genomes phase 1 version 3 panel (1KG phase 1 v3) was used as a common basis across 6 centres (GINIplus/LISA, GENR, GENEVA, YFS, PANIC, RAINE (Table S3.2.1). In ALSPAC, DNBC, COPSAC2000 and COPSAC 2010 the haplotype reference consortium (HRC v1.0 and v1.1) imputation panels were used (Table S3.2.1).

Each study performed routine quality control measures during genotyping, imputation and association testing (Table S3.2.2). Further pre-meta-analysis quality control was performed centrally using the EasyQC R package and accompanying 1KG phase1 v3 reference data [59]. Minor allele count (MAC) was derived as the product of minor allele frequency and site-specific number of alleles (twice the site-specific sample size). Variants were dropped which had a per-file MAC of 6 or lower, a site-specific sample size of 30 or lower, or an impute INFO score of less than 0.4. Sites which reported effect and non-effect alleles other than those reported in 1KG phase 1 v3 reference data were dropped. Following meta-analysis, sites with a weighted minor allele frequency (MAF) of less than 0.005were dropped, along with variants present in less than 50% of the total sample.

Statistical analysis

Association testing

Each cohort preformed GWA analysis using an additive genetic model. Caries status was modelled against genotype dosage whilst accounting for age at phenotypic assessment, age squared, sex and cryptic relatedness. Sex was accounted for by deriving phenotypic definitions and performing analysis separately within male and female participants, or by including sex as a covariate in association testing. Each study adopted approaches to account for cryptic relatedness and population stratification, as described in Table S3.2.2. In the GENR study parallel analyses were conducted for participants of European

ancestry (using the approach described in Table S3.2.2) and the entire study population, using a previously published method [48]. The software and exact approach used by each study is shown in Table S3.2.2.

Meta-analysis

Results of GWA analysis within each study were combined in two principal meta-analyses, representing caries status in primary teeth and caries status in permanent teeth. For primary teeth, parallel meta-analyses were performed, one using results of multi-ethnic analysis in the GENR study and the other using results of European ancestry analysis in the GENR study. The GENR study did not have phenotypic data for permanent teeth, therefore the analysis of permanent teeth contained only individuals of European ancestry. Fixed-effects meta-analyses was performed using METAL [60], with genomic control of input summary statistics enabled and I^2 test for heterogeneity. Meta-analysis was run in parallel in two centres and results compared. All available studies with genotype and phenotypic information were included in a one stage design, therefore there was no separate replication stage.

Meta-analysis heritability estimates

For each principal meta-analysis population stratification and heritability were assessed using linkage disequilibrium score regression (LDSR) [61]. Reference linkage disequilibrium (LD) scores were taken from HapMap3 reference data accompanying the LDSR package.

Within-sample heritability estimates

For comparison, heritability within the ALSPAC study was assessed using the GREML method [62], implemented in the GCTA software package [63], using participant level phenotype data and a genetic relatedness matrix estimated from common genetic variants (with MAF > 5.0%) present in HapMap3.

Hypothesis free cross trait lookup

We used PLINK 2.0 [64] to clump meta-analysis summary statistics based on LD structure in reference data from the UK10K project. We then performed hypothesis-free cross-trait lookup of independently associated loci using the SNP lookup function in the MRBase catalogue [65]. Proxies with an r^2 of 0.8 or higher were included where the given variant was not present in an outcome of interest. We considered performing hypothesis free cross-trait genetic correlation analysis using bivariate LD score regression implemented in LDhub [66].

Lookup in previously published pediatric caries GWAS

Previously published caries GWAS was performed within the GENEVA consortium, which is also represented in our meta-analysis. We therefore did not feel it would be informative to undertake lookup of associated variants in previously published results.

Lookup in GWAS for adult caries traits

This analysis was planned and conducted in parallel with analysis of quantitative traits measuring lifetime caries exposure in adults (manuscript in draft). The principal trait studied in the adult analysis was an index of decayed, missing and filled tooth surfaces (DMFS). This index was calculated from results of clinical dental examination, excluding third molar teeth. The DMFS index was age-and-sex standardized within each participating adult study before GWAS analysis was undertaken. Study-specific results files were then combined in a fixed-effects meta-analysis. In addition to DMFS, two secondary caries traits were studied in adults, namely number of teeth (a count of remaining natural teeth at time of study participation) and standardized DFS (derived as the number of decayed and filled surfaces divided by the number of natural tooth surfaces remaining at time of study participation). After age-and-sex standardization these secondary traits had markedly non-normal distribution and were therefore underwent rank-based inverse normal transformation before GWAS analysis and meta-analysis. We performed cross-trait lookup of lead associated variants in the pediatric caries meta-analysis against these three adult caries traits. As the unpublished analysis also contains samples which contributed to previously published GWAS, we did not feel it would be informative to undertake additional lookup in published data.

Gene prioritization, gene set enrichment and association with predicted gene transcription

Gene based testing of summary statistics was performed using MAGMA [67] with reference data for LD correction taken from the UK10K project and gene definitions based on a 50 kilobase window either side of canonical gene start:stop positions. Gene set enrichment analysis was considered using the software package DEPICT [68]. Tests for association between phenotype and predicted gene transcription were performed using S-PrediXcan [69], which is a summary-statistic implementation of the PrediXcan method. This method aims to assess the effects of tissue-specific gene transcription on phenotypes. Gene transcription models are trained in datasets with transcriptomic data, then used to predict gene expression in datasets with phenotypic data. This method was applied using the MetaXcan standalone software (https://github.com/hakyimlab/MetaXcan) and a transcription prediction model trained in whole blood (obtained from

the PedictDB data repository at http://predictdb.org/) [70]. Bonferroni correction was applied on the basis of approximately 7,000 independent gene-based tests for 2 caries traits, giving an experiment wide significance level of approximately $p < 3.6e-06$.

Power calculations

Post-hoc power calculations were performed using the free, web-based tool Genetic Association Study (GAS) Power Calculator and the software utility Quanto (v1.2.4) (https://csg.sph.umich.edu/ abecasis/gas_power_calculator/index.html, http://biostats.usc.edu/Quanto.html) [71]. Using the sample size and caries prevalence of the final meta-analysis samples, we calculated the minimum effect size required to have 80% discovery power at a significance level of 5.0e-08 for variants with MAF between 0.05 and 0.50. For primary teeth (17,037 individuals, 6,922 caries affected, prevalence 40.6%) we were able to detect variants with a minimal effect size (OR) between 1.13 and 1.37 for variants with MAF of 0.50 and 0.05, respectively (1.15 for MAF of 0.40) (Figure S3.2.4, Figure S3.2.5). For permanent teeth (13,353 individuals of which 5,875 were caries-affected, prevalence 44.0%) we had 80% power to detect variants with a minimal effect size (OR) between 1.15 and 1.43 for variants with MAF of 0.50 and 0.05, respectively (1.17 for MAF of 0.40) (Figure S3.2.4, Figure S3.2.5).

Results

Single variant results

Meta-analysis of caries in primary teeth in individuals of European ancestry included 17,037 individuals (6,922 affected) from 22 results files representing all 9 coordinating centres. After final quality control (QC), this meta-analysis included 8,640,819 variants, with mild deflation (genomic inflation factor (λ) = 0.994) (Figure S3.2.1). Meta-analysis of caries in primary teeth which included individuals of multiple ethnicities in the Generation R (GENR) study included 19,003 individuals (7,530 affected) from 22 results files representing all 9 coordinating centres. There were 8,699,928 variants after final QC, with mild deflation in summary statistics (λ = 0.986) (Figure S3.2.2). Analysis of caries status in permanent teeth included 13,353 individuals (5,875 affected) from 14 results files representing 7 coordinating centres. The sample size was smaller for permanent teeth as two coordinating centres did not have phentoype data for permanent teeth (RAINE and GENR), whilst the COPSAC group only had data for participants in the earlier birth cohort (COPSAC 2000). There were 8,734,121 variants after final QC, with mild deflation in summary statistics (λ = 0.999) (Figure S3.2.3).

The strongest evidence for association with caries in primary teeth was seen at rs1594318 (OR 0.85 for C allele, EAF 0.60, p = 4.13e-08) in the European ancestry meta-analysis (Figures 3.2.1, 3.2.2 and 3.2.3, Table 3.2.1). This variant is intronic within *ALLC* on 2p25, a locus which has not previously been reported for dental caries traits. In the meta-analysis combining individuals of all ancestories this variant no longer reached genome-wide significance, although suggestive evidence persisted at rs1594318 (OR 0.868 for C allele EAF 0.60 p = 3.78e-07) and other intronic variants within *ALLC* in high linkage disequilibrium (Figure 3.2.3). For the permanent dentition the strongest statistical evidence for association was seen between caries status and rs7738851 (OR 1.28 for A allele, EAF 0.85, p = 1.63e-08) (Figures 3.2.1, 3.2.2 and 3.2.4, Table 3.2.1). This variant is intronic within *NEDD9* on 6p24.

Estimated heritabiltiy

Using participant level data in ALSPAC heritability was estimated at 0.28 (95% CI 0.09:0.48) and 0.17 (95% CI 0.02:0.31) for primary and permanent teeth respectively. Using summary statistics at the meta-analysis level produced point estimates near zero heritability, with wide confidence intervals (Table 3.2.2).

Cross-phenotype comparisons

Genome-wide mean chi squared was too low to undertake genome-wide genetic correlation using the LDSR method for caries in either primary or permanent teeth. Hypothesis-free phenome wide lookup for rs1594318 included 885 GWAS where either rs1594318 or a proxy with $r^2 > 0.8$ was present. None of these traits showed evidence of association with rs1594318 at a Bonferroni-corrected alpha of 0.05. Lookup of rs7738851 and its proxies was performed against 662 traits, where similarly no traits reached a Bonferroni-corrected threshold. Hypothesis-driven lookup in adult caries traits revealed no strong evidence for persistent genetic effects into adulthood (Table 3.2.3).

Gene prioritization, gene set enrichment and association with predicted gene transcription

Gene based tests identified association between caries status in the primary dentition and a region of 7q35 containing *TCAF1*, *OR2F2* and *OR2F1* (p = 1.91e-06, 1.58e-06 and 1.29e-06, respectively). There were insufficient independently associated loci to perform gene set enrichment analysis using DEPICT for either of the principal meta-analyses. Association with predicted gene transcription was tested but no genes met

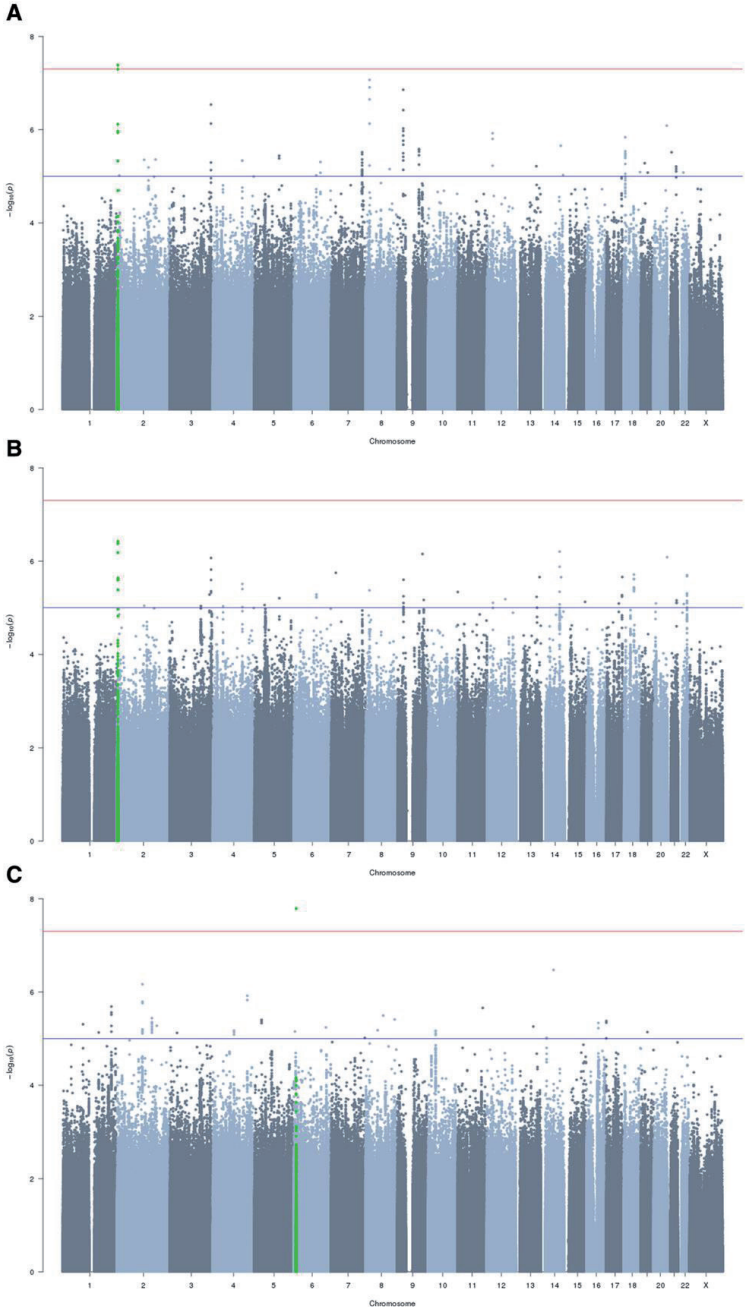


Figure 3.2.1. Manhattan plots for each principal meta-analysis.
(A) Caries in primary teeth (European ancestry), n samples = 17,036, n variants = 8,640,819, λ = 0.9944. Variants within 500Kb of rs1594318 are highlighted in green. **(B)** Caries in primary teeth (multi-ethnic analysis), n samples = 19,003, n variants = 8,699,928, λ =0.9861. **(C)** Caries in permanent teeth (European ancestry), n samples = 13,353, n variants = 8,734,121, λ =0.9991. Variants within 500Kb of rs7738851 are highlighted in green.

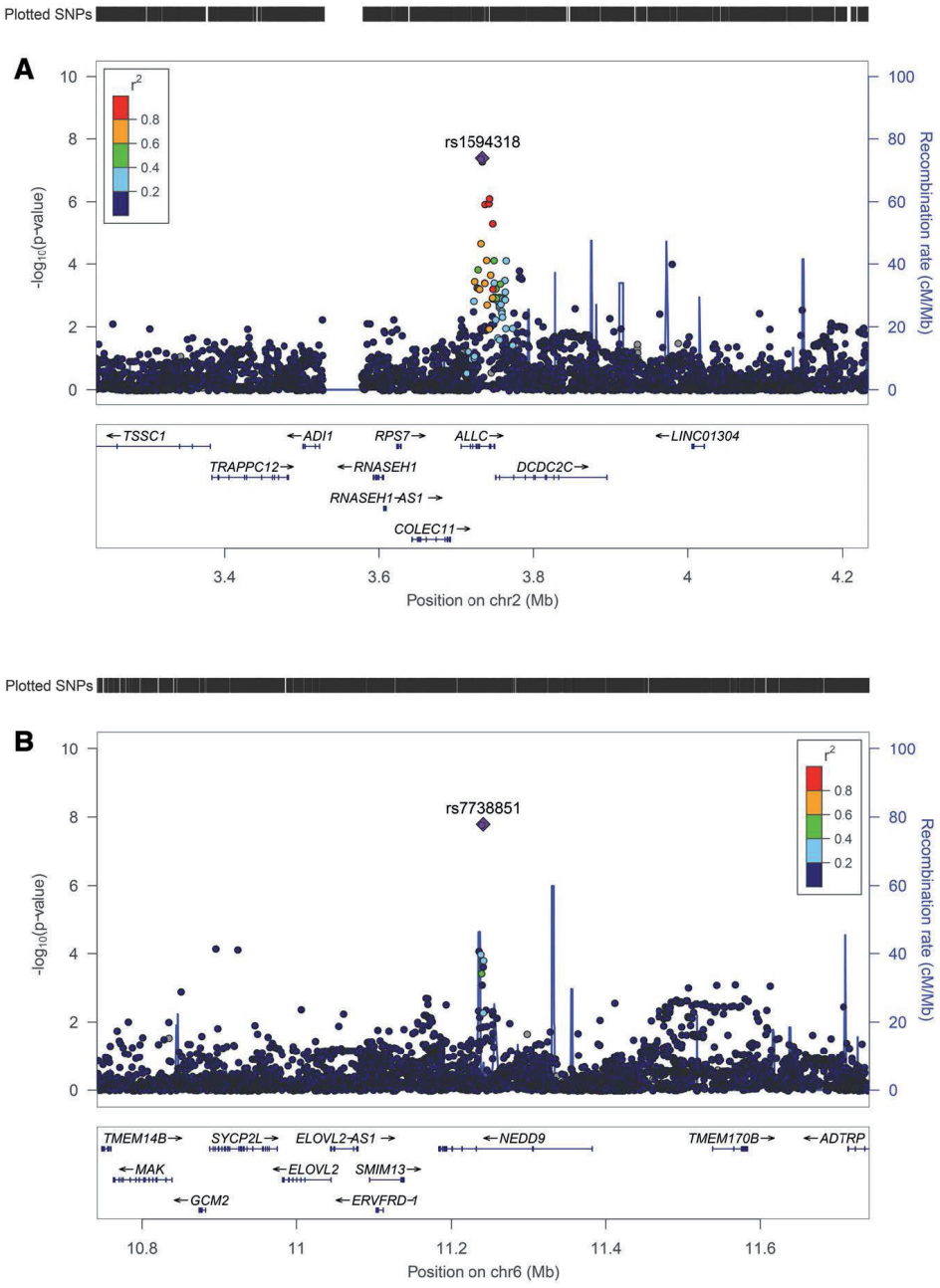


Figure 3.2.2. Regional association plots.
(A) Regional association plot for rs1594318 and caries in primary teeth (European ancestry meta-analysis).
(B) Regional association plot for rs7738851 and caries in permanent teeth.

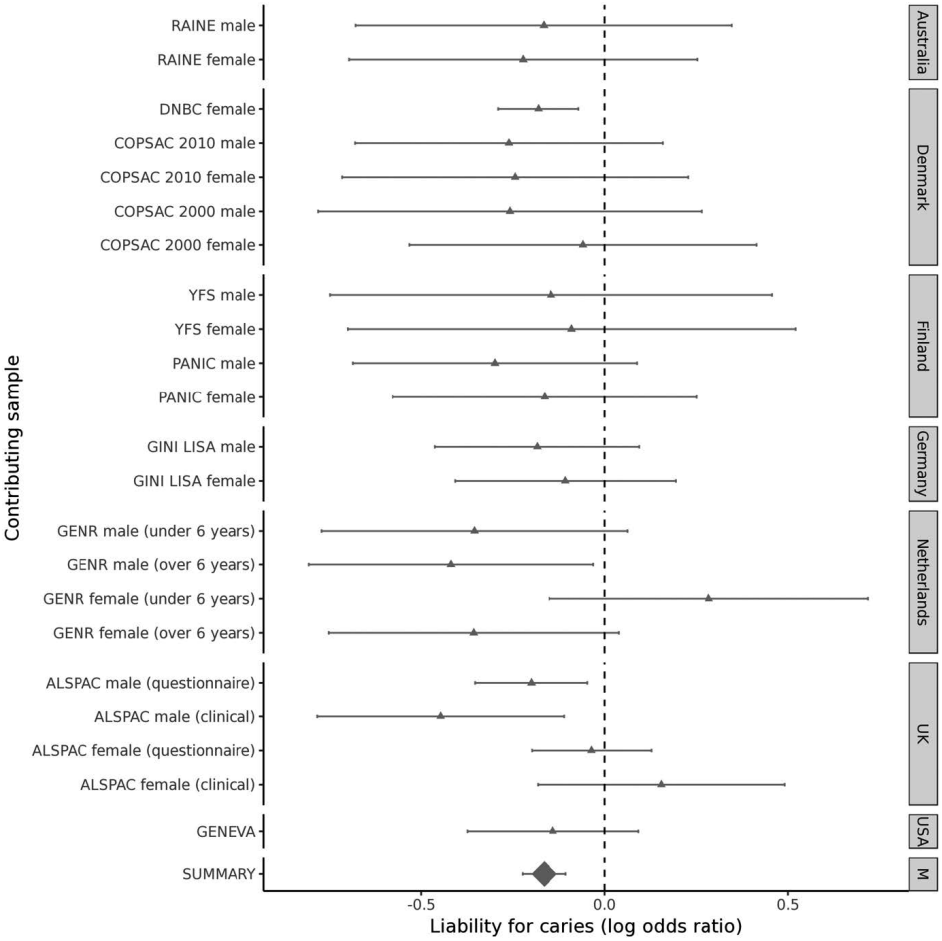


Figure 3.2.3. Forest plot for rs1594318 and caries in primary teeth.
Effect sizes are expressed on a log OR scale, grouped by geographical location. The summary estimate is from the fixed-effect meta-analysis of participants of European ancestry.

the threshold for association after accounting for multiple testing. The single greatest evidence for association was seen between increased predicted transcription of *CDK5RAP3* and increased liability for permanent caries (p = 3.94e-05). *CDK5RAP3* is known to interact with *PAK4* and $p14^{ARF}$, with a potential role in oncogenesis [18, 19].

Table 3.2.1. Lead associated single variants

Phenotype	Variant	Position	Effect allele	Other allele	EAF	Beta (SE)	Odds ratio	P-value	N	I^2	P-value for heterogeneity	Annotation
Caries in primary teeth (European ancestry analysis)	rs1594318	chr2:3733944	C	G	0.60	-0.165 (0.030)	0.848	4.13e-08	16,994	0.0	0.69	Intronic, *ALLC*
Caries in primary teeth (multi-ethnic analysis)*	rs1594318	chr2:3733944	C	G	0.60	-0.142 (0.028)	0.868	3.78e-07	18,960	0.0	0.61	Intronic, *ALLC*
Caries in primary teeth (multi-ethnic analysis)*	rs872877	chr2:3735826	A	G	0.59	-0.142 (0.028)	0.868	4.18e-07	18,958	17.5	0.68	Intronic, *ALLC*
Caries in permanent teeth	rs7738851	chr6:11241788	A	T	0.85	0.248 (0.044)	1.28	1.63e-08	13,353	13.3	0.20	Intronic, *NEDD9*

* No single variants were associated with dental caries status at the genome-wide level in the multi-ethnic analysis of primary teeth, however two variants are discussed in Results section and are included here for reference.

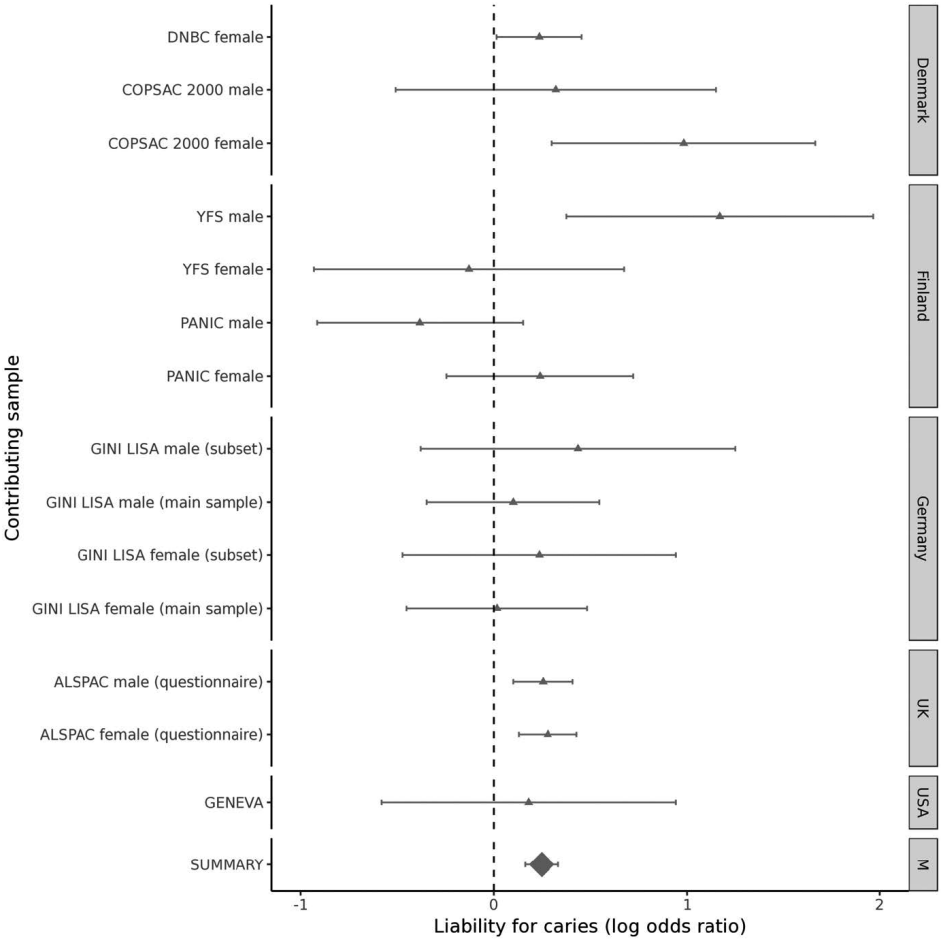


Figure 3.2.4. Forest plot for rs7738851 and caries in permanent teeth.
Effect sizes are expressed on a log OR scale, grouped by geographical location. The summary estimate is from fixed-effect meta-analysis.

Table 3.2.2. Within-sample and meta-analysis heritability estimates

Phenotype	Method		Estimated h^2 (95% CI)	N
Caries in primary teeth	GCTA GREML		0.28 (0.09:0.48)	7,230
	LDSR	All participants	0.01 (0.00:0.06)	19,003
		European ancestry only	0.01 (0.00:0.07)	17,036
Caries in permanent teeth	GCTA GREML		0.17 (0.02:0.31)	6,657
	LDSR		0.06 (0.00:0.12)	13,353

Table 3.2.3. Lookup of lead associated variants

Variant	Discovery trait	Risk increasing allele (discovery)	Cross trait lookup		P value	Effect per caries risk increasing allele (se)	N
rs1594318	Caries in primary teeth (European ancestry meta-analysis)	G	Adult caries traits	DMFS (standard deviation of residuals of caries-affected surfaces)	0.87	-0.0015 (0.0092)	26,790
				Number of teeth (inverse normal transformed residuals)	0.60	0.0051(0.0098)	27,947
				Standardized DFS (inverse normal transformed residuals)	0.033	-0.0195(0.0091)	26,532
			Hypothesis free	(no traits meeting threshold for multiple testing)			
rs7738851	Caries in permanent teeth	A	Adult caries traits	DMFS (standard deviation of residuals of caries-affected surfaces)	0.57	-0.007 (0.011)	26,791
				Number of teeth (inverse normal transformed residuals)	0.63	-0.0064 (0.013)	27,949
				Standardized DFS (inverse normal transformed residuals)	0.65	-0.0054 (0.012)	26,531
			Hypothesis free	(no traits meeting threshold for multiple testing)			

Adult caries traits were defined as follows. DMFS – a count of the number of decayed, missing or filled tooth surfaces. This count was residualized after regression on age and age squared and standard deviations of residuals calculated. Number of teeth – a count of the number of teeth in the mouth. This count was residualzed after regression on age and age squared and residuals underwent inverse normal transformation. Standardized DFS. The number of decayed and filled surfaces was divided by the total number of tooth surfaces in the mouth. This ratio was residualized after regression on age and age squared and residuals underwent inverse normal transformation.

Discussion

Dental caries in children and adolescents has not been studied to date using a large-scale, consortium-based genome-wide meta-analysis aproach. Based on previous knowledge of the heritability of caries in young populations and from our understanding of other complex diseases, we anticipated that common genetic variants would be associated with dental caries risk with consistent effects across different cohorts. We found evidence for association between rs1594318 and caries in primary teeth. This variant showed weaker evidence for association in the multi-ethnic meta-analysis, potentially relating to different alelle frequencies across the different ethnic groups included in analysis. Frequency of the G allele is reported to vary between 0.24 in Asian populations to 0.42 in populations of European ancestry based on 1KGP allele fequencies. *ALLC* (Allantoicase) codes the enzyme allantoicase, which is involved in purine metabolism and whose enzymatic activitiy is believed to have been lost during vertebrate evolution. Mouse studies suggest this loss of activity relates to low expression levels and low substrate affinity rather than total non-functionality [20]. Although there is some evidence that *ALLC* polymorphisms are associated with response to asthma treatment [21], there is limited understanding of the implications of variation in *ALLC* for human health, and it is possible that rs1594318 tags functionality elsewhere in the same locus.

For permanent teeth, we found evidence for association between caries status and rs7738851, an intronic variant with *NEDD9* (neural precursor cell-expressed, developmentally down-regulard gene 9). *NEDD9* is reported to mediate integrin-initiated signal transduction pathways and is conserved from gnathostomes into mammals [22, 23]. *NEDD9* appears to play a number of functional roles in disease and normal development, including regulation of neuronal differentiation, development and migration [22, 24-28]. One such function involves regulation of neural crest cell migration [26]. Disruption of neural crest signalling is known to lead to enamel and dentin defects in animal models [29, 30] and might provide a mechanism for variation at rs7738851 to influence dental caries susceptibiltiy.

Traditionally, risk assessment for dental caries in childhood has concentrated on dietary behaviours and other modifiable risk factors [31], with little focus on tooth quality. Although our understanding of the genetic risk factors for dental caries is incomplete, authors have noted that the evidence from previous genetic association studies tends to support a role for innate tooth structure and quality in risk of caries [32, 33]. If validated by future studies, the association with rs7738851 would provide further evidence for this argument, and may in the future enhance risk assessment in clinical practice.

The lookup of lead associated variants against adult caries traits provided no strong evidence for persistent association in adulthood. This might imply genetic effects which are specific to the near-eruption timepoint. An alternative explanation is that the variants identified in the present study represent false positive signals as the statistical evidence presented is not irrefutable and there is no formal replication stage in our study; yet, we see good consistency of effects across studies.

The meta-analysis heritabiltiy estimates were lower than anticipated from either previous within-study heritability estimates [34] or the the new within-study heritabiltiy estimates obtained for this analysis. There are several possible explanations for this phenomenom. First, the methods used in the present analysis are SNP based which consistently underestimate heritability of complex traits relative to twin and family studies [35]. Second, meta-analysis heritabiliy represents the heritability of genetic effects which are consistent across populations. In the event of genuine differences in genetic architecture of dental caries across strata of age, geography, environmental exposure or subtly different phenotypic meanings the meta-heritabiltiy estimate is not the same conceptually as the weighted average of heritabiltiy within each study.

More intuitively, genetic influences might be important within populations with relatively similar environments but not determine much of the overall diffrences in risk when comparing groups of people in markedly different environments. This view is consistent with existing literature from family based and candidate gene association studies suggesting the genetic architectre of dental caries is complex with multiple interactions. For example, gene-sex interactions are reported which change in magnitude between the primary and permanent dentition [36], genetic variants may have heterogeneous effects on the primary and permanent dentition [37] and environmental exposures such as fluoride may interact with genetic effects [38]. Finally, the aetiological relevance of specific microbiome groups appears to vary between different populations [39], suggesting genetic effects acting through the oral microbiome might also vary between populations. Unfortunately, this study lacks statistical power to perform meta-analyses stratified on these exposures, so does not resolve this particular question.

In line with any consortium based approach, the need to harmonize analysis across different collections led to some compromises.The phenotypic definitions used in this study do not contain information on disease extent or severity. Loss of information in creating these defintions may have contributed to the low statistical power of analysis. Our motivation for using simple definitions was based on the facts that a) case-control status simply represents a threshold level of an underlying continium of disease risk b) simple binary classifications facilitate comparison of studies with different assessment

protocols and population risks and c) simple classifications have been used sucsessully in a range of complex phenotypes.

Between participating centres there are differences in characteristics such as age at participation, phenotypic assesment and differences in the environment (such as nutrition, oral hygiene and the oral microbime) which might influence dental caries or its treatment, as reflected in the wide range of caries prevalence between different study centres. Varying phenotypic characteristics do not necessarily result in hetrogeneous genetic effects, as this variability may be uncorrelated with genetic effects. There was little evidence for heterogeneity in the top associated loci reported, however, the test for hetrogeneity in genetic effects (I^2) is limited by the small number of participting studies in meta-anlaysis [40] and wide confidence intervals for within-study genetic effect estimates. Given these limitations, it is possible that heterogeneity contributed to low study power and prevented more comprehensive single variant findings.

In the ALSPAC study we made extensive use of questionnaire derived data. This will systematically under-report true caries exposure compared to other studies as children or their parents are unlikely to be aware of untreated dental caries which would be evident to a trained assesor. We have explored some of these issues previously and shown that self-report measures at scale can be used to make meaningful inference about dental health in childhood [41]. We believe that misclassification and under-reporting in questionnaire data would tend to bias genetic effect estimates and heritability towards the null. Despite this we show evidence for heritabiltiy using these definitions and effect sizes at lead variants are comparable with effect sizes obtained using clinically assessed data (Figures 3.2.3, 3.2.4).

As our power calculations showed, the sample size was sufficient to detect the identified variants associated at a genome wide significant level with caries in the primary teeth (rs1594318) and in permanent teeth (rs872877), where we observed relatively large effect sizes. For smaller effect sizes we were underpowered to identify association, and did not detect any variants with effect sizes (expressed as per-allele increased odds) smaller than 15% or 17% in the primary and permanent teeth, respectively. Caries is highly influenced by environmental factors and it is likely that its susceptibility is polygenic in nature [32] with individual genetic variants conferring small effect sizes, as seen in other comparable complex traits [42]. Furthermore, some of the included studies had major differences in their caries prevalence, likely acting as a proxy for features affecting risk of caries. This may have introduced heterogeneity and reduced power to detect association, as discused further below.

One area of interest in the literature is the ability of genetics to guide personalized decisions on risk screening or identifying treatment modalities, and this is also true

in dentistry. The genetic variants identified in this study are unlikely to be useful on their own in this context, given the modest effect sizes and low total heritability observed in our meta-analysis. We would suggest clinicians should continue to consider environment and aggregate genetic effects (for example, knowledege of disease patterns of close relatives) rather than specific genetic variants at this moment in time. Nevertheless, the findings of our study contribute to a better understanding of the genetic and biological mechanisms underlying caries suceptibility.

References

[1] N.J. Kassebaum, E. Bernabe, M. Dahiya, B. Bhandari, C.J.L. Murray, W. Marcenes, Global Burden of Untreated Caries: A Systematic Review and Metaregression, J Dent Res. 94(5) (2015) 650-658.

[2] C.R. Vernazza, S.L. Rolland, B. Chadwick, N. Pitts, Caries experience, the caries burden and associated factors in children in England, Wales and Northern Ireland 2013, Br Dent J. 221(6) (2016) 315-320.

[3] J.T. van der Tas, L. Kragt, M.E.C. Elfrink, L.C.M. Bertens, V.W.V. Jaddoe, H.A. Moll, E.M. Ongkosuwito, E.B. Wolvius, Social inequalities and dental caries in six-year-old children from the Netherlands, Journal of Dentistry 62 (2017) 18-24.

[4] A.A. Schuller, P. van Dommelen, J.H.G. Poorterman, Trends in oral health in young people in the Netherlands over the past 20years: a study in a changing context, Community Dent Oral Epidemiol. 42(2) (2014) 178-184.

[5] N. Philip, B. Suneja, L.J. Walsh, Ecological Approaches to Dental Caries Prevention: Paradigm Shift or Shibboleth?, Caries Res. 52(1-2) (2018) 153-165.

[6] M.A. Peres, A. Sheiham, P. Liu, F.F. Demarco, A.E. Silva, M.C. Assunção, A.M. Menezes, F.C. Barros, K.G. Peres, Sugar Consumption and Changes in Dental Caries from Childhood to Adolescence, J. Dent. Res. 95(4) (2016) 388-394.

[7] A. Sheiham, W.P. James, Diet and Dental Caries: The Pivotal Role of Free Sugars Reemphasized, J. Dent. Res. 94(10) (2015) 1341-1347.

[8] R.J. Lynch, The primary and mixed dentition, post-eruptive enamel maturation and dental caries: a review, Int. Dent. J. 63(Suppl. 2) (2013) 3-13.

[9] J.C. Boraas, L.B. Messer, M.J. Till, A genetic contribution to dental caries, occlusion, and morphology as demonstrated by twins reared apart, J. Dent. Res. 67(9) (1988) 1150-1155.

[10] W.A. Bretz, P.M. Corby, N.J. Schork, M.T. Robinson, M. Coelho, S. Costa, M.R. Melo Filho, R.J. Weyant, T.C. Hart, Longitudinal analysis of heritability for dental caries traits, J. Dent. Res. 84(11) (2005) 1047-1051.

[11] C.S. Chung, R.H. Larson, Factors and inheritance of dental caries in the rat, J. Dent. Res. 46(3) (1967) 559-564.

[12] W.A. Bretz, P.M.A. Corby, M.R. Melo, M.Q. Coelho, S.M. Costa, M. Robinson, N.J. Schork, A. Drewnowski, T.C. Hart, Heritability estimates for dental caries and sucrose sweetness preference, Arch Oral Biol. 51(12) (2006) 1156-1160.

[13] J.R. Shaffer, X. Wang, E. Feingold, M. Lee, F. Begum, D. Weeks, K.T. Cuenco, M.M. Barmada, S.K. Wendell, D.R. Crosslin, C.C. Laurie, K.F. Doheny, E.W. Pugh, Q. Zhang, B. Feenstra, F. Geller, H.A. Boyd, H. Zhang, M. Melbye, J.C. Murray, R.J. Weyant, R. Crout, D.W. McNeil, S.M. Levy, R.L. Slayton, M.C. Willing, B. Broffitt, A.R. Vieira, M.L. Marazita, Genome-wide association scan for childhood caries implicates novel genes, J. Dent. Res. 90(12) (2011) 1457-1462.

[14] X. Wang, J.R. Shaffer, Z. Zeng, F. Begum, A.R. Vieira, J. Noel, I. Anjomshoaa, K.T. Cuenco, M.K. Lee, J. Beck, E. Boerwinkle, M.C. Cornelis, F.B. Hu, D.R. Crosslin, C.C. Laurie, S.C. Nelson, K.F. Doheny, E.W. Pugh, D.E. Polk, R.J. Weyant, R. Crout, D.W. McNeil, D.E. Weeks, E. Feingold, M.L. Marazita, Genome-wide association scan of dental caries in the permanent dentition, BMC Oral Health 12 (2012) 57.

[15] Z. Zeng, E. Feingold, X. Wang, D.E. Weeks, M. Lee, D.T. Cuenco, B. Broffitt, R.J. Weyant, R. Crout, D.W. McNeil, S.M. Levy, M.L. Marazita, J.R. Shaffer, Genome-wide association study of primary dentition pit-and-fissure and smooth surface caries, Caries Res. 48(4) (2014) 330-338.

[16] Z. Zeng, J.R. Shaffer, X. Wang, E. Feingold, D.E. Weeks, M. Lee, K.T. Cuenco, S.K. Wendell, R.J. Weyant, R. Crout, D.W. McNeil, M.L. Marazita, Genome-wide association studies of pit-and-fissure- and smooth-surface caries in permanent dentition, J. Dent. Res. 92(5) (2013) 432-437.

[17] J. Morrison, C.C. Laurie, M.L. Marazita, A.E. Sanders, S. Offenbacher, C.R. Salazar, M.P. Conomos, T. Thornton, D. Jain, C.A. Laurie, K.F. Kerr, G. Papanicolaou, K. Taylor, L.M. Kaste, J.D. Beck, J.R. Shaffer, Genome-wide association study of dental caries in the Hispanic Communities Health Study/Study of Latinos (HCHS/SOL), Hum. Mol. Genet. 25(4) (2016) 807-816.

[18] G.W.Y. Mak, W.L. Lai, Y. Zhou, M.T. Li, I.O.L. Ng, Y.P. Ching, CDK5RAP3 Is a Novel Repressor of p14(ARF) in Hepatocellular Carcinoma Cells, Plos One 7(7) (2012).

[19] G.W.Y. Mak, M.M.L. Chan, V.Y.L. Leong, J.M.F. Lee, T.O. Yau, I.O.L. Ng, Y.P. Ching, Overexpression of a Novel Activator of PAK4, the CDK5 Kinase-Associated Protein CDK5RAP3, Promotes Hepatocellular Carcinoma Metastasis, Cancer Research 71(8) (2011) 2949-2958.

[20] D. Vigetti, L. Pollegioni, C. Monetti, M. Prati, G. Bernardini, R. Gornati, Property comparison of recombinant amphibian and mammalian allantoicases, Febs Letters 512(1-3) (2002) 323-328.

[21] T.J. Park, J.S. Park, H.S. Cheong, B.L. Park, L.H. Kim, J.S. Heo, Y.K. Kim, K.U. Kim, S.T. Uh, H.S. Lee, J.O. Na, K.H. Seo, J.S. Choi, Y.H. Kim, M.S. Kim, C.S. Park, H.D. Shin, Genome-wide association study identifies ALLC polymorphisms correlated with FEV1 change by corticosteroid, Clinica Chimica Acta 436 (2014) 20-26.

[22] N. Tikhmyanova, J.L. Little, E.A. Golemis, CAS proteins in normal and pathological cell growth control, Cellular and Molecular Life Sciences 67(7) (2010) 1025-1048.

[23] M.K. Singh, D. Dadke, E. Nicolas, I.G. Serebriiskii, S. Apostolou, A. Canutescu, B.L. Egleston, E.A. Golemis, A novel Cas family member, HEPL, regulates FAK and cell spreading, Molecular Biology of the Cell 19(4) (2008) 1627-1636.

[24] S. Kumar, Y. Tomooka, M. Noda, Identification of a set of genes with developmentally down-regulated expression in the mouse brain, Biochemical and Biophysical Research Communications 185(3) (1992) 1155-1161.

[25] M.J. Latasa, A.M. Jimenez-Lara, J.M. Cosgaya, Retinoic acid regulates Schwann cell migration via NEDD9 induction by transcriptional and post-translational mechanisms, Biochimica Et Biophysica Acta-Molecular Cell Research 1863(7) (2016) 1510-1518.

[26] J.B. Aquino, F. Lallemend, F. Marmigere, Adameyko, II, E.A. Golemis, P. Ernfors, The retinoic acid inducible Cas-family signaling protein Nedd9 regulates neural crest cell migration by modulating adhesion and actin dynamics, Neuroscience 162(4) (2009) 1106-1119.

[27] A.S. Nikonova, A.V. Gaponova, A.E. Kudinov, E.A. Golemis, CAS Proteins in Health and Disease: An Update, Iubmb Life 66(6) (2014) 387-395.

[28] M.M. Riccomagno, L.O. Sun, C.M. Brady, K. Alexandropoulos, S. Seo, M. Kurokawa, A.L. Kolodkin, Cas Adaptor Proteins Organize the Retinal Ganglion Cell Layer Downstream of Integrin Signaling, Neuron 81(4) (2014) 779-786.

[29] S.K. Wang, Y. Komatsu, Y. Mishina, Potential contribution of neural crest cells to dental enamel formation, Biochemical and Biophysical Research Communications 415(1) (2011) 114-119.

[30] O. Duverger, A. Zah, J. Isaac, H.W. Sun, A.K. Bartels, J.B. Lian, A. Berdal, J. Hwang, M.I. Morasso, Neural Crest Deletion of Dlx3 Leads to Major Dentin Defects through Down-regulation of Dspp, Journal of Biological Chemistry 287(15) (2012) 12230-12240.

[31] K. Divaris, Predicting Dental Caries Outcomes in Children: A "Risky" Concept, J Dent Res. 95(3) (2016) 248-254.

[32] I.L.C. Chapple, P. Bouchard, M.G. Cagetti, G. Campus, M.-C. Carra, F. Cocco, L. Nibali, P. Hujoel, M.L. Laine, P. Lingström, D.J. Manton, E. Montero, N. Pitts, H. Rangé, N. Schlueter, W. Teughels, S. Twetman, C. Van Loveren, F. Van der Weijden, A.R. Vieira, A.G. Schulte, Interaction of lifestyle, behaviour or systemic diseases with dental caries and periodontal diseases: consensus report of group 2 of the joint EFP/ORCA workshop on the boundaries between caries and periodontal diseases, J Clin Periodontol. 44 (2017) S39-S51.

[33] L. Nibali, A. Di Iorio, Y.-K. Tu, A.R. Vieira, Host genetics role in the pathogenesis of periodontal disease and caries, J Clin Periodontol. 44 (2017) S52-S78.
[34] T.I. Wigen, N.J. Wang, Caries and background factors in Norwegian and immigrant 5-year-old children, Community Dentistry and Oral Epidemiology 38(1) (2010) 19-28.
[35] A.R. Docherty, A. Moscati, R. Peterson, A.C. Edwards, D.E. Adkins, S.A. Bacanu, T.B. Bigdeli, B.T. Webb, J. Flint, K.S. Kendler, SNP-based heritability estimates of the personality dimensions and polygenic prediction of both neuroticism and major depression: findings from CONVERGE, Translational Psychiatry 6 (2016).
[36] J.R. Shaffer, X.J. Wang, D.W. McNeil, R.J. Weyant, R. Crout, M.L. Marazita, Genetic Susceptibility to Dental Caries Differs between the Sexes: A Family-Based Study, Caries Res. 49(2) (2015) 133-140.
[37] M. Bayram, K. Deeley, M.F. Reis, V.M. Trombetta, T.D. Ruff, R.C. Sencak, M. Hummel, P.M. Dizak, K. Washam, H.F. Romanos, A. Lips, G. Alves, M.C. Costa, J.M. Granjeiro, L.S. Antunes, E.C. Kuchler, F. Seymen, A.R. Vieira, Genetic influences on dental enamel that impact caries differ between the primary and permanent dentitions, European Journal of Oral Sciences 123(5) (2015) 327-334.
[38] J.R. Shaffer, J.C. Carlson, B.O.C. Stanley, E. Feingold, M. Cooper, M.M. Vanyukov, B.S. Maher, R.L. Slayton, M.C. Willing, S.E. Reis, D.W. McNeil, R.J. Crout, R.J. Weyant, S.M. Levy, A.R. Vieira, M.L. Marazita, Effects of enamel matrix genes on dental caries are moderated by fluoride exposures, Human Genetics 134(2) (2015) 159-167.
[39] I. Johansson, E. Witkowska, B. Kaveh, P.L. Holgerson, A.C.R. Tanner, The Microbiome in Populations with a Low and High Prevalence of Caries, J Dent Res. 95(1) (2016) 80-86.
[40] P.T. von Hippel, The heterogeneity statistic I(2) can be biased in small meta-analyses, BMC Medical Research Methodology 15 (2015) 35.
[41] S. Haworth, T. Dudding, A. Waylen, S.J. Thomas, N.J. Timpson, Ten years on: Is dental general anaesthesia in childhood a risk factor for caries and anxiety?, Br Dent J. 222(4) (2017) 299-304.
[42] J.P. Kemp, J.A. Morris, C. Medina-Gomez, V. Forgetta, N.M. Warrington, S.E. Youlten, J. Zheng, C.L. Gregson, E. Grundberg, K. Trajanoska, J.G. Logan, A.S. Pollard, P.C. Sparkes, E.J. Ghirardello, R. Allen, V.D. Leitch, N.C. Butterfield, D. Komla-Ebri, A.-T. Adoum, K.F. Curry, J.K. White, F. Kussy, K.M. Greenlaw, C. Xu, N.C. Harvey, C. Cooper, D.J. Adams, C.M.T. Greenwood, M.T. Maurano, S. Kaptoge, F. Rivadeneira, J.H. Tobias, P.I. Croucher, C.L. Ackert-Bicknell, J.H.D. Bassett, G.R. Williams, J.B. Richards, D.M. Evans, Identification of 153 new loci associated with heel bone mineral density and functional involvement of GPC6 in osteoporosis, advance online publication (2017).
[43] A. Boyd, J. Golding, J. Macleod, D.A. Lawlor, A. Fraser, J. Henderson, L. Molloy, A. Ness, S. Ring, G.D. Smith, Cohort Profile: The 'Children of the 90s'-the index offspring of the Avon Longitudinal Study of Parents and Children, International Journal of Epidemiology 42(1) (2013) 111-127.
[44] H. Bisgaard, The Copenhagen Prospective Study on Asthma in Childhood (COPSAC): design, rationale, and baseline data from a longitudinal birth cohort study, Ann. Allergy Asthma Immunol. 93(4) (2004) 381-389.
[45] J. Olsen, M. Melbye, S.F. Olsen, T.I.A. Sorensen, P. Aaby, A.M.N. Andersen, D. Taxbol, K.D. Hansen, M. Juhl, T.B. Schow, H.T. Sorensen, J. Andresen, E.L. Mortensen, A.W. Olesen, C. Sondergaard, The Danish National Birth Cohort - its background, structure and aim, Scandinavian Journal of Public Health 29(4) (2001) 300-307.
[46] M.N. Kooijman, C.J. Kruithof, C.M. van Duijn, L. Duijts, O.H. Franco, M.H. van Ijzendoorn, J.C. de Jongste, C.C.W. Klaver, A. van der Lugt, J.P. Mackenbach, H.A. Moll, R.P. Peeters, H. Raat, E. Rings, F. Rivadeneira, M.P. van der Schroeff, E.A.P. Steegers, H. Tiemeier, A.G. Uitterlinden, F.C. Verhulst, E. Wolvius, J.F. Felix, V.W.V. Jaddoe, The Generation R Study: design and cohort update 2017, European Journal of Epidemiology 31(12) (2016) 1243-1264.

[47] J.T. van der Tas, L. Kragt, J.J.S. Veerkamp, V.W.V. Jaddoe, H.A. Moll, E.M. Ongkosuwito, M.E.C. Elfrink, E.B. Wolvius, Ethnic Disparities in Dental Caries among Six-Year-Old Children in the Netherlands, Caries Res. 50(5) (2016) 489-497.

[48] C. Medina-Gomez, J.F. Felix, K. Estrada, M.J. Peters, L. Herrera, C.J. Kruithof, L. Duijts, A. Hofman, C.M. van Duijn, A.G. Uitterlinden, V.W.V. Jaddoe, F. Rivadeneira, Challenges in conducting genome-wide association studies in highly admixed multi-ethnic populations: the Generation R Study, European Journal of Epidemiology 30(4) (2015) 317-330.

[49] M.C. Cornelis, A. Agrawal, J.W. Cole, N.N. Hansel, K.C. Barnes, T.H. Beaty, S.N. Bennett, L.J. Bierut, E. Boerwinkle, K.F. Doheny, B. Feenstra, E. Feingold, M. Fornage, C.A. Haiman, E.L. Harris, M.G. Hayes, J.A. Heit, F.B. Hu, J.H. Kang, C.C. Laurie, H. Ling, T.A. Manolio, M.L. Marazita, R.A. Mathias, D.B. Mirel, J. Paschall, L.R. Pasquale, E.W. Pugh, J.P. Rice, J. Udren, R.M. van Dam, X.J. Wang, J.L. Wiggs, K. Williams, K. Yu, G. Consortium, The Gene, Environment Association Studies Consortium (GENEVA): Maximizing the Knowledge Obtained from GWAS by Collaboration Across Studies of Multiple Conditions, Genetic Epidemiology 34(4) (2010) 364-372.

[50] Z. Zeng, E. Feingold, X. Wang, D.E. Weeks, M. Lee, K.T. Cuenco, B. Broffitt, R.J. Weyant, R. Crout, D.W. McNeil, S.M. Levy, M.L. Marazita, J.R. Shaffer, Genome-Wide Association Study of Primary Dentition Pit-and-Fissure and Smooth Surface Caries, Caries Res. 48(4) (2014) 330-338.

[51] D.E. Polk, R.J. Weyant, R.J. Crout, D.W. McNeil, R.E. Tarter, J.G. Thomas, M.L. Marazita, Study protocol of the Center for Oral Health Research in Appalachia (COHRA) etiology study, BMC Oral Health 8(1) (2008) 18.

[52] S.M. Levy, J.J. Warren, B. Broffitt, S.L. Hillis, M.J. Kanellis, Fluoride, beverages and dental caries in the primary dentition, Caries Res. 37(3) (2003) 157-165.

[53] H.R. Taal, B. St Pourcain, E. Thiering, S. Das, D.O. Mook-Kanamori, N.M. Warrington, M. Kaakinen, E. Kreiner-Moller, J.P. Bradfield, R.M. Freathy, F. Geller, M. Guxens, D.L. Cousminer, M. Kerkhof, N.J. Timpson, M.A. Ikram, L.J. Beilin, K. Bonnelykke, J.L. Buxton, P. Charoen, B.L.K. Chawes, J. Eriksson, D.M. Evans, A. Hofman, J.P. Kemp, C.E. Kim, N. Klopp, J. Lahti, S.J. Lye, G. McMahon, F.D. Mentch, M. Muller-Nurasyid, P.F. O'Reilly, I. Prokopenko, F. Rivadeneira, E.A.P. Steegers, J. Sunyer, C. Tiesler, H. Yaghootkar, M.M.B. Breteler, S. Debette, M. Fornage, V. Gudnason, L.J. Launer, A. van der Lugt, T.H. Mosley, S. Seshadri, A.V. Smith, M.W. Vernooij, A.I.F. Blakemore, R.M. Chiavacci, B. Feenstra, J. Fernandez-Banet, S.F.A. Grant, A.L. Hartikainen, A.J. van der Heijden, C. Iniguez, M. Lathrop, W.L. McArdle, A. Molgaard, J.P. Newnham, L.J. Palmer, A. Palotie, A. Pouta, S.M. Ring, U. Sovio, M. Standl, A.G. Uitterlinden, H.E. Wichmann, N.H. Vissing, C. DeCarli, C.M. van Duijn, M.I. McCarthy, G.H. Koppelman, X. Estivill, A.T. Hattersley, M. Melbye, H. Bisgaard, C.E. Pennell, E. Widen, H. Hakonarson, G.D. Smith, J. Heinrich, M.R. Jarvelin, V.W.V. Jaddoe, L.S. Adair, W. Ang, M. Atalay, T. van Beijsterveldt, N. Bergen, K. Benke, D. Berry, L. Coin, O.S.P. Davis, P. Elliott, C. Flexeder, T. Frayling, R. Gaillard, M. Groen-Blokhuis, L.K. Goh, C.M.A. Haworth, D. Hadley, J. Hedebrand, A. Hinney, J.N. Hirschhorn, J.W. Holloway, C. Holst, J.J. Hottenga, M. Horikoshi, V. Huikari, E. Hypponen, T.O. Kilpelainen, M. Kirin, M. Kowgier, H.M. Lakka, L.A. Lange, D.A. Lawlor, T. Lehtimaki, A. Lewin, C. Lindgren, V. Lindi, R. Maggi, J. Marsh, C. Middeldorp, I. Millwood, J.C. Murray, M. Nivard, E.A. Nohr, I. Ntalla, E. Oken, K. Panoutsopoulou, J. Pararajasingham, A. Rodriguez, R.M. Salem, S. Sebert, N. Siitonen, D.P. Strachan, Y.Y. Teo, B. Valcarcel, S. White, G. Willemsen, E. Zeggini, D.I. Boomsma, C. Cooper, M. Gillman, B. Hocher, T.A. Lakka, K.L. Mohlke, G.V. Dedoussis, K.K. Ong, E.R. Pearson, T.S. Price, C. Power, O.T. Raitakari, S.M. Saw, A. Scherag, O. Simell, T.I.A. Sorensen, J.F. Wilson, R. Schmidt, H.A. Vrooman, S. Sigurdsson, S. Ropele, L.H. Coker, W.T. Longstreth, W.J. Niessen, A.L. DeStefano, A. Beiser, A.P. Zijdenbos, M. Struchalin, C.R. Jack, M.A. Nalls, R. Au, H. Gudnason, T.B. Harris, W.M. Meeks, M.A. van Buchem, D. Catellier, B.G. Windham, P.A. Wolf, H. Schmidt, E. Cohorts Heart Aging Res Genetic, E. Early Genetics Lifecourse, E.G.G.C. Early Growth Genetics, Common variants at 12q15 and 12q24 are associated with infant head circumference, Nature Genetics 44(5) (2012) 532-+.

[54] A. von Berg, U. Kramer, E. Link, C. Bollrath, J. Heinrich, I. Brockow, S. Koletzko, A. Grubl, B. Filipiak-Pittroff, H.E. Wichmann, C.P. Bauer, D. Reinhardt, D. Berdel, G.I.S. Grp, Impact of early feeding on childhood eczema: development after nutritional intervention compared with the natural course - the GINIplus study up to the age of 6 years, Clinical and Experimental Allergy 40(4) (2010) 627-636.

[55] A. Zutavern, I. Brockow, B. Schaaf, G. Bolte, A. von Berg, U. Diez, M. Borte, O. Herbarth, H.E. Wichmann, J. Heinrich, L.S. Grp, Timing of solid food introduction in relation to atopic dermatitis and atopic sensitization: Results from a prospective birth cohort study, Pediatrics 117(2) (2006) 401-411.

[56] A.M. Eloranta, V. Lindi, U. Schwab, T. Tompuri, S. Kiiskinen, H.M. Lakka, T. Laitinen, T.A. Lakka, Dietary factors associated with overweight and body adiposity in Finnish children aged 6-8 years: the PANIC Study, International Journal of Obesity 36(7) (2012) 950-955.

[57] O.T. Raitakari, M. Juonala, T. Ronnemaa, L. Keltikangas-Jarvinen, L. Rasanen, M. Pietikainen, N. Hutri-Kahonen, L. Taittonen, E. Jokinen, J. Marniemi, A. Jula, R. Telama, M. Kahonen, T. Lehtimaki, H.K. Akerblom, J.S.A. Viikari, Cohort Profile: The Cardiovascular Risk in Young Finns Study, Int J Epidemiol. 37(6) (2008) 1220-1226.

[58] L. Straker, J. Mountain, A. Jacques, S. White, A. Smith, L. Landau, F. Stanley, J. Newnham, C. Pennell, P. Eastwood, Cohort Profile: The Western Australian Pregnancy Cohort (Raine) Study-Generation 2, Int J Epidemiol. 46(5) (2017) 1384-1385j.

[59] T.W. Winkler, F.R. Day, D.C. Croteau-Chonka, A.R. Wood, A.E. Locke, R. Mägi, T. Ferreira, T. Fall, M. Graff, A.E. Justice, J.a. Luan, S. Gustafsson, J.C. Randall, S. Vedantam, T. Workalemahu, T.O. Kilpeläinen, A. Scherag, T. Esko, Z. Kutalik, I.M. Heid, R.J.F. Loos, C. The Genetic Investigation of Anthropometric Traits, Quality control and conduct of genome-wide association meta-analyses, Nat. Protocols 9(5) (2014) 1192-1212.

[60] C.J. Willer, Y. Li, G.R. Abecasis, METAL: fast and efficient meta-analysis of genomewide association scans, Bioinformatics 26(17) (2010) 2190-2191.

[61] B.K. Bulik-Sullivan, P.-R. Loh, H.K. Finucane, S. Ripke, J. Yang, N. Patterson, M.J. Daly, A.L. Price, B.M. Neale, G. Schizophrenia Working, LD Score regression distinguishes confounding from polygenicity in genome-wide association studies, Nature Genetics 47(3) (2015) 291-295.

[62] J.A. Yang, B. Benyamin, B.P. McEvoy, S. Gordon, A.K. Henders, D.R. Nyholt, P.A. Madden, A.C. Heath, N.G. Martin, G.W. Montgomery, M.E. Goddard, P.M. Visscher, Common SNPs explain a large proportion of the heritability for human height, Nature Genetics 42(7) (2010) 565-U131.

[63] J.A. Yang, S.H. Lee, M.E. Goddard, P.M. Visscher, GCTA: A Tool for Genome-wide Complex Trait Analysis, American Journal of Human Genetics 88(1) (2011) 76-82.

[64] C.C. Chang, C.C. Chow, L.C.A.M. Tellier, S. Vattikuti, S.M. Purcell, J.J. Lee, Second-generation PLINK: rising to the challenge of larger and richer datasets, GigaScience 4 (2015) 7.

[65] G. Hemani, J. Zheng, K.H. Wade, C. Laurin, B. Elsworth, S. Burgess, J. Bowden, R. Langdon, V. Tan, J. Yarmolinsky, H.A. Shihab, N. Timpson, D.M. Evans, C. Relton, R.M. Martin, G. Davey Smith, T.R. Gaunt, P.C. Haycock, MR-Base: a platform for systematic causal inference across the phenome using billions of genetic associations, bioRxiv (2016).

[66] J. Zheng, A.M. Erzurumluoglu, B.L. Elsworth, J.P. Kemp, L. Howe, P.C. Haycock, G. Hemani, K. Tansey, C. Laurin, B. St Pourcain, N.M. Warrington, H.K. Finucane, A.L. Price, B.K. Bulik-Sullivan, V. Anttila, L. Paternoster, T.R. Gaunt, D.M. Evans, B.M. Neale, L. Early Genetics, LD Hub: a centralized database and web interface to perform LD score regression that maximizes the potential of summary level GWAS data for SNP heritability and genetic correlation analysis, Bioinformatics 33(2) (2017) 272-279.

[67] C.A. de Leeuw, J.M. Mooij, T. Heskes, D. Posthuma, MAGMA: Generalized Gene-Set Analysis of GWAS Data, Plos Computational Biology 11(4) (2015).

[68] T.H. Pers, J.M. Karjalainen, Y. Chan, H.J. Westra, A.R. Wood, J. Yang, J.C. Lui, S. Vedantam, S. Gustafsson, T. Esko, T. Frayling, E.K. Speliotes, M. Boehnke, S. Raychaudhuri, R.S.N. Fehrmann, J.N. Hirschhorn, L. Franke, A.T. Genetic Invest, Biological interpretation of genome-wide association studies using predicted gene functions, Nature Communications 6 (2015) 9.

[69] A.N. Barbeira, S.P. Dickinson, J.M. Torres, R. Bonazzola, J. Zheng, E.S. Torstenson, H.E. Wheeler, K.P. Shah, T. Edwards, T. Garcia, D. Nicolae, N.J. Cox, H.K. Im, Exploring the phenotypic consequences of tissue specific gene expression variation inferred from GWAS summary statistics, bioRxiv (2017).

[70] G.M. Im Lab, Department of Medicine, The University of Chicago, PredictDB Data Repository, 2017.

[71] A. Skol, L. Scott, G. Abecasis, M. Boehnke, Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies, 38(2) (2006) 209-213.

Chapter 3.3

Social inequalities and dental caries in six-year-old children from the Netherlands

Justin T. van der Tas | Lea Kragt | Marlies E. Elfrink | Loes C. Bertens | Vincent W. Jaddoe | Henriëtte A. Moll | Edwin M. Ongkosuwito | Eppo B. Wolvius

Journal of Dentistry, 2017, 62, 18-24

Abstract

The purpose of our study was to investigate the association of different socioeconomic and sociodemographic factors with dental caries in six-year-old children. Furthermore, we applied a district-based approach to explore the distribution of dental caries among districts of low and high socioeconomic position (SEP). In our cross-sectional study 5,189 six-year-olds were included. This study was embedded in a prospective population-based birth cohort study in Rotterdam, the Netherlands, the Generation R Study. Parental education level, parental employment status, net household income, single parenting, and teenage pregnancy were considered as indicators for SEP. Dental caries was scored on intraoral photographs by using the decayed, missing, and filled teeth (dmft) index. We compared children without caries (dmft = 0) to children with mild caries (dmft = 1–3) or severe caries (dmft > 3). Multinomial logistic regression analyses and binary logistic regression analyses were performed to study the association between SEP and caries, and between district and caries, respectively. Only maternal education level remained significantly associated with mild caries after adjusting for all other SEP-indicators. Paternal educational level, parental employment status, and household income additionally served as independent indicators of SEP in children with severe caries. Furthermore, living in more disadvantaged districts was significantly associated with higher odds of dental caries. Dental caries is more prevalent among six-year-old children with a low SEP, which is also visible at the district level. Maternal educational level is the most important indicator of SEP in the association with caries. Our results should raise concerns about the existing social inequalities in dental caries and should encourage development of dental caries prevention strategies. New knowledge about the distribution of oral health inequalities between districts should be used to target the right audience for these strategies.

Introduction

Dental caries in children leads not only to tooth pain, but also leads to significant health losses in a population, affecting the quality of life of both children and their parents [1-3]. Moreover, it leads to considerable costs in the short and long term [4]. Identifying high-risk populations for developing dental caries can help to lower the incidence of these health issues by targeting the right audience with preventive strategies.

It is well known that a lower socioeconomic position (SEP) is related to poorer health outcomes [5]. This also seems to apply for oral health outcomes. For example, a recent meta-analysis by Schwendicke et al. [6] showed a low socioeconomic position (SEP) to be significantly associated with a higher risk of having dental caries in both children and adults. Various other studies in oral health research also presented a higher caries prevalence in children with a low SEP [7-9]. Possible mediating pathways for the association between SEP and caries, however, have been suggested but have not been studied well yet. Moreover, among these studies SEP was not measured in a uniform way. Often only one or two indicators for SEP are chosen, i.e. parental educational level, household income or parents' employment status. In the Netherlands, maternal educational level or residential neighborhood are most commonly used as indicator for SEP [10-12]. However, different studies have advised using more than one or two indicators of SEP. This may increase comparability and may avoid residual confounding [6, 13]. Therefore, we wanted to study all advised indicators for family SEP in relation to dental caries [13].

Recently, dental caries was found to be clustered in more deprived areas within a country and even within a city [14-16]. This could be associated with clustering of low SEP families within a particular district. Less access to dental care within a district due to a lower density of available (pediatric) dentists could also be an explanation. It is important to distinguish between these explanations, since this information could help to improve targeting of preventive strategies. Moreover, possible weaknesses within a living area, perpetuating health inequalities, could be identified. Therefore, we also wanted to study the distribution of caries in the city of Rotterdam, the Netherlands. With this information we hoped to answer the question whether possible differences could be only explained by differences in social background or also by characteristics of the district itself.

Summarizing, the purpose of our study was to investigate the association of different socioeconomic and sociodemographic factors on dental caries in six-year-old children. Furthermore, we applied a district approach to explore the distribution of dental caries within the city of Rotterdam, the Netherlands. With this information we also tried to identify possible explanations of the identified differences between districts.

Methods

Study samples

We conducted a cross-sectional study, embedded in the Generation R Study, situated in the city of Rotterdam, the Netherlands. The Generation R Study is a prospective population-based cohort study and was designed to identify environmental and genetic determinants of growth, development and health. The design of this cohort is described in detail elsewhere. For this purpose, children have been followed from fetal life until adulthood in Rotterdam, the Netherlands [17]. The Medical Ethics Committee of Erasmus Medical Center, Rotterdam, approved this study (MEC-2007-413). All participants of this study gave written informed consent.

Study population

All pregnant women with a delivery date between April 2002 and January 2006 and who lived in the study area of Rotterdam, the Netherlands, were eligible for participation in the Generation R Study. From all included children, 8,305 mothers gave consent to participate in the school aged period (5 years onwards) of the Generation R Study. Ultimately, 6,690 children had actually visited the research center. From these, we excluded all participants with incomplete information on dental caries (n = 1,367) and all twin participants (n = 134), leaving a total study population of 5,189 children.

Socioeconomic position

Since young children cannot have yet established their own socioeconomic level, they were classified according to their parents' socioeconomic position. We named this family SEP. We considered the following socioeconomic and sociodemographic factors as indicators for family SEP: parental education level, parental employment status (paid job vs. unpaid job), net household income, parenting, and teenage pregnancy. Educational level was categorized based on the Dutch Standard Classification of Education [18]. We defined four educational levels that could be obtained by a parent: low (no education, primary school, lower vocational training, intermediate general school, or three years or less general secondary school), mid-low (more than three years general secondary school, intermediate vocational training, or first year of higher vocational training), mid-high (higher vocational training), and high (university or PhD degree). Paternal and maternal employment status was defined as paid job or no paid job. Net household income was divided into three categories; < 2,000 euro/month, 2,000–3,200 euro/month and > 3,200 euro/month. Teenage pregnancy was defined as pregnancy in girls aged

19 years or younger and was based on maternal age at enrolment. All information on socioeconomic and sociodemographic factors were obtained by questionnaires [17].

Dental caries

We scored the presence of dental caries on intraoral photographs by using the decayed, missing, and filled teeth index (dmft index). We took these pictures with either the Poscam USB intraoral (Digital Leader PointNix) or Sopro 717 (Acteon) autofocus camera. Both cameras had a resolution of 640x480 pixels and a minimal scene illumination of 1.4 and 30 lx. The usage of intraoral photographs, compared to ordinary oral examination, in scoring dental caries with a dmft index has been described elsewhere and showed to have high sensitivity and specificity (85.5% and 83.6% respectively) [19]. Furthermore, we evaluated intra-observer reliability (K = 0.98) and inter-observer reliability (K = 0.89), both indicating an almost perfect agreement [20].

For the analyses, we categorized the children as having no dental caries (dmft = 0), having mild caries (dmft = 1–3), or having severe caries (dmft > 3). The cut-off values for mild and severe caries were based on the mean dmft index of five-year-old Dutch children obtained from a recent report by Schuller et al. [21].

Covariates

We considered children's age, sex, ethnicity, and oral health behavior as potential confounders in the relationship between dental caries and SEP. Children's ethnicity was based on the birth country of both parents [22]. If one of the parents was born in another country than the Netherlands, the child's ethnicity was defined as the birth country of that parent. Birth country of the mother was conclusive if both parents were born in another country. For this study we categorized children into two different groups; Dutch/Western and non-Western. Mothers had to fill in questionnaires on oral health behavior of their children at age six. Oral health related behavior was measured by age at first dental visit (0–3 years, > 3 years, or never), dental visits in the past year (yes or no), and tooth brushing frequency (once a day, twice a day, or more than twice a day).

District approach

To compare caries prevalence between districts with different levels of SEP, the four-digit postal codes were collected from the mothers when the children were six years of age. The postal code had to be their current living area at the moment of dental caries measurement. We only considered children living in the city of Rotterdam, the Netherlands, for analysis (n = 3,942). In general, Rotterdam has fourteen different

districts (Centrum, Prins Alexander, Pernis, Overschie, Noord, Kralingen-Crooswijk, IJsselmonde, Hoogvliet, Hoek van Holland, Hillegersberg-Schiebroek, Feijenoord, Delfshaven, Charlois, and Rozenburg), however we excluded Pernis, Hoek van Holland, and Rozenburg due to low sample sizes within these districts (n < 20). We ranked the districts from socioeconomically weakest to strongest district, using individual based indicators of SEP. For this, we calculated the prevalence of low SEP, for each SEP indicator, within a district. Afterwards, we took the sum of these to calculate a total prevalence of low SEP indicators per district. By using these numbers, each district could be ranked from lowest to highest total prevalence. We used this ranking to compare our data to the municipality of Rotterdam, the Netherlands, and to visually depict the districts from weakest to strongest. Moreover, the ranking was used to assess the reference district for regression analysis (socioeconomically strongest district). Caries prevalence was calculated for each district by dividing the total number of children with a dmft > 0 by the total number of children living in that district. We used the open-source desktop Geographic Information System (GIS), QGIS 2.8.4-Wien, for Mac, for geo-mapping our ranking of the districts and caries prevalence per district. The difference between the highest and lowest total prevalence of low SEP indicators was divided by four to construct four even quartiles. We applied the same method for caries prevalence.

Statistical analyses

Characteristics of the study population were calculated and presented as absolute numbers with percentages for categorical variables and median values, with 90% range, for continuous variables.

A multinomial regression analysis was performed to analyze the association between all SEP indicators and dental caries, expressed in odds ratio's (OR's) with 95% confidence intervals (CI's). Children were categorized into three groups based on their dental caries experience (dmft = 0 versus dmft = 1–3 or dmft > 3). Three different regression models were constructed. The first model was adjusted for children's age, sex and ethnicity only. The second model was additionally adjusted for children's oral health behavior. The third model was additionally adjusted for all other SEP indicators, to investigate their mediating effects on the association between every single SEP indicator and caries. Moreover, we checked whether the presence of Hypomineralized Second Primary Molars (HSPMs) altered the association between SEP and dental caries [23]. This was not the case and therefore the presence of HSPM was not included in the third model.

Logistic regression analysis was used to analyze the association between the social ranking of a district and dental caries. In this analysis, the district with the highest

social ranking (Hillegersberg-Schiebroek, Rotterdam, The Netherlands) was used as the reference category. First, a crude model, including only the districts as independent variables was built. Second, this model was adjusted for child's age, sex, and ethnicity. Third, this model was additionally adjusted for all SEP indicators.

Children with complete data on all SEP indicators were compared with children with missing data on at least one SEP indicator by using a Pearson Chi-Square test (Table S3.3.1). Parents of children with missing information on at least one indicator of SEP were lower educated ($p < 0.001$), were more often unemployed ($p < 0.005$), had a lower household income ($p < 0.001$), were more often single ($p < 0.001$), and had more often a teenage pregnancy ($p < 0.001$). To handle the missing data, we performed multiple imputation by using the Markov Chain Monte Carlo method [24]. Ten independent datasets were generated by this method after which the pooled effect estimates were calculated [25]. The relationship between all variables, that have been used in this study, served as a predictor in the multiple imputation models. We used the Statistical Package of Social Sciences version 22.0 for Mac (IBM Corp, Armonk, NY, USA) for our statistical analyses. A p-value < 0.05 was considered to be statistically significant.

Non-response analysis and stratified analysis

We compared the children which were excluded from analysis (incomplete information on caries, n = 1,334) with the children that were included in the study (n = 5,189) on all indicators of SEP (Table S3.3.2). There were no significant differences of all indicators between participants with incomplete information on dental caries and participants with complete information ($p > 0.05$). Moreover, since ethnicity is related to SEP, we performed a stratified analysis under Dutch children only for the association between SEP and dental caries [26, 27].

Results

Population characteristics

In Table 3.3.1 the characteristics of the study population are presented. The children had a median age (90% range) of 6.03 years (5.68 to 7.83). The prevalence of caries in our study population was 31.7%. About 28.6% of the mothers and 33.7% of the fathers had a high level of education. The majority of mothers and fathers had a paid job (75.4% and 93.9%, respectively) and almost half of the parents reported a household income of more than 3,200 euros per month (49.7%). The household was run by a single parent in 21.0% of the families.

Table 3.3.1. Characteristics of the study population (n = 5,189)

		Total	Missing
		n (%)	n (%)
Maternal educational level	High	1,260 (28.6)	784 (15.1)
	Mid-high	1,215 (27.6)	
	Mid-low	1,386 (31.5)	
	Low	544 (12.3)	
Paternal educational level	High	1,348 (33.7)	1,192 (23.0)
	Mid-high	913 (22.8)	
	Mid-low	1,085 (27.1)	
	Low	651 (16.3)	
Maternal employment status	Paid job	3,143 (75.3)	1,015 (19.6)
	No paid job	1,031 (24.7)	
Paternal employment status	Paid job	3,644 (93.8)	1,305 (25.1)
	No paid job	240 (6.20)	
Household income	> €,3200	2,075 (49.7)	1,016 (19.6)
	€2,000–€3200	1,081 (25.9)	
	< €2,000	1,017 (24.4)	
Single parenting	No	3,518 (79.0)	738 (14.2)
	Yes	933 (21.0)	
Teenage pregnancy	No	5,044 (97.2)	-
	Yes	145 (2.80)	
Ethnic background	Dutch/Western	3,446 (68.0)	125 (2.41)
	Non-Western	1,618 (32.0)	
Child's sex	Boy	2,601 (50.1)	-
	Girl	2,588 (49.9)	
Child's age	Median (90% range)	6.03 (5.68–7.83)	-
Tooth brushing	Once a day	884 (20.7)	920 (17.7)
	Twice or more a day	3,385 (79.3)	
Age first dental visit	0–3 years	2,389 (54.1)	770 (14.8)
	Older than 3 years	1,863 (42.2)	
	Never been	167 (3.70)	
Dental visit in past year	Yes	3,967 (92.4)	897 (17.3)
	No	325 (7.60)	
Index dmft	0	3,524 (68.3)	-
	1–3	995 (19.2)	
	> 3	652 (12.6)	

Table is based on non-imputed data set. Percentages are based on the number of valid cases.

SEP and caries

Table 3.3.2 shows the results of the multinomial logistic regression models. Maternal mid-low or low, as well as paternal low educational level were significantly associated with mild caries (OR 1.55, 95% CI 1.22 to 1.96; OR 2.13, 95% CI 1.64 to 2.77; OR 1.59, 95% CI 1.28 to 1.98 respectively). Also, both maternal and paternal unemployment were significantly associated with mild caries (OR 1.40, 95% CI 1.18 to 1.65; OR 1.48, 95% CI 1.04 to 2.12 respectively). No other indicators were associated with mild caries. However, all SEP indicators were significantly associated with severe caries. After adjusting for the other indicators, only maternal education remained significantly associated with mild caries. For the association with severe caries, however, paternal educational level, parental employment status, and household income additionally served as independent indicators of SEP. No different associations between SEP indicators and dental caries were observed in the stratified analysis under 2,863 Dutch children (Table S3.3.3).

Districts and caries

The greatest differences between districts were seen in the prevalence of low parental educational levels. Based on the crude model, all odds for having a higher caries prevalence were significantly higher in socially lower ranked districts compared to Hillegersberg-Schiebroek (reference). However, significance of the associations between districts and caries disappeared after adjusting for child characteristics and all SEP indicators in the third model for almost all districts (Table 3.3.3). To visually support these findings, the social ranking and the caries prevalence of each district in the city of Rotterdam, the Netherlands are shown in Figure 3.3.1.

Table 3.3.2. Multinomial Logistic regression models for the association between Socioeconomic Position (SEP) indicators and dmft indices

		dmft						
		Model 1			Model 2		Model 3	
		OR [95% CI]			OR [95% CI]		OR [95% CI]	
(n = 5,189)		Index 0	Index 1–3	Index > 3	Index 1–3	Index > 3	Index 1–3	Index > 3
Maternal educational level	High	reference	reference	reference	reference	reference	reference	reference
	Mid-high	reference	1.20 [0.95–1.52]	1.37 [0.74–2.56]	1.21 [0.96–1.53]	1.40 [0.74–2.65]	1.18 [0.92–1.50]	1.13 [0.64–2.00]
	Mid-low	reference	**1.55 [1.22–1.96]**	**2.83 [1.55–5.15]**	**1.56 [1.23–1.98]**	**2.91 [1.56–5.43]**	**1.46 [1.10–1.93]**	1.83 [1.00–3.36]
	Low	reference	**2.13 [1.64–2.77]**	**6.05 [3.36–10.9]**	**2.16 [1.62–2.87]**	**6.38 [3.33–12.2]**	**1.85 [1.31–2.62]**	**3.10 [1.60–6.02]**
Paternal educational level	High	reference	reference	reference	reference	reference	reference	reference
	Mid-high	reference	1.14 [0.90–1.46]	1.39 [0.72–2.70]	1.15 [0.91–1.45]	1.42 [0.74–2.69]	1.03 [0.82–1.31]	1.12 [0.75–1.67]
	Mid-low	reference	1.20 [0.95–1.50]	**2.07 [1.28–3.37]**	1.20 [0.96–1.51]	**2.11 [1.30–3.42]**	0.96 [0.75–1.23]	1.30 [0.84–2.00]
	Low	reference	**1.59 [1.28–1.98]**	**4.04 [2.67–6.12]**	**1.60 [1.28–2.04]**	**4.18 [2.59–6.74]**	1.09 [0.83–1.43]	**1.83 [1.13–2.96]**
Maternal employment status	Paid job	reference	reference	reference	reference	reference	reference	reference
	No paid job	reference	**1.40 [1.18–1.65]**	**2.51 [2.01–3.13]**	**1.38 [1.17–1.63]**	**2.52 [1.97–3.22]**	1.12 [0.92–1.35]	**1.60 [1.25–2.04]**

Paternal employment status	Paid job	reference	reference	reference	reference	reference	reference	reference	reference
	No paid job	reference	**1.48 [1.04–2.12]**	**2.08 [1.39–3.10]**	1.46 [1.01–2.11]	**2.05 [1.39–3.03]**	1.30 [0.89–1.89]	**1.57 [1.04–2.37]**
Household income	> €3,200	reference	reference	reference	reference	reference	reference	reference	reference
	€2,000–€3,200	reference	1.22 [0.97–1.54]	**2.28 [1.39–3.74]**	1.22 [0.98–1.52]	**2.28 [1.42–3.67]**	1.06 [0.86–1.32]	**1.66 [1.18–2.33]**
	< €2,000	reference	**1.55 [1.21–1.99]**	**3.16 [1.92–5.19]**	**1.55 [1.22–1.97]**	**3.19 [1.91–5.32]**	1.20 [0.91–1.59]	1.42 [0.88–2.29]
Single parenting	No	reference	reference	reference	reference	reference	reference	reference	reference
	Yes	reference	1.17 [0.98–1.40]	**1.56 [1.24–1.97]**	1.16 [0.97–1.39]	**1.56 [1.23–1.99]**	0.93 [0.76–1.13]	1.00 [0.76–1.33]
Teenage pregnancy	No	reference	reference	reference	reference	reference	reference	reference	reference
	Yes	reference	1.04 [0.67–1.61]	**1.85 [1.20–2.84]**	1.02 [0.66–1.60]	**1.85 [1.18–2.88]**	0.80 [0.51–1.25]	1.12 [0.72–1.73]

Model 1 = basic model, adjusted for age, sex and child's ethnicity only;
Model 2 = additionally adjusted oral health related behaviour (Tooth brushing frequency, age at first dental visit, dental visit in past year);
Model 3 = additionally adjusted for all other SEP indicators;
Significant associations are **bold**.

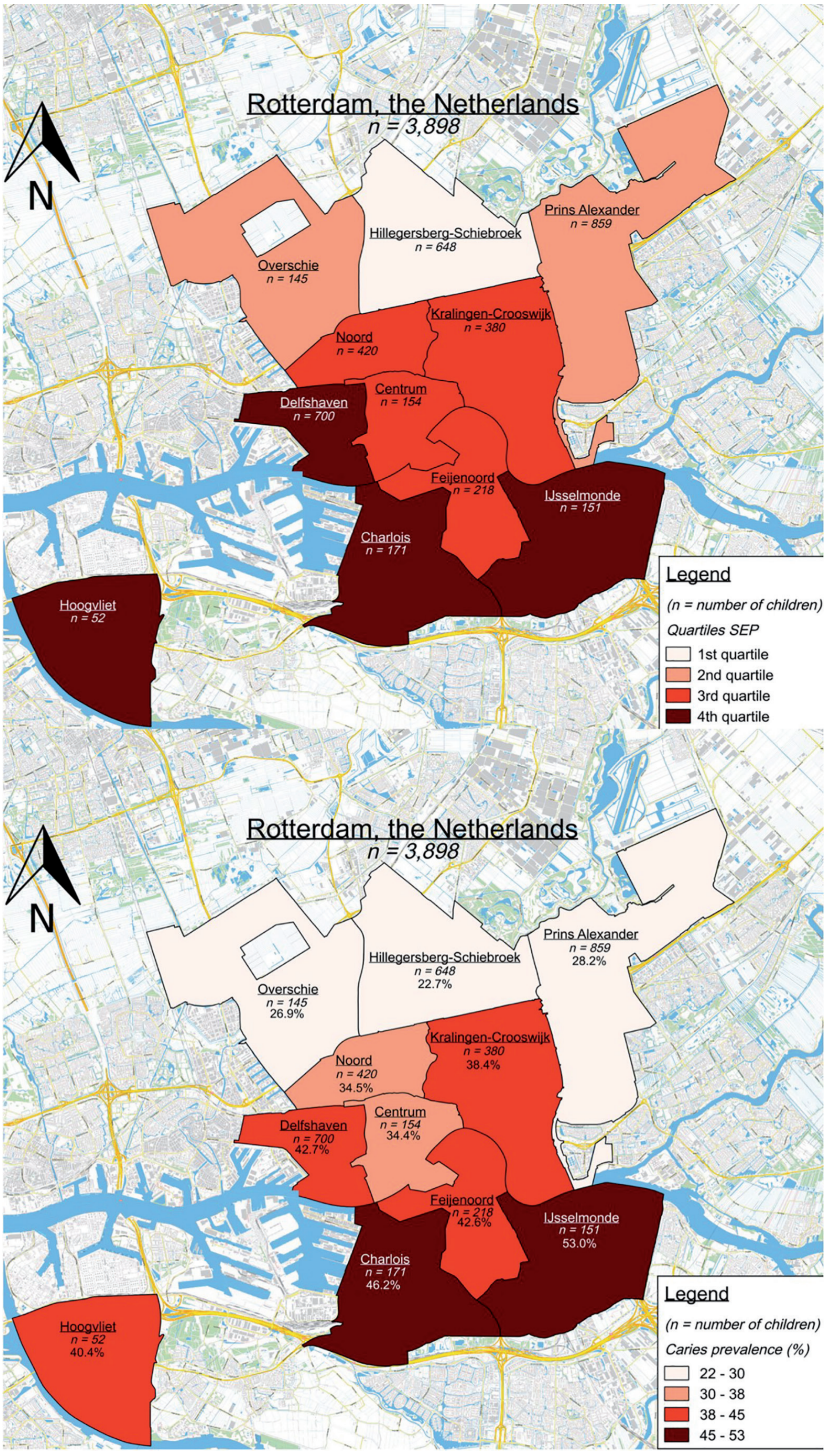


Figure 3.3.1. Ranking of each district (upper) and caries prevalence per district (lower).

Table 3.3.3. Binary logistic regression for the association between districts and dental caries*

District (n = 3,898) (n with caries / n without caries)	Crude model OR [95% CI] dmft-index > 0	Model 2 OR [95% CI] dmft-index > 0	Model 3 OR [95% CI] dmft-index > 0
1. Hillegersberg/Schiebroek (147 / 501)	reference	reference	reference
2. Prins Alexander (242 / 617)	**1.34 [1.06–1.69]**	1.23 [0.97–1.57]	1.03 [0.80–1.32]
3. Overschie (39 / 106)	1.25 [0.83–1.89]	1.13 [0.74–1.71]	0.90 [0.58–1.39]
4. Kralingen-Crooswijk (146 / 234)	**2.13 [1.61–2.80]**	**1.62 [1.22–2.16]**	1.33 [0.99–1.79]
5. Noord (145 / 275)	**1.80 [1.37–2.36]**	**1.37 [1.04–1.82]**	1.06 [0.79–1.42]
6. Centrum (53 / 101)	**1.79 [1.22–2.62]**	1.32 [0.89–1.96]	0.98 [0.65–1.48]
7. Feijenoord (92 / 126)	**2.49 [1.80–3.45]**	**1.46 [1.03–2.07]**	1.12 [0.78–1.61]
8. Delfshaven (299 / 401)	**2.54 [2.01–3.22]**	**1.62 [1.25–2.10]**	1.19 [0.91–1.56]
9. Hoogvliet (21 / 31)	**2.31 [1.29–4.14]**	1.43 [0.78–2.62]	1.08 [0.58–2.02]
10. IJsselmonde (80 / 71)	**3.84 [2.66–5.55]**	**2.45 [1.67–3.60]**	**1.85 [1.24–2.76]**
11. Charlois (79 / 92)	**2.93 [2.06–4.16]**	**1.66 [1.37–2.01]**	1.21 [0.83–1.79]

* Districts were ranked from lowest prevalence of SEP indicators, that are associated with dental caries, to highest prevalence.
Model 2 = Adjusted for age, sex, and child's ethnicity.
Model 3 = Additionally adjusted for all SEP indicators (parental educational level, parental employment status, household income, single parenting, and teenage pregnancy).
Significant associations are **bold**.

Discussion

Our results demonstrate a higher caries prevalence in children with a low family SEP. Maternal education level served as the most important indicator of family SEP in the association with dental caries. Furthermore, we observed major inequalities in caries prevalence between the districts with different socioeconomic levels. However, those inequalities were mostly explained by family SEP of the population.

In agreement with other studies, we have found a higher caries prevalence in children of parents with a low educational level, without a paid job, and with a low household income [6, 28]. As mentioned before different studies used diverse indicators for SEP. This lowers the comparability and may increase the chance of residual confounding. Our study is one of the first that studied all recommended indicators of family SEP in relation to dental caries simultaneously.

Schwendicke et al. [6] already proposed possible mediating pathways for the association between caries and SEP. Parents with a low education level would have worse health literacy, worse dietary and oral health behavior and lower health service utilization [6].

We did not find oral health behavior to explain the association between SEP and dental caries. Although we did not take dietary habits into account, we do believe that this plays an important role in explaining high caries prevalence among children with a low SEP. Especially, since colleagues found an association between low SEP and a more frequent "Western" dietary pattern (containing high intake of sugar-containing beverages) at the age of 14 months in our population [29]. Adherence to this more cariogenic diet may track from this age to childhood and therefore cause more caries [29-31]. Furthermore, maternal educational level was the most influential on the odds of having mild or severe caries. Recently, van den Branden et al. [32] observed more desirable oral health behavior (brushing frequency and visits to the dentist) and lower consumption of in-between sugared drinks among five-year-old children from mothers with high educational backgrounds. This was in agreement with the results of Schwendicke et al. [6]. Hence, both differences in oral health behavior and the amount of sugar consumption can be reasonable explanations for the association between parental educational levels and caries.

Interestingly, lower household income was another strong predictor of severe caries prevalence in children of our population. Costs for basic dental care are reimbursed for all children until the age of 18 in The Netherlands, according to the basic health insurance. Therefore, financial reasons probably should not play a role in parents not providing their children with adequate dental care in the Netherlands. Unlike parents from other countries, where dental care is not or only partly insured [33]. Likewise, the Dutch Central Agency for Statistics (CBS) recently observed no disparities in dental visit frequency of children between different groups of income in the Netherlands [34]. Adults with a low income, however, do tend to visit a dentist less often than adults with a higher income [34]. As a result, we speculate that their children will not visit the dentist either. Partly because of financial reasons and partly due to disinterest or ignorance. Still, dental visit in the past year at the age of six did not explain the association between dental caries and SEP in our population. Our data, however, was parent-reported and the total number of cavities and fillings can develop in more than one year. Therefore, objectifying dental health service utilization frequency over a longer time period in future research is needed to make a more valid statement.

Furthermore, social participation, support, stability, and cohesion are also influenced by SEP and may affect health [6]. Duijster et al. [35] recently found that a lower SEP was significantly associated with lower dental self-efficacy, a more external locus of control and poorer parenting practices. It is thus likely that these factors, along with other possible unmeasured and unknown mediators, play a role in the complex interaction between SEP and caries. Future research should further explore this complex interaction, so that social disparities in caries prevalence can be tackled in the future.

We have not only measured SEP on a family level, but also studied the association between district and dental caries. In correspondence with the results of Truin et al. [36], we observed a higher caries prevalence in more disadvantaged districts. Disparities between the districts in our study were mostly explained by socioeconomic differences and not by district characteristics. This finding is new and can be used by policy makers to efficiently target prevention programs. In development of preventive methods, evidence suggests not only to provide easier access to health care, but also to intervene more upstream at risks, beliefs, and behavior [37]. Suggestions for development or improvement of these interventions are provision of clear oral health education using a positive approach, early referral to a dental practice, dietary regulations at school and a multidisciplinary approach in providing parental support [35]. Further research should focus on developing the most effective methods on resolving inequalities in oral health.

During interpretation of our results, attention needs to be paid to some limitations. We have not performed an intraoral examination on the children for diagnosing dental caries, which is known as the gold standard [38]. Instead, we have used intraoral photographs. This method has shown to perform moderately [19]. Therefore, non-differential misclassification could have occurred in our data, which may have led to a possible underestimation of the association between SEP indicators and dental caries prevalence. Subsequently, use of intraoral examination would have resulted in stronger associations. Furthermore, occurrence of response bias could not have been completely avoided due to the use of questionnaires. Especially for the parent-reported oral health behavior questions it is likely that we received socially desirable answers. Although we were able to adjust for a broad range of confounding factors, residual confounding should still be considered. The municipality of Rotterdam published for each district a social index score based on capacities, living environment, participation, and social bonding [39]. However, we made a ranking of the districts ourselves. This turned out to be representative of the ranking of the municipality of Rotterdam, the Netherlands, except for Hoogvliet [39]. Therefore, caution needs to be taken when interpreting the results for this district. As a final point, use of a multilevel model would have given us the association between SEP and caries independent of district characteristics. Influence of district characteristics on the caries distribution, however, was not likely from the logistic regression analysis. Therefore, we chose not to perform a multilevel logistic regression analysis for our study.

The major strength of this study was the large population of children with different socioeconomic backgrounds included. Furthermore, we performed adequate statistical methods and considered different approaches to answer our research questions. Another strength is that we have also included SEP on a community level per district.

Geo-mapping of these results gave us a clear insight of where the disparities are the greatest and where preventive strategies should be targeted at.

In conclusion, dental caries is more prevalent among children with a lower SEP and this is even visible at the district level. The magnitude of inequalities in oral health among districts was previously unknown and should be unacceptable in a developed country such as The Netherlands. These new findings will help the development of health strategies to be better targeted and to efficiently reduce caries prevalence. Future research should address the pathways that are responsible for the high prevalence of dental caries among socially disadvantaged groups.

References

[1] M.C. Gomes, T.C. Pinto-Sarmento, E.M. Costa, C.C. Martins, A.F. Granville-Garcia, S.M. Paiva, Impact of oral health conditions on the quality of life of preschool children and their families: a cross-sectional study, Health Qual. Life Outcomes. 12 (2014) 55.

[2] L. Kragt, J.T. van der Tas, H.A. Moll, M.E. Elfrink, V.W. Jaddoe, E.B. Wolvius, E.M. Ongkosuwito, Early Caries Predicts Low Oral Health-Related Quality of Life at a Later Age, Caries Res. 50(5) (2016) 471-479.

[3] W. Marcenes, N.J. Kassebaum, E. Bernabé, A. Flaxman, M. Naghavi, A. Lopez, C.J. Murray, Global burden of oral conditions in 1990-2010: a systematic analysis, J. Dent. Res. 92(7) (2013) 592-597.

[4] L.C.J. Slobbe, J.M. Smit, J. Groen, M.J.J.C. Poos, G.J. Kommer, Costs of illness in the Netherlands 2007; trends in Dutch health expenditures 1999-2010, 2011. www.rivm.nl/dsresource?objectid=rivmp:61294&type=org&disposition=inline&ns_nc=1. (Accessed 31.10.2016).

[5] M. Marmot, J. Allen, R. Bell, E. Bloomer, P. Goldblatt, Consortium for the European Review of Social Determinants of Health and the Health Divide, WHO European review of social determinants of health and the health divide, Lancet 380(9846) (2012) 1011-1029.

[6] F. Schwendicke, C.E. Dörfer, P. Schlattmann, L. Foster Page, W.M. Thomson, S. Paris, Socioeconomic inequality and caries: a systematic review and meta-analysis, J. Dent. Res. 94(1) (2015) 10-18.

[7] L.B. Christensen, S. Twetman, A. Sundby, Oral health in children and adolescents with different socio-cultural and socio-economic backgrounds, Acta Odontol. Scand. 68(1) (2010) 34-42.

[8] M. Maliderou, S. Reeves, C. Noble, The effect of social demographic factors, snack consumption and vending machine use on oral health of children living in London, Br. Dent. J. 201(7) (2006) 441-444.

[9] K. Pieper, S. Dressler, M. Heinzel-Gutenbrunner, A. Neuhäuser, M. Krecker, K. Wunderlich, A. Jablonski-Momeni, The influence of social status on pre-school children's eating habits, caries experience and caries prevention behavior, Int. J. Public Health 57(1) (2012) 207-215.

[10] A.A. Schuller, P. van Dommelen, J.H. Poorterman, Trends in oral health in young people in the Netherlands over the past 20 years: a study in a changing context, Community Dent. Oral. Epidemiol. 42(2) (2014) 178-184.

[11] G.J. Truin, J.E. Frencken, J. Mulder, A.J. Kootwijk, E. de Jong, Prevalentie van tandcariës en tanderosie bij Haagse schoolkinderen in de periode 1996-2005, Ned. Tijdschr. Tandheelkd. 114 (2007) 335-342.

[12] G.H. Verrips, H. Kalsbeek, M.A. Eijkman, Ethnicity and maternal education as risk indicators for dental caries, and the role of dental behavior, Community Dent. Oral. Epidemiol. 21(4) (1993) 209-214.

[13] B. Galobardes, M. Shaw, D.A. Lawlor, J.W. Lynch, G. Davey Smith, Indicators of socioeconomic position (part 1), J. Epidemiol. Community Health 60(1) (2006) 7-12.

[14] A. Kämppi, T. Tanner, J. Päkkilä, P. Patinen, M.R. Järvelin, L. Tjäderhane, V. Anttonen, Geographical distribution of dental caries prevalence and associated factors in young adults in Finland, Caries Res. 47(4) (2013) 346-354.

[15] M.C. Priesnitz, R.K. Celeste, M.J. Pereira, C.A. Pires, C.A. Feldens, P.F. Kramer, Neighbourhood Determinants of Caries Experience in Preschool Children: A Multilevel Study, Caries Res. 50(5) (2016) 455-461.

[16] D. Duijster, C. van Loveren, E. Dusseldorp, G.H. Verrips, Modelling community, family, and individual determinants of childhood dental caries, Eur. J. Oral. Sci. 122(2) (2014) 125-133.

[17] M.N. Kooijman, C.J. Kruithof, C.M. van Duijn, O.H. Franco, M.H. van IJzendoorn, J.C. de Jongste, C.C. Klaver, A. van der Lugt, J.P. Mackenbach, H.A. Moll, R.P. Peeters, H. Raat, E.H. Rings, F. Rivadeneira, M.P. van der Schroeff, E.A. Steegers, H. Tiemeier, A.G. Uitterlinden, F.C. Verhulst, E. Wolvius, J.F. Felix, V.W. Jaddoe, E.J.E. 1243., The Generation R Study: design and cohort update 2017, Eur. J. Epidemiol. 31(12) (2016) 1243-1264.

[18] R. Schaart, S. Westerman, M.B. Moens, The Dutch standard classification of education SOI 2006, Statistics Netherlands, 2008. www.cbs.nl/en-gb/background/2008/24/the-dutch-standard-classification-of-education-soi-2006. (Accessed 01.10.2016).

[19] M.E. Elfrink, J.S. Veerkamp, I.H. Aartman, H.A. Moll, J.M. Ten Cate, Validity of scoring caries and primary molar hypomineralization (DMH) on intraoral photographs, JM Eur. Arch. Paediatr. Dent. 10(Suppl. 1) (2009) 5-10.

[20] J.R. Landis, G.G. Koch, The Measurement of Observer Agreement for Categorical Data, Biometrics 33(1) (1977) 159-174.

[21] A.A. Schuller, I.P.F. van Kempen, J.H.G. Poorterman, G.H.W. Verrips, Kies voor tanden: Een onderzoek naar mondgezondheid en preventief tandheelkundig gedrag van jeugdigen, 2013. www.tno.nl/media/1167/kiesvoortanden_tnols2013r10056.pdf. (Accessed 05.09.2016).

[22] CBS, Centraal Bureau voor de Statistiek: Jaarrapport Integratie 2014, 2014.http://www.cbs.nl/NR/rdonlyres/4735C2F5-C2C0-49C0-96CB-0010920EE4A4/0/jaarrapportintegratie2014pub.pdf. (Accessed 10.10.2016).

[23] M.E. Elfrink , A.A. Schuller, J.S. Veerkamp, J.H. Poorterman, H.A. Moll, B.J. ten Cate, Factors increasing the caries risk of second primary molars in 5-year-old Dutch Children, Int. J. Paediatr. Dent. 20(2) (2010) 151-157.

[24] J.A.C. Sterne, I.R. White, J.B. Carlin, M. Spratt, P. Royston, M.G. Kenward, A.M. Wood, J.R. Carpenter, Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls, BMJ 338 (2009) b2393.

[25] D.B. Rubin, N. Schenker, Multiple imputation in health-care databases: an overview and some applications, Stat. Med. 10(4) (1991) 585-598.

[26] P.A. Braveman, C. Cubbin, S. Egerter, S. Chideya, K.S. Marchi, M. Metzler, S. Posner, Socioeconomic status in health research: one size does not fit all, JAMA 294(22) (2005) 2879-2888.

[27] T.L. Cheng, E. Goodman, Committee on Pediatric Research, Race, ethnicity, and socioeconomic status in research on child health, Pediatrics 135(1) (2015) e225-237.

[28] L. Christensen, S. Twetman, A. Sundby, Oral health in children and adolescents with different socio-cultural and socio-economic backgrounds, Acta Odontol Scand 68(1) (2010) 34-42.

[29] J.C. Kiefte-de Jong, J.H. de Vries, S.E. Bleeker, V.W. Jaddoe, A. Hofman, H. Raat, H.A. Moll, Socio-demographic and lifestyle determinants of 'Western-like' and 'Health conscious' dietary patterns in toddlers, Br. J. Nutr. 109(1) (2013) 137-147.

[30] M.A. Peres, A. Sheiham, P. Liu, F.F. Demarco, A.E. Silva, M.C. Assunção, A.M. Menezes, F.C. Barros, K.G. Peres, Sugar Consumption and Changes in Dental Caries from Childhood to Adolescence, J. Dent. Res. 95(4) (2016) 388-394.

[31] W. Sohn, B.A. Burt, M.R. Sowers, Carbonated soft drinks and dental caries in the primary dentition, J. Dent. Res. 85(3) (2006) 262-266.

[32] S. van den Branden, S. van den Broucke, R. Leroy, D. Declerck, K. Hoppenbrouwers, Oral health and oral health-related behaviour in preschool children: evidence for a social gradient, Eur. J. Pediatr. 172(2) (2013) 231-237.

[33] S.O. Griffin, L.K. Barker, L. Wei, C.H. Li, M.S. Albuquerque, B.F. Gooch, Use of dental care and effective preventive services in preventing tooth decay among U.S. Children and adolescents--Medical Expenditure Panel Survey, United States, 2003-2009 and National Health and Nutrition Examination Survey, United States, 2005-2010, MMWR Suppl. 63(2) (2014) 54-60.

[34] CBS, Met hoger inkomen meer naar tandarts en mondhygiënist, 2016. https://www.cbs.nl/nl-nl/nieuws/2016/11/met-hoger-inkomen-meer-naar-tandarts-en-mondhygienist. (Accessed 20.08.2016).
[35] D. Duijster, M. de Jong-Lenters, C. de Ruiter, J. Thijssen, C. van Loveren, E. Verrips, Parental and family-related influences on dental caries in children of Dutch, Moroccan and Turkish origin, Community Dent. Oral. Epidemiol. 43(2) (2015) 152-162.
[36] G.J. Truin, J.E. Frencken, A.J. Mulder, A.J. Kootwijk, E. de Jong, Tandcariës en tanderosie bij de Haagse schooljeugd in de periode 2002-2008, Epidem. Bull. 44(1) (2009) 2-9.
[37] J. Steele, J. Shen, G. Tsakos, E. Fuller, S. Morris, R. Watt, C. Guarnizo-Herreño, J. Wildman, The Interplay between socioeconomic inequalities and clinical oral health, J. Dent. Res. 94(1) (2015) 19-26.
[38] T. Gimenez, C. Piovesan, M.M. Braga, D.P. Raggio, C. Deery, D.N. Ricketts, K.R. Ekstrand, F.M. Mendes, Visual Inspection for Caries Detection: A Systematic Review and Meta-analysis, J. Dent. Res. 94(7) (2015) 895-904.
[39] W. Vleugels, J. van Gelder, T. van Doveren, P. Koppelaar, C. Ergun, L. van Dun, Rotterdam sociaal gemeten: 3e meting Sociale Index, 2010. http://www.rotterdam.nl/COS/publicaties/Vanaf%202005/09-3100.Sociale%20Index%202010.pdf. (Accessed 05.08.2016 2016).

Chapter 3.4

Caries experience among children born after a complicated pregnancy

Justin T. van der Tas | Eppo B. Wolvius | Lea Kragt |
Fernando Rivadeneira | Henriëtte A. Moll | Eric A.P. Steegers |
Sarah Schalekamp-Timmermans

Submitted for publication

4

Chapter 4

Validation of using quantitative light-induced fluorescence photographs for assessing dental caries and enamel hypomineralization

Justin T. van der Tas | Lea Kragt | Fernando Rivadeneira | Brunilda Dhamo | Eppo B. Wolvius

Submitted for publication

5

General discussion

This thesis contributes to the existing epidemiological knowledge of dental enamel hypomineralization and dental caries. In this chapter, the main findings together with their general interpretation and subsequent implications will be discussed. Hereafter, methodological considerations on limitations and strengths are point of discussion. Finally, future research directions will be proposed, and the chapter ends with general conclusions.

Main findings' interpretation and their clinical implications

Here we focus on those findings requiring additional discussion, in a more general context than the one provided in each chapter.

Mineral metabolism and dental enamel hypomineralization

Chapter 2 focuses on determining new risk factors for dental enamel hypomineralization, with mineral metabolism as the main and shared interest. Considering that there is an inverse association between enamel hypomineralization and some parameters of bone accrual in childhood (Chapter 2.1); a logical next step was to evaluate in Chapter 2.2. whether 25(OH) vitamin D levels, which play an essential role in calcium metabolism, could have an effect on the risk of having dental enamel hypomineralization [1-5]. We measured 25(OH)D concentrations at three points in time; prenatal in mothers, early postnatal (umbilical cord blood) and late postnatal (at the age of six). However, no significant association between the 25(OH)D status at any of the points in time and dental enamel hypomineralization at the age of six was found.

Before the study from Chapter 2.1, there were no studies published about the association between bone health and dental enamel hypomineralization. There was only one research group that has studied the association between Bone Mineral Density (BMD) and dental caries, which is interesting because children with dental enamel hypomineralization are more prone to develop caries [6, 7]. Fabiani et al. found a positive relationship between a low BMD and dental caries in 12-year-old children [8]. Since Fabiani et al. did not adjust for dental enamel hypomineralization within their analyses, part of the reported association could be explained by a skewed distribution of children affected by dental enamel hypomineralization within their population. However, because of the relatively low prevalence of dental enamel hypomineralization and older age of their population this effect is expected to be minimal. Apparently, children with worse low bone health parameters are more prone to develop enamel diseases as Hypomineralized Second Primary Molars (HSPM's) and dental caries than

children with high bone health parameters. The reason why low Bone Mineral Content (BMC) is associated with HSPM and not with Molar Incisor Hypomineralization (MIH) remains to be elucidated. It is well-established that the first permanent molars need a longer time period to develop fully than the primary second molar [9, 10]. Therefore, it is plausible that within this longer period of development, the mineralization capacity is of less importance for the eventual mineral density of a permanent tooth than that of a primary tooth.

What is the clinical relevance of the finding that children with a low BMC are more frequently affected by HSPM than children with a high BMC? Somehow, the general ability to mineralize tissue in children with HSPM seems to be affected. Tissue mineralization is dependent of cation/ion availability throughout the body, with calcium as the most important element of the hydroxyapatite mineral ($Ca_{10}(PO_4)_6(OH)_2$), which forms the basis of dental enamel and bone [11, 12]. The availability of this cation is dependent of several factors like calcium intake and vitamin D status. How calcium intake affects its cation availability is straightforward, but behind the regulation of the calcium serum concentration by vitamin D hides a more complex interplay of different processes. Vitamin D affects the serum calcium concentration by three different activities; it enhances intestinal calcium absorption, together with parathyroid hormone (PH) it mobilizes calcium from bone by activating osteoclasts if the calcium blood serum concentration is too low, and together with PH it enhances the reabsorption of calcium from the distal renal tube [13]. Hence, the question is whether the mineralization ability is affected by a too low calcium intake, by a too low vitamin D serum concentration, or are there any other unknown etiological factors accountable?

Within the studies is was not possible to adjust for calcium intake in a reliable way, but in Chapter 2.2 the relationship between vitamin D serum concentrations and dental enamel hypomineralization was further explored. However, no significant association was found, although previous research had suggested that 25(OH)D status is a significant factor in the development of MIH as well as for dental caries [14-18]. It was suggested that high 25(OH)D concentrations could have led to enhanced enamel mineralization which prevents the development of dental caries [16]. However, this possible mechanism of enhanced enamel mineralization, was not evident in the study population of this thesis as less dental enamel hypomineralization. Another mechanism may be found through an immunological pathway, in which 25(OH)D enhances the expression of cathelicidin. Cathelicidin is an antimicrobial peptide, that can be found in epithelial and immunological cells, and destroys the cell membrane of microbes [5]. This may rationalize the significant relationship between 25(OH)D and dental caries (which is an infectious disease), and no relationship between 25(OH)D and enamel

hypomineralization (which is a developmental disease). Severe Vitamin D deficiency (set at below 10 nmol/L) has been shown to have undeniable biologic deleterious consequences on the skeletal system either due to "environmental vitamin D deficiency" resulting in rickets in children and osteomalacia in adults; or due to genetic mutations resulting from hereditary vitamin D-dependent rickets or 1α-Hydroxylase deficiency [19]. The phenotypic presentation of these rare genetic diseases is the consequence of alterations in calcium metabolism, extended to tooth alterations and alopecia (findings also corroborated by mutant mice models). In fact, in utero development in all cases is considered to be normal due to maternal normocalcemia (as is the case with children born normal from mothers with osteomalacia). This is in line with the finding of no effect in relation to maternal vitamin D status. Whereas in children from Generation R, the low number of children with very low vitamin D levels also explains the lack of association with dental enamel hypomineralization propensity. Still, more research is needed to fully understand a possible role of vitamin D in the development of dental enamel hypomineralization.

From a clinical perspective, the Health Council of the Netherlands advises all children up to the age of four to use vitamin D supplementation of 10mcg on a daily basis in order to prevent the occurrence of rickets [20]. Based on our findings, we cannot advise to add dental enamel hypomineralization as a possible extra indication for vitamin D supplementation in children. Neither are we able to make any recommendations concerning calcium intake in children as a preventive measure for dental enamel hypomineralization. Moreover, the existing evidence suggests a multifactorial etiology for HSPMs and MIH [21-23]. Therefore, it is unlikely that the association between BMC and dental enamel hypomineralization is fully explained by a deficit in mineral metabolism by vitamin D only. More research is needed to further investigate these associations, to deliberate more on new hypotheses, or to test any preventive measures for dental enamel hypomineralization.

Ethnic and genetic determinants on caries

Dental caries, the most common form of enamel disease, was previously shown by our group to be found more often in children with dental enamel hypomineralization [6, 7]. Nevertheless, it was still not established whether genetic susceptibility plays a role. First, we explored in one of our studies (Chapter 3.1) whether ethnic disparities in caries prevalence existed in our study population. The odds to have dental caries (defined as a decayed-, missing, and filled teeth (dmft) > 0) were almost two-times higher in Surinamese Hindustani children and more than three-times higher in Turkish and Moroccan as compared to Dutch children. Both Turkish and Moroccan children

even had five-time higher odds to experience severe dental caries (dmft > 3) than Dutch children. These children from ethnic minority groups were vulnerable to develop dental caries, but the origin of these disparities remain to be elucidated. The literature proposes a few hypotheses like poor oral hygiene, frequent sugar consumption, and/or genetic factors [24-26]. I elaborated on these genetic factors in Chapter 3.2, where a Genome Wide Association Study (GWAS) for caries was carried out within a consortium of nine different research groups around the world. Using the GWAS data we could determine that the heritability of dental caries in the primary and permanent dentition was low. From the GWAS meta-analysis of caries in primary teeth, a marker (rs1594318) on chromosome 2 (chr2: 3733944) was suggestive of genome wide significance. The meta-analysis for caries in the permanent dentition revealed another locus associated with dental caries, with the strongest signal (rs7738851) mapping to chromosome 6 (chr6:11241788). While environmental factors are expected to play a prominent role, the heritable component suggests that a highly polygenic architecture underlies the etiology of dental caries, as recently corroborated by the discovery of 47 GWAS signals in the UK-Biobank study including almost 490,000 participants [27].

Impact of caries in a sociodemographic context

The next step was to typify further the environmental factors influencing the risk of developing caries. In Chapter 3.1 I also showed that an important part of the association between ethnicity and dental caries was explained by mother's educational level and household income. In line with the influence of sociodemographic factors, I studied in Chapter 3.3 how social inequalities could influence caries prevalence in children. Moreover, we explored the distribution of caries within the city of Rotterdam, the Netherlands. The most important indicator of socioeconomic position (SEP) in relation to dental caries was maternal educational level. Furthermore, paternal educational level, parental employment status, and household income additionally served as independent indicators of SEP in children with severe caries (dmft > 3). Examining the distribution of dental caries within Rotterdam showed clustering of dental caries within certain districts. Especially children in the districts IJsselmonde and Charlois were highly affected by caries. The significant relationship between the districts and the caries prevalence, however, disappeared after adjustment for all included SEP indicators.

The findings of this thesis suggest that caries may "run in a family" with certain genetic factors as a plausible underlying reason. However, in this thesis only a few genetic loci were identified that led to a decreased or increased caries risk in the primary and permanent dentition respectively, and the heritability of dental caries was estimated to be low. Clinicians should still reckon the environmental risk factors (highly frequent

sugar consumption, low fluoride use, and bad oral hygiene) as the most indicative rather than single genetic variants. Information towards patients should take this into account as well; dental caries risk is most probably the consequence of the patient's own behavior and actions, more than it is determined by their genetic background which is characteristic for a multifactorial disease.

In light of the findings above, we found no environmental factors that fully explained the association between ethnicity, SEP, and dental caries. However, we did not adjust for sugar consumption which might explain the worse oral health in children with an ethnic minority background and/or from a mother with a low educational level [28, 29]. Following the caries prevention recommendations of "het Ivoren Kruis", there is already ample attention for nutritional influences on dental caries [30]. In their core nutritional advise, they recommend no more than three main meals, no more than four snacks in-between meals, to avoid sugary drinks, to avoid intake of acidy products one hour before tooth brushing, and to avoid eating or drinking of sugar-containing beverages after the last moment of tooth brushing in the evening (Table 5.1). If this guideline would be followed strictly by dental professionals, every patient should be familiar with these nutritional advices. Patients presenting with caries activity at a dental professional, should be informed again about the core advises or should get

Table 5.1. Core advice caries prevention[1] "Ivoren Kruis"

1. Core advice oral hygiene	2. Core advice fluoride	3. Core advice nutrtion
• Brush the teeth twice a day for 2 minutes with an appropriate amount of toothpaste • Children from 0 up to 1 year brush the teeth once a day with toothpaste for toddlers • Parents are advised to brush the teeth again after the children brushed the teeth themselves up to the age of ten before going to bed	• <u>0 and 1 year of age:</u> From the eruption of the fist tooth brush once a day with toothpaste containing 500–750 ppm fluoride • <u>2, 3, and 4 years of age:</u> Brush the teeth twice a day with toothpaste containing 500–750 ppm fluoride • <u>5 years of age and older:</u> Brush the teeth twice a day with toothpaste containing 1000–1500 ppm fluoride • <u>For all ages:</u> Consult the dentist or oral hygienist for any other form of fluoride use	• Only eat or drink something 7 times a day (3 main meals and a maximum of 4 snacks) with an interval of minimal 2 hours • Do not eat or drink acidy products one hour before toothbrushing • Do not eat or drink anything after the last moment of toothbrushing and do not bring anything to bed

[1] B.M. van Amerongen, C.M.M. Berendsen-Wolters, N.G. Blanksma, S.J. Fokkema, M.C.D.N.J.M. Huysmans, C. van Loveren, A.A. Schuller, G. Stel, Advies Cariëspreventie, 2011. https://www.ivorenkruis.nl/userfiles/File/IvK_Advies_Cari_spreventie.pdf.

additional recommendations concerning their oral hygiene, fluoride use, and/or their nutritional behavior [30]. Despite this guideline, disparities in caries prevalence among six-year-old children from different ethnic groups and a different SEP exist. Question remains why these caries prevention recommendations of "het Ivoren Kruis" are less known/less followed among children from an ethnic minority group or from a low SEP. A dental professional should be aware of suboptimal adherence or knowledge to the core recommendations in an early stage, so that guidance to a better adherence or more informative counseling can be started. Therefore, it is encouraged for a dental professional to check the parents' and/or patients' knowledge and insight on oral health on a regular basis. Another recommendation would be to start with oral health training at the "Centrum voor Jeugd en Gezin" already. We know that early education of the parents, as used in the non-operative caries treatment program (NOCTP), at the age of 8 months is an effective measure in caries prevention [31, 32]. However, I found that the probability of seeing children at this age at the dentist is low. Almost 50% of the children visited the dentist above three years of age for the first time or never visited the dentist at the age of six. Therefore, if the age of first dental visit remains this high, the likelihood of reaching children and their parents at a younger age is the highest at the "Centrum voor Jeugd en Gezin". The first steps for this idea are already taken; we are sharing thoughts with the municipality of Rotterdam and the "Centrum voor Jeugd en Gezin" to implement oral health education to parents through coaching during their regular visits at the center by discussing our findings and sharing our concerns.

Early origins of dental caries

Chapter 3.4 describes the relation between a complicated pregnancy course and dental caries later in childhood. Our hypothesis was that children, born from a complicated pregnancy, have high prenatal stress exposure and therefore a weaker mineralization potential resulting in less caries resistant enamel. We assessed four different measures for a complicated pregnancy course and a combination of those four: SGA, sPTB, GH, PE, and placental syndrome respectively. We did not find a significant relationship between a complicated pregnancy course and caries in six-year-old children.

In Chapter 2.2 the early origin topic was already mentioned on prenatal vitamin D exposure and dental enamel hypomineralization prevalence in childhood. My colleague dr. Elfrink already has explored other early origin factors, like those derived from adverse pregnancy outcomes, in relation to dental enamel hypomineralization and dental caries. [33]. She found that a low birth weight, the ethnic background, alcohol consumption of the mother during pregnancy, and any history of fever in the first year of the child's life may play a role in the development of HSPM's [33]. Within this thesis, however, no

association was found between adverse pregnancy outcomes (SGA, sPTB, GH, PE) and dental caries in six-year-old children. Previous literature on the association between adverse pregnancy outcomes and dental caries risk later in childhood was inconclusive. Some groups found no association between sPTB, SGA and/or PE with dental caries in childhood, while others did find a significant association between sPTB and SGA with dental caries [34-38]. Highly different populations, residual confounding, or true absence of any relationship could be explanatory factors for the contradictory findings.

One research group assessed a longitudinal relationship between caries increment and adverse pregnancy outcomes [39]. They found that children with a low birth weight < 2,500 grams, had a higher caries increment in a lifespan of four years compared to children with a normal birth weight [39]. It is reasonable that the influence of adverse pregnancy outcomes on eventual enamel strength is better captured by caries increment than total caries experience as used in Chapter 3 and by other researchers. Therefore, it would be interesting to explore the potential association with rising prevalence of caries in future research. But for now, I believe the evidence is still too weak to draw definitive conclusions and/or formulate any clinical recommendations about caries risk in children born from complicated pregnancies. Again, the findings of this thesis underscores that the focus of a clinician should be on the patient's own behavior and responsibility for maintaining a low caries risk (sustainable oral health), disrespect of their early life-events or their genetic background.

Digital assessment of dental enamel hypomineralization and caries

In all presented studies, enamel hypomineralization and dental caries were assessed from intra-orally made photographs with conventional white light. In the last chapter a research project is presented, with which I aimed validating Quantitative Light Fluorescence (QLF)-photographs in the setting of epidemiologic research. QLF photography is a different method of oral photography using not only conventional with light, but fluorescent blue light as well which may improve identification of dental caries and tooth-colored restoration material. We assessed the reliability of diagnosing both conditions in children and adolescents by comparing its performance with visual examination by a dental professional. Based on the results of that study, we found a QLF-camera to be a reliable tool to evaluate caries experience and MIH in a population aged between nine and eighteen years. Moreover, the reliability of scoring dental caries increased by having extra fluorescent photographs next to the white light photographs. There was no difference in added reliability between scoring decay or fillings. For MIH the added value of fluorescent photographs was absent.

The extra fluorescent photographs from the QLF-camera enhanced the reliability for assessing dental caries compared to using white light photographs for assessment only. Likely, the red fluorescence on decayed surfaces and the more intense blue fluorescence tooth-colored composite resin restorations are helpful for the examiner to identify a caries lesion or restoration correctly. From a clinical perspective these are interesting results. It enables research groups to implement a QLF-based method as an assessment tool for dental caries and MIH in their study. This tackles the logistical and financial problem of appointing a dental professional for clinical examination in any study, provides the option of easy data storage, and the examiner is not limited by time or any location to assess the clinical photographs. Moreover, data can be easily assessed in a standardized way which is preferable in research settings. Using a QLF-method for a study that is only interested in dental enamel hypomineralization as the primary outcome, the added value of fluorescent pictures may be debatable and other (cheaper) options may perform equally. Especially in severe cases the effects of MIH are obvious and less subtle than in caries lesions or tooth-colored restorations. This may explain the absence of any added value of QLF in diagnosing MIH. The setting in which our study was performed, including the capturing of the intra-oral photographs, was probably different from most (epidemiological) studies. This limitation should be taken into account when a research group decides to use a QLF-method. Still, when dental caries only or both entities are outcomes of interest within a future study, a QLF-camera is a recommendable and reliable option when tele-assessment is desired.

Methodological limitations, considerations, and strengths

Before describing the implications of my research for professionals and patients, the methodological limitations and considerations of the studies contained in my thesis should be discussed. Furthermore, I will emphasize several strengths of our studies as well.

Phenotype assessment

One limitation arises from the fact that all diagnoses of dental enamel hypomineralization and dental caries were based on the assessment from intra-oral photographs. Elfrink et al. showed that assessments from intra-oral photographs to be a valid method for diagnosing dental enamel hypomineralization and dental caries [40]. The sensitivity for scoring enamel hypomineralization reached 72.3% and a specificity of even 92.8%. This low-sensitivity represents a relatively high number of false-negatives in

presence of high specificity resulting in a relatively low number of false-positives for identifying enamel hypomineralization. Therefore, slight underestimation of the enamel hypomineralization prevalence might have occurred and the use of intra-oral photographs for enamel hypomineralization diagnoses may have led to loss of power in our studies. Moreover, the sensitivity and specificity were quite similar for scoring dental caries on intra-oral photographs, being 85.5% and 83.6% respectively [40]. Therefore, the number of false-negatives and false-positives is probably equally distributed within our research in Generation R. If this misclassification is equally distributed and independent of any outcome measure (non-differential misclassification) like ethnicity or SEP, the influence of using intra-oral photographs on our effect estimates in analyses for dental caries is rather minimal. Furthermore, because of measuring caries at only one time point, we only had information about caries prevalence/experience and not about caries activity. Caries activity is defined as an increase in the number or size of the discolorations or decalcifications of the dental enamel, or newly formed or progressed (root)caries compared to the previous periodic dental examination [30]. Subsequently, caries activity may be a better indicator for a child's current caries risk than caries prevalence/experience which is used in this thesis.

Selection bias and missing data

Another limitation that applies to most of the studies embedded in Generation R, is the possibility of selection bias, where the identified effect estimates of exposure on outcome do not represent the true magnitude of those who were eligible for the study [41]. The Generation R study is especially prone to two types of selection bias i.e., bias due to differential loss to follow up and due to non-response/missing data. Loss to follow-up within the Generation R Study was 10%, while 80% of the participating children visited the research center at the age of six years (n = 6,690) [42, 43], resulting in children from mothers with a higher educational background, with older age, and Dutch ethnicity who have been recruited more frequently than what was anticipated from the study area. This degree of selection bias might have resulted in loss of power in the studies about the relationship between ethnicity, SEP and caries; and should be considered in the interpretation of the findings. Furthermore, we had to deal with nonresponse and missing data bias, where not all children who visited the research center had available oral photographs for the assessment of enamel hypomineralization and dental caries. Several factors were responsible for this loss of information; i.e. refusal of intra-oral photography by the participant/caretakers, the research employee ran out of time at the research center, or the photograph quality was too low to score. However, the chance of nonresponse/missing data bias to be differential is low, considering that

sensitivity analyses revealed no significant difference in baseline characteristics between the participants with or without missing data on the outcome. Furthermore, we made use of multiple imputation methods to account for missing data on the covariates. This is a widely applied and accepted method to lower the risk of influencing the effect estimates by selection bias [44]. Therefore, we deem the effects of selection bias and missing data on our results to be low.

Confounding

Residual confounding is another limitation needing to be considered in my thesis. Confounding means that an apparent association is actually explained by a determinant that is related to both the exposure and the outcome but is not in the causal pathway. For example, if boys tend to consume more sugar than girls and boys seem to have more dental caries in general, sex is a confounder of the association between sugar consumption and dental caries. Absence of control for this confounder results in biased or so called "spurious" associations. In this thesis, our models were adjusted for many different potential confounders. In the literature, the term residual confounding is often used for two forms of biases combined; residual confounding and unmeasured confounding. Residual confounding occurs when there is a measurement error in the confounder included in the model and unmeasured confounding occurs if a confounder is not included in the model due to omission of no measurement of the confounder at all [45]. In all studies within our thesis, both forms of biases may be present. The Generation R study has collected by design a comprehensive set of health determinants that allows extensive correction for confounders [42]. Still, we were not able to measure all possible confounding factors. For example, we missed data on sugar consumption in children and the possibility remains that these unmeasured confounders exist. Moreover, the occurrence of erroneous measurement of confounders cannot be completely ruled out within a big cohort study with thousands of participants. Though assumed to be limited, there is always a possibility in participants making mistakes in questionnaires, the occurrence of mistakes in data processing, or participants giving socially acceptable answers concerning health behavior (response bias, i.e. smoking habits or tooth brushing frequency).

Study design

The cross-sectional setting in which most studies are performed within this thesis, poses another limitation. Within an observational setting it is not possible to randomize participants on exposure and confounding plays an important role, particularly with a

cross-sectional study where all measurements of a participant are taken at one point in time [46]. Therefore, we assessed differences in exposure variables between children with and without a dental trait and adjusted the analyses for the exposure variables that were significantly different between the two groups. This may have overcome the disadvantage of lack of randomization for a part, but we still lacked information about the temporal relationship between exposure and the outcome. Therefore, we should be cautious about drawing any conclusions on causality from our cross-sectional studies [46, 47]. For the findings of this thesis it means that we were only able to identify "associations", and any inference about "causation" needs to be addressed in future research drawn within Generation R. Because dental enamel hypomineralization is already present after eruption of the affected tooth and does not develop over time, the cross-sectional measurement is of less relevance in light of our limitations.

Strengths

The large study population is one of the biggest strengths of the Generation R Study, where most of the studies of my thesis were embedded. This study, located in the city of Rotterdam, the Netherlands, is a large multi-ethnic prospective population-based pregnancy-cohort with a relatively low percentage of loss to follow-up [42]. Embedding the studies within the Generation R Study made it possible to study a relatively large sample of children affected by dental enamel hypomineralization and dental caries. Worldwide, our study is one of the largest in which dental enamel hypomineralization and dental caries is studied [48].

Furthermore, we were the first to have studied dental health problems across seven major ethnic minority groups in the Netherlands [49]. Other Dutch studies were limited to distinguish only two or three ethnic groups [48, 50] and frequently also drawing conclusions across studies. Instead, the Generation R Study allows for relative matching across socioeconomic, health access, schooling and regional exposures (like physical activity, sunlight, etc.) among other determinants. This led to a more specific understanding in which ethnic minority groups need more intensive guidance in learning good oral health behavior. Like advised, we based the child's ethnicity on the birth country of both parents; if one parent was born abroad, the child was classified within a non-native Dutch group and if both parents were born abroad the mother's origin was used as the child's ethnicity [51]. In fact, the cultural background and behavior is better accounted by using the mother's origin as the determinative factor. Further division in first, second, or third generation immigrants is commonly used as well. However, we did not take this ethnic classification into account as this may have resulted in a mismatch between the assigned ethnicity group and the self-perceived

ethnical background and culture of the children. The likelihood, however, of a first- or second-generation immigrant to be raised completely by a native/Dutch culture is considered to be low.

Concerning the SEP of our participants we were able to include many different indicators for SEP, as compared to those typically reported in the literature. Where other studies assess the SEP with only one or two indicators, all studies in my thesis used at least seven different ones. This way, we were able to better determine the SEP as advised by Galobardes et al., and also determine the most important SEP indicators influencing childhood caries [52, 53]. The construct of SEP is a highly complex term that can only be assessed by using variables related to SEP, and a clear measurable definition for SEP is lacking [52, 53]. We believe that our studies approximated the children's true SEP very close by including the most important ones in relationship to dental enamel hypomineralization and dental caries.

Future research

In general, I encourage to replicate some of the findings within this thesis, typifying associations with rarely studied outcomes. For example, we were the first to study the association between bone health and dental enamel hypomineralization and one out of few groups, studying the association between vitamin D and dental enamel hypomineralization. More evidence, especially in the rarely studied associations may lead to new and clearer insights. Eventually and hopefully, this might be translated to better patient oral health and improved care. Moreover, a larger GWAS on dental enamel hypomineralization could be a promising opportunity to help find new biological pathways in explaining the etiology of this enamel disease. For sufficient power, collaboration is needed between well-designed (prospective) cohort studies like was done for the GWAS on dental caries included in this thesis.

A major finding in my thesis was the great disparity in dental caries prevalence between children from different ethnic groups and different social classes. The explanation behind these disparities, however, remains poorly understood. Within this thesis we provided some hypotheses and colleagues already found a possible mediator of these inequalities in the parent's attitude towards oral health being dependent on extrinsic or intrinsic factors, but more research is needed to really get a grip on the underlying problem [54]. Other study designs like qualitative research may be suitable for answering these open research questions, but within Generation R there are still opportunities to draw additional studies seeking better understanding of this problem. Children are still being followed-up and currently they are visiting the

research center for data collection on multiple dimensions of oral health and assessing many other parameters, recently completed at a mean age of thirteen and which will be collected again around the age of seventeen years. To better understand the ethnic and socioeconomic disparities in caries prevalence, it would be interesting to test the children's and their parents' knowledge on the caries prevention recommendations of "het Ivoren Kruis" with open questions followed by the question on how many days per week they feel have succeeded in following the recommendations. This will give insight into whether this is a problem of knowledge/education or whether it is a motivational problem, once a month disregarding biological susceptibility (ethnic or genetic). Afterwards, stratification or correction for ethnicity or SEP may help us to further grasp the cause of disparities in caries prevalence.

The data collection at the age of thirteen, provides us with new data on oral health. Because the children have probably passed the second transitional period at this, we will be able to study caries confined to the permanent dentition. Combining this information together with the caries experience at the age of six, we can measure caries activity instead of caries experience, which is an important measure in terms of risk factor identification. Following the Dutch guideline of "het Ivoren Kruis", the choice of preventive measures is based on caries activity rather than on previous caries experience [30]. Thus, caries activity may better capture current oral health behavior than caries prevalence/experience. However, because children at the age of six are not at risk for developing caries for a long time yet and probably had similar oral health behavior throughout their life, it is plausible that caries prevalence/experience may be a good proxy for caries activity in young children. Highly different findings from using caries activity within this thesis, instead of caries experience/prevalence, are therefore not expected. Moreover, all questions asked within this thesis are as relevant for the permanent dentition as they are for the primary dentition, especially since permanent teeth have to endure a lifetime. Permanent teeth already start developing in utero and need a longer period of time to develop fully. Hence, environmental factors can even have longer influence on the development of permanent teeth than on primary teeth. Moreover, all children will have their permanent first molar at the age of thirteen (if there are no cases of agenesis or early extractions). This will result in a greater population of children in which the presence of MIH can be assessed and thus more power to identify new potential risk factors for MIH of which the exact etiology remains unknown [55]. Based on the findings of this thesis, continuation of dental enamel hypomineralization and dental caries assessment with QLF-based photography is highly encouraged due to its positive characteristics of high reliability and ease of use.

Besides embedding new research within the Generation R Study, we encourage researchers to look for methods to decrease the existing sociodemographic disparities in caries prevalence. For example, the NOCTP studied by Vermaire et al. may be a promising method, but still has to be validated in a population with a more mixed socioeconomic background [31]. Within this program the focus lies on the patient's responsibility for self-care and the duration between periodic visits is based on the patient's level of self-care, the eruption period of the permanent teeth and the caries progression within the dentition, especially in the permanent first molars [31]. Furthermore, our before mentioned initiative at the "Centrum voor Jeugd en Gezin" may be a good opportunity to reach children and their parents at a young age. Well-designed interventional studies, preferably randomized controlled trials (RCT's), have to show which methods are most effective in our ultimate goal to decrease the caries prevalence in minority groups. Like is advocated for in a recent Cochrane Review as well [56]. For example, children with a mixed socioeconomic background could be randomized between a group receiving preventive care according to the NOCTP or a group receiving care according to the traditional preventive dental care at the dentist. Comparing the caries progression over time between these groups of children, would show the efficacy of NOCTP in a mixed population. Moreover, caries progression could be compared between children randomized over a group receiving extra oral health education at the "Centrum voor Jeugd en Gezin" at a young age or receiving only the regular care and education (not involving oral health education) at the "Centrum voor Jeugd en Gezin".

General conclusion

From this thesis we can conclude that children who have a low BMC more often have HSPMs, but this association is not explained by differences in vitamin D serum concentrations. Vitamin D serum concentrations measured at three time points (prenatal, umbilical cord blood, and at the age of six) were neither associated with the presence of HSPMs nor with MIH at the age of six. I encourage other researchers to replicate our findings, since we were one of the first to study those associations and it may increase the power of our findings. Moreover, research should still focus on finding the biological pathway of the development of enamel hypomineralization which remains unknown. This may help the development of preventive measures for enamel hypomineralization. For example, a GWAS might be a useful instrument to discover new biological pathways.

Furthermore, we found great disparities in caries prevalence among different ethnic and socioeconomic groups. Particularly, Surinamese-Hindustani, Turkish,

Moroccan children, and children from parents with a low SEP were significantly more affected by dental caries than Dutch children and children from parents with a higher SEP. Although we did propose several hypotheses, the underlying mechanisms behind these disparities are unknown. Still, higher awareness among these groups for poor oral health and impact on the long term is the first step of prevention. Therefore, this thesis is not only a stimulus for more research, but also for development of strategies to successfully lower the existing oral health inequalities.

I have showed in this thesis that genetic variants and prenatal factors only have modest or no influence on caries susceptibility in children. Nevertheless, limitations of our study (i.e. power limitations) should be kept in mind, but from our results I believe behavioral factors are probably most important in caries development.

Finally, the QLF-camera seemed to be a reliable method in caries and dental enamel hypomineralization assessment. Moreover, the addition of fluorescent photographs did seem to have added value in caries assessment in terms of reliability. Future epidemiological studies may benefit from this new technology for digital assessment of these enamel conditions.

Unfortunately, from this thesis no preventive measures for dental enamel hypomineralization could yet be proposed. However, a persistent challenge for caries prevention, however, might be found in reaching and engaging the children from the identified risk groups. An opportunity to educate them and their parents early in life could be pursued at the "Centrum voor Jeugd en Gezin". Another opportunity could arise from implementing the NOCTP at the general dental practice. Future research has to tell which method is most effective or whether they could be synergetic. Eradicating caries completely, remains a utopia; developing one cavity can be as inevitable as getting the flu. However, decreasing disparities in caries prevalence and severity between population groups should be a priority in a well-developed country as the Netherlands, during the upcoming decade. Hopefully, initiatives derived from the findings of this thesis can help reaching this goal.

In conclusion, there are still large areas pending to be investigated, but "Advances in Epidemiological Research of Dental Enamel Hypomineralization and Caries" have definitely been achieved.

References

[1] T. Winzenberg, S. Powell, K. Shaw, G. Jones, Vitamin D supplementation for improving bone mineral density in children, Cochrane Database Syst Rev, 2010.

[2] K. Josephsen, Y. Takano, S. Frische, J. Praetorius, S. Nielsen, T. Aoba, O. Fejerskov, Ion transporters in secretory and cyclically modulating ameloblasts: a new hypothesis for cellular control of preeruptive enamel maturation, Am J Physiol Cell Physiol 299(6) (2010) 1299-1307.

[3] F. Lézot, V. Descroix, D. Hotton, N. Mauro, S. Kato, A. Berdal, Vitamin D and tissue non-specific alkaline phosphatase in dental cells, Eur J Oral Sci 114 Suppl 1 (2006) 178-182.

[4] P. Lips, Vitamin D physiology, Prog Biophys Mol Biol 92(1) (2006) 4-8.

[5] D. Vandamme, B. Landuyt, W. Luyten, L. Schoofs, A comprehensive summary of LL-37, the factotum human cathelicidin peptide, Cell Immunol 280(1) (2012) 22-35.

[6] M.E. Elfrink , A.A. Schuller, J.S. Veerkamp, J.H. Poorterman, H.A. Moll, B.J. ten Cate, Factors increasing the caries risk of second primary molars in 5-year-old Dutch Children, Int. J. Paediatr. Dent. 20(2) (2010) 151-157.

[7] K. Weerheijm, B. Jälevik, S. Alaluusua, Molar-incisor hypomineralisation, Caries Res 35(5) (2001) 390-391.

[8] L. Fabiani, G. Mosca, D. Giannini, A. Giuliani, G. Farello, M. Marci, E. Ballatori, Dental caries and bone mineral density: a cross sectional study, Eur J Paediatr Dent 7(2) (2006) 67-72.

[9] W.R. Profitt, H.W. Fields, D.M. Sarver, Contemporary Orthodontics, Elsevier - Health Sciences Division2012, p. 67.

[10] W.R. Profitt, H.W. Fields, D.M. Sarver, Contemporary Orthodontics, Elsevier - Health Sciences Division2012, p. 83.

[11] A. Nanci, Ten Cate's Oral Histology, Elsevier - Health Sciences Division 2012, pp. 122-148.

[12] K. Zhu, R. Prince, Calcium and bone, Clin Biochem 45(12) (2012) 936-942.

[13] H.F. DeLuca, Overview of general physiologic features and functions of vitamin D, Am J Clin Nutr 80(Suppl 6) (2004) 1689S-1896S.

[14] J. Kühnisch, E. Thiering, J. Kratzsch, R. Heinrich-Weltzien, R. Hickel, J. Heinrich, Elevated Serum 25(OH)-Vitamin D Levels Are Negatively Correlated with Molar-Incisor Hypomineralization, J Dent Res 94(2) (2015) 381-387.

[15] P. Hujoel, Vitamin D and dental caries in controlled clinical trials: systematic review and meta-analysis, Nutr Rev 71(2) (2013) 88-97.

[16] R. Schroth, C. Lavelle, R. Tate, S. Bruce, R. Billings, M. Moffatt, Prenatal vitamin D and dental caries in infants, Pediatrics 133(5) (2014) 1277-1284.

[17] R. Schroth, R. Rabbani, G. Loewen, M. Moffatt, Vitamin D and Dental Caries in Children, J Dent Res 95(2) (2016) 173-179.

[18] R.J. Schroth, J.A. Levi, E.A. Sellers, J. Friel, E. Kliewer, M.E. Moffatt, Vitamin D status of children with severe early childhood caries: a case-control study, BMC Pediatr 13(174) (2013).

[19] B. Wharton, N. Bishop, Rickets, The Lancet 362(9393) (2003) 1389-1400.

[20] The Hague: Health Council of the Netherlands, Evaluation of the dietary reference values for vitamin D, publication no. 2012/15 (2012).

[21] M.J. Silva, N.M. Kilpatrick, J.M. Craig, D.J. Manton, P. Leong, D. Burgner, K.J. Scurrah, Etiology of Hypomineralized Second Primary Molars: A Prospective Twin Study, Journal of Dental Research (2018) 0022034518792870.

[22] M.J. Silva, K.J. Scurrah, J.M. Craig, D.J. Manton, N. Kilpatrick, Etiology of molar incisor hypomineralization - A systematic review, Community Dent. Oral Epidemiol. 44(4) (2016) 342-353.

[23] M.E. Elfrink, H.A. Moll, J.C. Kiefte-de Jong, V.W. Jaddoe, A. Hofman, J.M. ten Cate, J.S. Veerkamp, Pre- and postnatal determinants of deciduous molar hypomineralisation in 6-year-old children. The generation R study, PLos One, 2014, p. e91057.

[24] M.A. Peres, A. Sheiham, P. Liu, F.F. Demarco, A.E. Silva, M.C. Assunção, A.M. Menezes, F.C. Barros, K.G. Peres, Sugar Consumption and Changes in Dental Caries from Childhood to Adolescence, J. Dent. Res. 95(4) (2016) 388-394.
[25] I. Johansson, P. Holgerson, N. Kressin, M. Nunn, A. Tanner, Snacking habits and caries in young children, Caries Res 44 (2010) 421-430.
[26] J. Morrison, C.C. Laurie, M.L. Marazita, A.E. Sanders, S. Offenbacher, C.R. Salazar, M.P. Conomos, T. Thornton, D. Jain, C.A. Laurie, K.F. Kerr, G. Papanicolaou, K. Taylor, L.M. Kaste, J.D. Beck, J.R. Shaffer, Genome-wide association study of dental caries in the Hispanic Communities Health Study/Study of Latinos (HCHS/SOL), Human Molecular Genetics 25(4) (2016) 807-816.
[27] D. Shungin, S. Haworth, K. Divaris, C.S. Agler, Y. Kamatani, M. Keun Lee, K. Grinde, G. Hindy, V. Alaraudanjoki, P. Pesonen, A. Teumer, B. Holtfreter, S. Sakaue, J. Hirata, Y.-H. Yu, P.M. Ridker, F. Giulianini, D.I. Chasman, P.K.E. Magnusson, T. Sudo, Y. Okada, U. Völker, T. Kocher, V. Anttonen, M.-L. Laitala, M. Orho-Melander, T. Sofer, J.R. Shaffer, A. Vieira, M.L. Marazita, M. Kubo, Y. Furuichi, K.E. North, S. Offenbacher, E. Ingelsson, P.W. Franks, N.J. Timpson, I. Johansson, Genome-wide analysis of dental caries and periodontitis combining clinical and self-reported data, Nature Communications 10(1) (2019) 2773.
[28] S. van den Branden, S. van den Broucke, R. Leroy, D. Declerck, K. Hoppenbrouwers, Oral health and oral health-related behaviour in preschool children: evidence for a social gradient, Eur. J. Pediatr. 172(2) (2013) 231-237.
[29] F. Schwendicke, C. Dörfer, P. Schlattmann, L. Foster Page, W. Thomson, S. Paris, Socioeconomic inequality and caries: a systematic review and meta-analysis., J Dent Res 94(1) (2015) 10-18.
[30] B.M. van Amerongen, C.M.M. Berendsen-Wolters, N.G. Blanksma, S.J. Fokkema, M.C.D.N.J.M. Huysmans, C. van Loveren, A.A. Schuller, G. Stel, Advies Cariëspreventie, 2011. https://www.ivorenkruis.nl/userfiles/File/IvK_Advies_Cari_spreventie.pdf. (Accessed 29.04.2019.
[31] J.H. Vermaire, J.H.G. Poorterman, L. van Herwijnen, C. van Loveren, A Three-Year Randomized Controlled Trial in 6-Year-Old Children on Caries-Preventive Strategies in a General Dental Practice in the Netherlands, Caries Research 48(6) (2014) 524-533.
[32] K.R. Ekstrand, M.E.C. Christiansen, Outcomes of a Non-Operative Caries Treatment Programme for Children and Adolescents, Caries Research 39(6) (2005) 455-467.
[33] M.E.C. Elfrink, Deciduous molar hypomineralisation, its nature and nurture, Faculty of Dentistry (ACTA), 2012.
[34] J.D. Shulman, Is There an Association between Low Birth Weight and Caries in the Primary Dentition?, Caries Res. 39(3) (2005) 161-167.
[35] K. Tanaka, Y. Miyake, Low birth weight, preterm birth or small-for-gestational-age are not associated with dental caries in young Japanese children, BMC Oral Health 14 (2014) 38-38.
[36] T. Sayyed, M. Kandil, O. Bashir, H. Alnaser, The relationship between term pre-eclampsia and the risk of early childhood caries, The Journal of Maternal-Foetal & Neonatal Medicine 27(1) (2014) 62-65.
[37] M.C. Saraiva, H. Bettiol, M.A. Barbieri, A.A. Silva, Are intrauterine growth restriction and preterm birth associated with dental caries?, Community Dent. Oral. Epidemiol. 35(5) (2007) 364-376.
[38] A. Nirunsittirat, W. Pitiphat, C. McKinney, T.A. DeRouen, N. Chansamak, O. Angwaravong, P. Patcharanuchat, T. Pimpak, Adverse Birth Outcomes and Childhood Caries: A Cohort study, Community Dent. Oral Epidemiol. 44(3) (2016) 239-247.
[39] E. Bernabé, H. MacRitchie, C. Longbottom, N.B. Pitts, W. Sabbah, Birth Weight, Breastfeeding, Maternal Smoking and Caries Trajectories, J. Dent. Res. 96(2) (2016) 171-178.
[40] M.E. Elfrink, J.S. Veerkamp, I.H. Aartman, H.A. Moll, J.M. Ten Cate, Validity of scoring caries and primary molar hypomineralization (DMH) on intraoral photographs, JM Eur. Arch. Paediatr. Dent. 10(Suppl. 1) (2009) 5-10.

[41] M.A. Hernán, S. Hernández-Díaz, J.M. Robins, A Structural Approach to Selection Bias, Epidemiology 15(5) (2004) 615-625.
[42] M.N. Kooijman, C.J. Kruithof, C.M. van Duijn, O.H. Franco, M.H. van IJzendoorn, J.C. de Jongste, C.C. Klaver, A. van der Lugt, J.P. Mackenbach, H.A. Moll, R.P. Peeters, H. Raat, E.H. Rings, F. Rivadeneira, M.P. van der Schroeff, E.A. Steegers, H. Tiemeier, A.G. Uitterlinden, F.C. Verhulst, E. Wolvius, J.F. Felix, V.W. Jaddoe, E.J.E. 1243., The Generation R Study: design and cohort update 2017, Eur. J. Epidemiol. 31(12) (2016) 1243-1264.
[43] V. Jaddoe, C. van Duijn, O. Franco, A. van der Heijden, M. van Iizendoorn, J. de Jongste, A. van der Lugt, J. Mackenbach, H. Moll, H. Raat, e. al., The Generation R Study: design and cohort update 2012, Eur J Epidemiol 27(9) (2012) 739–756.
[44] J.A.C. Sterne, I.R. White, J.B. Carlin, M. Spratt, P. Royston, M.G. Kenward, A.M. Wood, J.R. Carpenter, Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls, BMJ 338 (2009) b2393.
[45] Z. Fewell, G. Davey Smith, J.A.C. Sterne, The Impact of Residual and Unmeasured Confounding in Epidemiologic Studies: A Simulation Study, American Journal of Epidemiology 166(6) (2007) 646-655.
[46] P. Sedgwick, Cross sectional studies: advantages and disadvantages, BMJ : British Medical Journal 348 (2014) g2276.
[47] M.S. Thiese, Observational and interventional study design types; an overview, Biochemia medica 24(2) (2014) 199-210.
[48] A. Schuller, E. Vermaire, I. van Kempen, P. van Dommelen, H. Hofstetter, G. Verrips, Kies voor tanden: Tussenmeting 2014, een vervolg op de reeks TJZ- en KvT-onderzoeken, http://www.mondhygienisten.nl/wp-content/uploads/2016/07/SignalementMondzorg2016.pdf (2016).
[49] CBS, Bevolking per maand; leeftijd, geslacht, migratieachtergrond, generatie, 2018. https://statline.cbs.nl/StatWeb/publication/?PA=71090ned. (Accessed 29.04.2019.
[50] G.H. Verrips, H. Kalsbeek, M.A. Eijkman, Ethnicity and maternal education as risk indicators for dental caries, and the role of dental behavior, Community Dent. Oral. Epidemiol. 21(4) (1993) 209-214.
[51] J. Ooijevaar, C. Bloemendal, A. Boerdam, R. Schaart, Jaarrapport Integratie 2016, 2016. https://www.cbs.nl/-/media/_pdf/2016/47/2016b5_jaarrapport_integratie_2016_web.pdf. (Accessed 29.04.2019.
[52] B. Galobardes, M. Shaw, D.A. Lawlor, J.W. Lynch, G. Davey Smith, Indicators of socioeconomic position (part 1), J. Epidemiol. Community Health 60(1) (2006) 7-12.
[53] B. Galobardes, M. Shaw, D.A. Lawlor, J.W. Lynch, G. Davey Smith, Indicators of socioeconomic position (part 2), Journal of Epidemiology and Community Health 60(2) (2006) 95.
[54] D. Duijster, M. de Jong-Lenters, C. de Ruiter, J. Thijssen, C. van Loveren, E. Verrips, Parental and family-related influences on dental caries in children of Dutch, Moroccan and Turkish origin, Community Dent Oral Epidemiol 43(2) (2015) 152-162.
[55] S. Alaluusua, Aetiology of Molar-Incisor Hypomineralisation: A Systematic Review, Eur Arch Paediat Dent 11(4) (2010) 53-58.
[56] E. Riggs, N. Kilpatrick, L. Slack-Smith, B. Chadwick, J. Yelland, M.S. Muthu, J.C. Gomersall, Interventions with pregnant women, new mothers and other primary caregivers for preventing early childhood caries, Cochrane Database of Systematic Reviews (11) (2019).

6

Summary
Samenvatting

Summary

Chapter 1 introduces the three dental enamel diseases on which we focused within this thesis; Hypomineralized Second Primary Molars (HSPM), Molar Incisor Hypomineralization (MIH), and dental caries. Children affected by HSPM and/or MIH have brittle dental enamel due to less mineralization, which may result in a higher caries susceptibility or even early tooth loss. Somewhere in the complex process of tooth development, the cells involved with enamel mineralization show problems to facilitate mineralization of the extracellular matrix. Factors causing these problems, however, are largely unknown. It is speculated that early childhood diseases may cause these mineralization issues, but the exact etiology of HSPM and MIH is still unknown. On the contrary, the etiology of dental caries has been well established. Dental caries is a multifactorial disease. Major risk factors are a low frequency of fluoride use and a high frequency of sugar intake. Preventive strategies focus most on those two risk factors and are quite successful. Yet, it is expected that the caries prevalence in children can reach even lower numbers. Identification of risk groups and/or new risk factors may help to develop more effective caries prevention strategies. Aims of this thesis were to further explore the etiology of dental enamel hypomineralization, to identify major risk groups and risk factors for dental caries, and to assess the use of relatively new instrument, a Quantitatve Light induced Fluorescent (QLF)-camera, for diagnosing dental enamel hypomineralization and dental caries within an epidemiological study setting. All studies, except the last study, were embedded within a population-based cohort study following children from fetal life until adulthood, the Generation R Study. Dental enamel hypomineralization and dental caries were assessed at the children's age of six from intra-oral photographs.

In **Chapter 2** we tried to further unravel the etiology of dental enamel hypomineralization. First, we assessed the association between the children's bone mass and dental enamel hypomineralization. Since the same minerals play a crucial role in both bone development and enamel maturation, we hypothesized that children's bone mass could be an indicative factor for having dental enamel hypomineralization. Bone mass was measured using a Dual-energy X-ray Absorptiometry scan at the age of six (DXA-scan). Eventually, we found a low child's bone mineral content (BMC) to be associated with a higher presence of HSPM. No significant association was found between BMC and MIH.

Furthermore, in the search for etiologic factors of dental enamel hypomineralization, we assessed the association between vitamin D status and HSPM/MIH. Vitamin D is an important mediator in bone metabolism, vitamin D receptors are found on ameloblasts, and may therefore (partly) explain the found association between a child's BMC and the presence of HSPM. We measured vitamin D serum concentrations at three points in time; mid-gestational from the mother's blood sample, early postnatal from umbilical

cord blood, and at the age of six from the child's blood sample. After analyzing the results, however, the vitamin D serum concentrations were neither associated with the presence of HSPM at the age of six, nor with MIH. Based on our findings, we were not able to identify a new risk factor for dental enamel hypomineralization nor to explain the found association between a child's bone mass and the presence of HSPM in children.

In **Chapter 3** we focused on dental caries. First, we explored the presence of ethnic disparities in dental caries among the children from the Generation R Study, independent of SEP. For this study we distinguished seven different ethnic groups, including the most important ethnic minority groups within the Netherlands: Dutch, Surinamese-Hindustani, Surinamese-Creole, Turkish, Dutch Antillean, Moroccan, and Cape Verdean. Compared to native Dutch children, children with a Surinamese-Hindustani, Surinamese-Creoles, Turkish, Moroccan, and Cape Verdean background had significantly higher odds for dental caries. Especially the Surinamese-Hindustani, Turkish, and Moroccan group had significantly higher odds for severe dental caries (more than three teeth affected by decay, missing teeth and/or filings due to caries). Household income and educational level of the mother explained up to 43% of the association between ethnicity and dental caries. Public health strategies can apply this new knowledge and specifically focus on the reduction of ethnic disparities in oral health.

Second, we assessed whether dental caries traits in children and adolescents are partially heritable. For this purpose, we performed a genome-wide association study (GWAS) within an international consortium of nine different cohort studies with participants aged from 2.5 up to 18.0 years. Phenotype definitions were created for the presence or absence of treated or untreated caries, stratified by primary and permanent dentition. Analysis included up to 19,003 individuals (7,530 affected) for primary teeth and 13,353 individuals (5,875 affected) for permanent teeth. Eventually, we found two different single variants to be associated with dental caries in the primary and permanent dentition (rs1594318-C and rs7738851-A, respectively). However, consortium-wide estimated heritability of caries was low compared to corresponding within-study estimates or previously published estimates. Phenotypic heterogeneity between cohorts and limited statistical power might have contributed to the low number of found single variants and the relatively low heritability. These findings could also reflect complexity not captured by our study design, such as genetic effects which are conditional on environmental exposure.

Third, we explored social inequalities in dental caries prevalence among six-year-old children from the Generation R Study. We did this by assessing the association between seven different indicators for socioeconomic position (SEP) and by using a

district-based approach to explore the distribution of dental caries among districts of low and high SEP within the city of Rotterdam. Statistical analyses showed that dental caries is more prevalent among six-year-old children with a low SEP, which is also visible at a district level. This should raise concerns about the existing social inequalities in dental caries and should encourage development of dental caries prevention strategies. New knowledge about the distribution of oral health inequalities between districts should be used to target the right audience for these strategies.

In the last part of **Chapter 3**, we studied the prevalence of dental caries in children born from a complicated pregnancy course compared to children born from an uncomplicated one. Since enamel formation already starts in utero, pregnancy course and outcome may play a role in the eventual enamel strength and caries susceptibility. Again, we included the six-year-old children from the Generation R study. Four different adverse pregnancy outcomes were studied individually; being small for gestational age (SGA), spontaneous preterm birth (sPTB), gestational hypertension (GH), and pre-eclampsia (PE). Moreover, we studied all four outcomes combined as the placental syndrome. However, statistical analyses did not reveal any association between the adverse pregnancy outcomes and dental caries in six-year-old children. Although it was expected that prenatal stress can be a risk factor for caries development later in life, our study did not support this hypothesis, despite the well-powered setting of our study.

Because the efficient and cost saving aspects of digital assessment, we examined whether QLF-photographs may help in assessment of dental caries and MIH within a study setting in **Chapter 4**. We compared scoring dental caries and enamel hypomineralization from QLF-photographs with visual examination by a dentist as the gold standard. The study was situated in a dental practice in the Netherlands, included participants from 9 up to and including 18 years old. Eventually, 113 children/adolescents participated in the study. We found dental photographs, made by the Qraycam™, to be reliable enough to score dental caries and MIH in children/adolescents aged 9 up to and including 18 years compared to visual examination. Moreover, QLF-photographs seemed to enhance the reliability of diagnosing dental caries compared to assessment from conventional white light photographs alone. Therefore, the use of a Qraycam™, might be a convenient tool for dental caries and enamel hypomineralization assessment within study settings.

Chapter 5 covers a general discussion about the main findings of all our studies within this thesis, which are put into perspective of the existing literature. Furthermore, the most important limitations of our studies are mentioned like potential biases, limitations regarding the data collection, but the great sample size, it's advantages, and other strengths are discussed as well. Moreover, we tried to translate our findings to a

clinical setting and therewith to deliberate on their clinical implications. Afterwards, we proposed different future research directions for unanswered and newly raised research questions by this thesis. We concluded the general discussion with a summary of our conclusions that:

- Children who have a low BMC more often have HSPMs, this association is probably not explained by differences in vitamin D serum concentrations,
- Surinamese-Hindustani, Turkish, Moroccan children, and children from parents with a low SEP were significantly more affected by dental caries than Dutch children and children from parents with a higher SEP,
- We found no common genetic variants that influenced dental caries with modest effects on susceptibility in the primary dentition, but a single variant did influence the presence of dental caries in the permanent dentition,
- Placental dysfunction outcomes did not influence the caries risk in six-year-old children,
- A QLF-camera is a reliable system to assess dental caries and enamel hypomineralization, and the addition of fluorescent photographs did seem to have added value in caries experience assessment in terms of reliability.

Samenvatting

Hoofdstuk 1 introduceert de drie tandglazuuraandoeningen waarop wij focussen binnen dit proefschrift; Hypomineralized Second Primary Molars (HSPM), Molar Incisor Hypomineralization (MIH) en cariës. Het tandglazuur van kinderen met HSPM en/of MIH is broos door een lagere mineraaldichtheid en kan zorgen voor een hogere cariësgevoeligheid of zelfs voor vroeg tandverlies. Ergens in het complexe proces van de tandontwikkeling, lijken de cellen die betrokken zijn bij de mineralisatie van het glazuur te falen in het mineraliseren van de extracellulaire matrix. Echter, duidelijke oorzaken hiervoor zijn tot op heden nog niet gevonden. In de literatuur zijn vroege kinderziekten geassocieerd met glazuur hypomineralisatie, maar verder onderzoek is nodig naar de exacte etiologie van HSPM en MIH. Tegenovergesteld, is de etiologie van cariës duidelijk bekend. Deze is zeer multifactorieel van aard, maar twee belangrijke risicofactoren zijn een lage fluoridefrequentie en veelvuldige suikerconsumptie per dag. Preventieprogramma's focussen dan ook vooral op deze twee beïnvloedbare risicofactoren en zijn daarmee redelijk succesvol. De cariësprevalentie onder kinderen is echter nog relatief hoog, zeker omdat lagere prevalentiecijfers te verwachten zijn bij preventie. Identificatie van risicogroepen en/of nieuwe risicofactoren kunnen mogelijk helpen bij het ontwikkelen van nog effectievere preventieprogramma's. De belangrijkste doelstellingen voor dit proefschrift waren om de etiologie van glazuurhypomineralisatie verder te ontrafelen, om belangrijke risicogroepen en mogelijk nieuwe risicofactoren voor cariës te identificeren en om de betrouwbaarheid te onderzoeken van een relatief nieuwe methode, Quantitatve Light induced Fluorescent (QLF)-foto's, om glazuurhypomineralisatie en cariës te diagnosticeren binnen een onderzoekssetting. Al onze studies, behalve die over de QLF-camera, zijn uitgevoerd binnen het Generation R onderzoek. Dit is een op de populatie gebaseerd cohortonderzoek, waarbinnen kinderen worden gevolgd vanaf het moment voor de geboorte tot aan de volwassen leeftijd. Glazuurhypomineralisatie en cariës hebben wij bij de kinderen geëvalueerd toen zij zes jaar waren op basis van intra-orale lichtfoto's.

Hoofdstuk 2 begint met de eerste doelstelling; het verder ontrafelen van de etiologie van glazuurhypomineralisatie. Ten eerste onderzochten we het verband tussen de botmassa van een kind en het hebben van glazuurhypomineralisatie. Omdat dezelfde mineralen een rol spelen in het mineraliseren van zowel bot als glazuur, was onze hypothese dat de botmassa van een kind indicatief kan zijn voor de aanwezigheid van glazuurhypomineralisatie. Botmassa werd bij alle kinderen gemeten door middel van een "Dual-energy X-ray Absorptiometry scan" op dezelfde leeftijd als dat we glazuurhypomineralisatie hadden beoordeeld, zes jaar. Uit de analyses bleek dat een laag botmineraal gehalte (BMC) significant geassocieerd was met de aanwezigheid van HSPM. Deze associatie vonden wij echter niet met MIH.

In de verdere zoektocht naar een etiologische verklaring voor het ontstaan van glazuurhypomineralisatie, onderzochten wij tevens de relatie tussen vitamine D-status en HSPM/MIH. Vitamine D is een belangrijke factor in botmetabolisme en vitamine D receptoren zijn te vinden op ameloblasten (glazuur aanmakende cellen). Mogelijk dat vitamine D dus een mediërende rol speelt in de relatie tussen botmassa en HSPM onder kinderen. Vitamine D serumconcentraties werden gemeten op drie momenten; op de helft van de zwangerschap in moeders' bloed, vroeg na de zwangerschap in bloed van de navelstreng en op zesjarige leeftijd in bloed van de kinderen zelf. Na de analyses, echter, vonden wij geen van deze Vitamine D serumconcentraties significant geassocieerd met de aanwezigheid van HSPM of MIH op zesjarige leeftijd. Uit onze bevindingen kunnen wij dus geen nieuwe risicofactor voor glazuurhypomineralisatie aandragen, noch kunnen wij de relatie verklaren tussen een verminderde BMC en het meer voorkomen van HSPM onder kinderen.

In **Hoofdstuk 3** zoomen we in op de derde glazuuraandoening van dit proefschrift, cariës. Ten eerste onderzochten we etnische verschillen in cariësprevalentie onder de kinderen uit de Generation R Study, onafhankelijk van de SEP. Alle kinderen werden ingedeeld in zeven verschillende etnische groepen, inclusief de grootste groepen etnische minderheden binnen Nederland: Nederlands, Surinaams-Hindoestaans, Surinaams-Creools, Turks, Antilliaans, Marokkaans en Kaapverdiaans. Vergeleken met Nederlandse kinderen, kinderen met een Surinaams-Hindoestaanse, Surinaams-Creoolse, Turkse, Marokkaanse en Kaapverdiaanse achtergrond hadden grotere kans op het hebben van cariës. Vooral de Surinaams-Hindoestaanse, Turkse en Marokkaanse groep hadden een significant hogere kans op ernstige cariës (meer dan drie tanden met actieve cariës, meer dan drie missende tanden en/of vullingen door cariës). Inkomen van de ouders en opleidingsniveau van moeder verklaarden slechts 43% van de gevonden associatie. Deze nieuwe kennis zou kunnen worden meegenomen in het opstellen van nieuwe volksgezondheidmaatregelen om de etnische ongelijkheden in mondgezondheid onder kinderen te verkleinen.

In het tweede deel hebben we getracht antwoord te geven op de vraag of cariës in zowel kinderen als adolescenten deels erfelijk is. Hiervoor hebben we een "genome-wide association study" (GWAS) uitgevoerd in een internationaal consortium van negen verschillende cohortstudies met deelnemers van tussen de 2,5 en 18 jaar oud. De definitie van het fenotype cariës werd gebaseerd op de aan- of afwezigheid van behandelde of onbehandelde cariës, gestratificeerd voor het melk- en het blijvende gebit. Uit de resultaten kwamen twee verschillende genetische varianten naar voren die werden gecorreleerd aan de aanwezigheid van cariës in het melk- en het blijvende gebit (rs1594318-C en rs7738851-A, respectievelijk). De maat voor erfelijkheid over

het gehele cohort was echter laag vergeleken met de waarden gevonden binnen de studies of uit andere gepubliceerde studies. Mogelijk dat fenotypische heterogeniteit tussen de cohortstudies en een matige statistische "power" hebben bijgedragen aan het beperkte aantal gevonden genetische varianten en de relatief lage waarde voor erfelijkheid. Onze resultaten tonen waarschijnlijk ook de mate van complexiteit die niet gevangen kon worden met de huidige studieopzet, zoals genetische effecten die afhankelijk zijn omgevingsfactoren.

Ten derde exploreerden wij sociale ongelijkheden in cariësprevalentie onder kinderen van de Generation R studie. Dit hebben wij gedaan door zeven verschillende indicatoren voor socio-economische positie (SEP) te includeren in de analyses en door op wijkniveau de cariësprevalentie te bekijken in zowel de Rotterdamse wijken met een lage SEP als die met een hoge SEP. Uiteindelijk vonden wij een hogere cariësprevalentie onder kinderen met een lage SEP ten opzichte van kinderen met een hoge SEP. Dit verschil was ook te zien op wijkniveau. Zulke ongelijkheden roepen zorgen op en moet de ontwikkeling van preventieve programma's aanmoedigen. De kennis over de distributie van cariës over de sociale groepen en wijken kan als hulpmiddel worden ingezet om te focussen op het publiek dat dit het meeste nodige heeft.

In het laatste deel van **Hoofdstuk 3**, hebben we onderzocht of kinderen geboren uit een gecompliceerde zwangerschap meer cariës hebben dan kinderen geboren uit een ongecompliceerde zwangerschap. De ontwikkeling van het glazuur begint namelijk al in de baarmoeder. Het zwangerschapsverloop en de uitkomst kunnen dus mogelijk een effect hebben om de glazuursterkte en daarmee op cariësgevoeligheid. Opnieuw includeerden we de zesjarigen uit het Generation R onderzoek. Vier verschillende zwangerschapsuitkomsten werden onderzocht; klein zijn voor de draagtijd (SGA), spontane vroeggeboorte (sPTB), zwangerschapshypertensie (GH) en pre-eclampsie (PE). Bovendien hebben we alle vier de uitkomsten gecombineerd in een variabele, het placentaire syndroom. De analyses toonden echter geen associatie tussen een enkele zwangerschapsuitkomst en cariës in zesjarigen. Ondanks onze studie met voldoende "power" en de gefundeerde hypothese dat prenatale stress een risicofactor kan zijn voor cariësontwikkeling later in het leven, ondersteunen onze bevindingen deze niet.

Vanwege het efficiënte en kostenbesparende aspect van digitale beoordelingen, onderzochten wij in **Hoofdstuk 4** of QLF-foto's een rol kunnen spelen bij het digitaal diagnosticeren van cariës en MIH in onderzoeksverband. Wij vergeleken het scoren van cariës en glazuurhypomineralisatie via QLF-foto's met visuele inspectie door een tandarts als de gouden standaard. Deze studie hebben wij uitgevoerd in een Nederlandse tandartspraktijk. Uiteindelijk includeerden we 113 deelnemers van 9 tot en met 18 jaar oud. Gebitsfoto's, gemaakt met de Qraycam™, waren betrouwbaar genoeg

om cariës en MIH te diagnosticeren ten opzichte van visuele inspectie. Bovendien leken de fluorescentiefoto's, voor het diagnosticeren van cariës, van toegevoegde waarde te zijn ten opzichte van witlicht foto's alleen. Daarom kan de Qraycam™ een geschikt hulpmiddel zijn voor digitale beoordelingen van cariës en glazuurhypomineralisatie in onderzoeksverband.

Hoofdstuk 5 is een algemene discussie over de belangrijkste bevindingen uit al onze studies van dit proefschrift en worden in de context van de bestaande literatuur gezet. Verder bespreken we de beperkingen van onze studies zoals mogelijke "biases", beperkingen omtrent de dataverzameling, maar ook de grote studie populatie, de voordelen hiervan, en andere sterke punten worden uiteengezet. Bovendien hebben we geprobeerd onze bevindingen te vertalen naar de kliniek en daarmee hun klinische implicaties behandeld. Hierna hebben we een voorstel gedaan voor toekomstige onderzoeksrichtingen om onbeantwoorde en nieuw ontstane onderzoeksvragen uit dit proefschrift te kunnen beantwoorden. We sloten de algemene discussie af met een samenvatting van onze conclusies dat:

- Kinderen met een lage BMC vaker HSPM hebben, maar dat deze associatie waarschijnlijk niet verklaard wordt door verschillen in vitamine D concentraties,
- Surinaams-Hindoestaanse, Turkse, Marokkaanse kinderen en kinderen van ouders met een lage SEP significant vaker cariës hebben dan Nederlandse kinderen en kinderen van ouders met een hogere SEP,
- We geen genetische varianten hebben gevonden die effect hebben op cariësgevoeligheid in het melkgebit, maar wel een variant die de aanwezigheid van cariës beïnvloedt in het blijvende gebit,
- Placentaire dysfunctie uitkomsten het cariësrisico onder zesjarigen niet beïnvloeden,
- Een QLF-camera een betrouwbaar systeem is om cariës en glazuurhypomineralisatie digitaal te beoordelen in onderzoeksverband en dat fluorescentie foto's van toegevoegde waarde zijn voor het beoordelen van cariëservaring.

7

Appendices

Chapter 7.1

Curriculum Vitae

About the author

Justin Thomas van der Tas was born in Rotterdam on the 28th of July 1992 and grew up in Mijnsheerenland with his parents, brother, and twin sister. In 2010 he obtained a "gymnasium-diploma" at the "Rijks Scholengemeenschap Hoeksche Waard, Oud-Beijerland". Furthermore, he followed an extracurricular program, the Junior Med School at the Erasmus University MC, during his pre-university education. This resulted in acceptance for medical school at the Erasmus University Rotterdam which he started with in 2010. In 2013, Justin got accepted for a second master, "Clinical Research", at the Netherlands Institute for Health Sciences in Rotterdam. For this master he got in touch with the head of the department of Oral and Maxillofacial Surgery, Special Dental Care and Orthodontics. There he received the opportunity to start with research within the Generation R Study, which eventually led to a PhD project and this thesis. In 2017, Justin obtained his master's degree in Medicine and Clinical Research. Moreover, he got accepted for following his residency at the department of Oral and Maxillofacial Surgery, Special Dental Care and Orthodontics to become an Oral- and Maxillofacial surgeon. Before starting his residency, he started with the "Tandheelkundige Opleiding Voor Artsen" at the Radboud University in Nijmegen in 2017. After two years in Nijmegen he started his residency in Rotterdam and he received his master's degree in Dentistry in October 2020. Justin lives in Rotterdam together with his fiancée Fleur.

PERSONALIA

Name:	Justin Thomas van der Tas
Address:	Nicolaas Ruyschstraat 8 02L
ZIP code:	3039 WR
City:	Rotterdam
Birth date:	28-07-1992
Birthplace:	Rotterdam
Nationality:	Dutch
Sex:	Male
E-mailaddress:	j.vandertas@erasmusmc.nl
Phone numberr:	+31 6 2929 3542

COMPUTER SKILLS

Statistics (SPSS and R)
Microsoft Office (Word, Excel and PowerPoint)

LANGUAGES

Dutch, mother tongue
English, fluently
German, good

INTERESTS

Kitesurfing
Sailing
Wakeboarding
Fitness
Reading
Snowboarding

EDUCATION

Sept 2017 – Oct 2020	***Dentistry*** (MSc) Radboud University, Nijmegen
Sept 2013 – Aug 2017	***Clinical Research*** (MSc) Erasmus MC Netherlands Institute for Health Sciences, Rotterdam
Sept 2013 – July 2017	***Medicine*** (MSc) Erasmus University Rotterdam, Rotterdam
Sept 2010 – June 2013	***Medicine*** (BSc) Erasmus University Rotterdam, Rotterdam
Sept 2007 – Oct 2009	***Junior Med School*** Erasmus University Rotterdam, Rotterdam
Sept 2004 – June 2010	***Gymnasium*** RSG Hoeksche Waard, Oud-Beijerland

WORK EXPERIENCE

Aug 2019 – current	***Resident Oral & Maxillofacial Surgery*** Department of Oral and Maxillofacial Surgery, Special Dental Care and Orthodontics Erasmus MC, Rotterdam
Sept 2017 – current	***Scientific Researcher*** Department of Oral and Maxillofacial Surgery, Special Dental Care and Orthodontics Erasmus MC, Rotterdam
Oct 2011 – Aug 2017	***Student team*** Medium Care & Intensive Care Cardiologie, supportive nursing tasks Erasmus MC, Rotterdam
Sept 2008 – Sept 2011	***Mailman*** TNT Post, Numansdorp

SCIENTIFIC PUBLICATIONS

Publications

van der Tas JT, Kragt L, Veerkamp JJ, Jaddoe VW, Moll HA, Ongkosuwito EM, Elfrink ME, Wolvius EB. Ethnic Disparities in Dental Caries among Six-Year-Old Children in the Netherlands. Caries Res. 2016;50(5):489-497.

Kragt L, **van der Tas JT**, Moll HA, Elfrink ME, Jaddoe VW, Wolvius EB, Ongkosuwito EM. Early Caries Predicts Low Oral Health-Related Quality of Life at a Later Age. Caries Res. 2016;50(5):471-479.

van der Tas JT, Elfrink ME, Vucic S, Heppe DH, Veerkamp JS, Jaddoe VW, Rivadeneira F, Hofman A, Moll HA, Wolvius EB. Association between Bone Mass and Dental Hypomineralization. J Dent Res. 2016;95(4):395-401.

van der Tas JT, Kragt L, Elfrink ME, Bertens LC, Jaddoe VW, Moll HA, Ongkosuwito EM, Wolvius EB. Social Inequalities in Dental Caries at the Age of Six. J Dent. 2017;62:18-24.

van der Tas JT, Elfrink ME, Heijboer AC, Rivadeneira F, Jaddoe VW, Tiemeier H, Schoufour JD, Moll HA, Ongkosuwito EM, Wolvius EB, Voortman T. Foetal, neonatal and child vitamin D status and enamel hypomineralization. Community Dent Oral Epidemiol. 2018;46(4):343-351.

Haworth S*, Shungin D*, **van der Tas JT,** … Wolvius EB. Franks OW. Johansson I. Timpson NJ, Consortium-based genome-wide meta-analysis for childhood dental caries traits. Human Mol Genet. 2018;27(17):3113-3127.

Jonker BP, Wolvius EB, **van der Tas JT**, Pijpe J. The effect of resorbable membranes on one-stage ridge augmentation in anterior single-tooth replacement: A randomized, controlled clinical trial. Clin Oral Implants Res. 2018;29(2):235-247.

Under review

van der Tas JT, Kragt L, Rivadeneira F, Dhamo B, Wolvius EB. Validation of using Quantitative Light-induced Fluorescence Photographs for Assessing Dental Caries and Enamel Hypomineralization.

van der Tas JT, Wolvius EB, Kragt L, Rivadeneira F, Steegers EA, Schalekamp-Timmermans S. Caries among Children Born from a Complicated Pregnancy Course.

Chapter 7.2

Portfolio

Name PhD student:	Justin T. van der Tas
Erasmus MC department:	Oral & Maxillofacial Surgery, Special Dental Care and Orthodontics/ The Generation R Study Group
Research school:	Netherlands Institute for Health Sciences (NIHES)
PhD period:	September 2016 –
Promotors:	Prof. dr. Eppo B. Wolvius
	Dr. Fernando Rivadeneira
Copromotor:	Dr. Lea Kragt-de Roos

PhD training and teaching activities	Year	Workload (ECTS)
1. PhD Training		
Master's degree Clinical Research, NIHES, Erasmus University Rotterdam, The Netherlands		
Principles of Research in Medicine	2014	0.7
Clinical Decision Analysis	2014	0.7
Methods of Clinical Research	2014	0.7
Social Epidemiology	2015	0.7
Markers and Prognostic Research	2015	0.7
The Practice of Epidemiologic Analysis	2015	0.7
Study Design	2014	4.3
Biostatistical Methods I: Basic Principles	2014	5.7
Repeated Measurements in Clinical Studies	2016	1.4
Biostatistical Methods II: Classical Regression Models	2014	4.3
Advanced Topics in Decision-making in Medicine	2014	1.9
Pharmaco-epidemiology and Drug Safety	2014	1.9
Intervention Research and Clinical Trials	2015	0.9
Diagnostic Research	2015	0.9
Advanced Topics in Clinical Trials	2015	1.9
Advanced Analysis of Prognosis Studies	2015	0.9
Prognosis Research	2015	0.9
Principles of Epidemiologic Data-analysis	2015	0.7
Quality of life Measurement	2016	0.9
Summercourses at Johns Hopkins (USA)	2016	3.0
Clinical Research Introduction	2014	3.0
Medical Demography	2017	4.3
Principles of Genetic Epidemiology	2016	2.8
Genomics in Molecular Medicine	2015	1.1
Conceptual Foundation of Epidemiologic Study Design	2015	0.7
Case-control Studies	2015	0.7
Logistic Regression	2016	1.4
Genome Wide Association Studies	2016	0.7
History of Epidemiologic Ideas	2014	0.7

PhD training and teaching activities	Year	Workload (ECTS)
Pharmaco-epidemiology	2016	0.7
Health Economics	2015	0.7
Scientific Writing in English for Publication	2017	2.0
Courses for the Quantitative Researcher	2017	1.4
Extracurricular courses, Erasmus University Rotterdam/ Erasmus University Medical Center, Rotterdam, The Netherlands		
Stralingshygiëne 5R	2016	0.3
Basiscursus Regelgeving en Organisaties voor klinisch Onderzoek (BROK)	2017	1.0
Wetenschappelijke Integriteit	2017	0.3
Seminars and workshops		
Research seminars	2014–2017	1.0
Generation R research meetings	2016–2017	0.5
Research meetings Department of Oral & Maxillofacial Surgery, Special Dental Care and Orthodontics	2014–2020	1.0
Conferences and presentations		
Research meeting Department of Oral & Maxillofacial Surgery, Special Dental Care and Orthodontics, oral presentation	2017	0.5
Generation R research meeting, oral presentation	2017	0.5
Research defense Clinical Research, NIHES, Erasmus University Rotterdam, the Netherlands	2017	0.5
SCEM Symposium Mondzorg bij het Kind, Bunnik, the Netherlands, oral presentation	2017	0.7
2. Teaching activities		
Supervision of master student 'Vitamin D Status and Dental Caries in Six-year-old Children'	2017–2018	0.5

Chapter 7.3

Dankwoord

Als een rode draad was het werk voor dit proefschrift verweven met de afgelopen studiejaren en het begin van mijn opleiding. Daaraan lijkt nu een einde gekomen te zijn. Spijtig, maar ik hoop in de toekomst nauw betrokken te blijven bij dit onderzoek. Dit dankwoord is dan ook niet alleen om te danken voor wat geweest is, maar te meer om stil te staan bij wat komen gaat. Hieronder staan heel wat personen die ik zwart op wit bedankt wil hebben. Ongetwijfeld zal ik iemand vergeten zijn, maar weet; u ook bedankt!

In de eerste plaats wil ik alle deelnemers van Generation R en hun ouders bedanken. Jullie bereidbaarheid, enthousiasme en ongelooflijke inzet om mee te blijven doen aan dit cohort zijn bewonderenswaardig. Zonder jullie deelname had dit onderzoek nooit bestaan! Ook wil ik alle patiënten via deze weg bedanken, die wilden deelnemen aan de validatiestudie!

Alle medewerkers van Generation R; de onderzoeksleiders, het bureau, de directie, het datamanagement, de onderzoeksmedewerkers en de promovendi, bedankt voor jullie tomeloze inzet om Generation R draaiende te houden. Uniek om te zien hoe zo'n groot cohort al zo vele jaren een hoog follow-up percentage weet te behouden. Dat kan alleen door jullie enorme inspanningen. Bedankt dat ik hier deel van uit heb mogen maken!

Beste professor Wolvius, dank voor het door u gestelde vertrouwen in mij. Als derdejaars geneeskundestudent kwam ik bij u om te praten over onderzoek voor mijn Research Master. Dat gesprek was het begin van dit proefschrift. Bedankt voor de kansen die ik bij u heb gekregen, alle feedback op onze manuscripten en uw rol als promotor. Ik zie de komende jaren opleiding met plezier tegemoet!

Beste Fernando, ook jij was vroeg betrokken bij mijn onderzoek en voorzag de meeste stukken van scherp en zeer deskundig commentaar. Ik herinner me een telefoongesprek over de statistiek van m'n eerste manuscript toen ik in de auto zat te wachten op de parkeerplaats van het Admiraal de Ruyter Ziekenhuis te Goes voor mijn coschap KNO. Tekenend voor de laagdrempeligheid waarmee er overleg gevoerd kon worden. Dank daarvoor en voor jouw rol als promotor voor dit proefschrift!

Beste Lea, jouw promotietraject startte ongeveer dezelfde periode als toen ik betrokken raakte bij Generation R. Inmiddels ben jij al gepromoveerd en werk je als postdoc verder voor onze afdeling. Ik waardeer je scherpte, punctualiteit en collegialiteit. Dank voor al je werk en rol als co-promotor. Ik hoop dat je nog lang als postdoc bij de afdeling betrokken blijft!

Beste professor Jaddoe en professor Raat, bedankt voor het beoordelen van mijn proefschrift als leden van de leescommissie en nogmaals dank voor uw werk voor Generation R.

Beste professor ten Cate, dank dat u als extern lid deel uit wilde maken van de leescommissie voor de beoordeling van mijn proefschrift. Ik zie uit naar uw vragen op 4 november.

Beste co-auteurs, een artikel schrijf je niet alleen. Jullie zijn er met te veel om apart te benoemen, maar dank voor jullie feedback, harde werk aan de artikelen en daarmee jullie contributie aan dit proefschrift.

Kamergenoten van de 29ste verdieping, dank voor de tijd die ik als "vreemde eend in de bijt" bij jullie op de kamer heb mogen doorbrengen. Als student-onderzoeker/promovendus was ik maar even aanwezig en alweer snel vertrokken naar Nijmegen. Desalniettemin waren het een aantal waardevolle maanden met serieus werk welke werden afgewisseld met voetbaltafel intermezzo's. Ik zie uit naar jullie promoties!

Alle collegae van de afdeling; stafleden, AIOS, poli-assistenten, OK-assistenten en het secretariaat, dank voor jullie interesse en de prettige werksfeer. Door jullie kijk ik terug op een zeer geslaagd eerste opleidingsjaar en zie de komende drie met nog meer plezier tegemoet!

Bart en Jeff, dank dat jullie naast me willen staan tijdens de verdediging. Mooi om te zien waar jullie mee bezig zijn, goed zijn in wat jullie doen en behoorlijk aan de weg timmeren.

Lieve familie en schoonfamilie, dank voor jullie aanwezigheid en jullie interesse in mijn werk. Indirect en direct heb ik ontzettend veel aan jullie te danken en ik verheug me op alles wat nog komen gaat met elkaar!

Paul nog even in het bijzonder, dank voor het ontwerpen van de kaft. Ik ben ontzettend blij met het eindresultaat!

Lieve pap en mam, tijdens het dankwoord na de uitreiking van m'n bul geneeskunde vergat ik jullie als enige op te noemen. Hier kwam ik later pas achter toen ik de beelden zag… Gelukkig staat het bij deze zwart op wit! Het is ook lastig in woorden of tekst uit te drukken hoeveel wij aan jullie te danken hebben. Een klein en tekenend voorbeeld is te vinden in hoofdstuk 4 van dit proefschrift. Bewonderenswaardig hoeveel steun en mogelijkheden jullie ons bieden en geboden hebben om ons te kunnen ontwikkelen tot wie en wat we willen worden. Steeds meer raak ik me er bewust van hoe bijzonder dit is. Wijsheid komt met de jaren… Bedankt voor alles en voor de mooie momenten die nog komen gaan! Ik hou van jullie!

Lieve Fleur, vaak vraag ik je gekscherend; Waar zou ik toch zijn zonder jou? Het antwoord op deze vraag is echter simpel, nergens. Dank voor alles en op een mooie toekomst samen! Ik hou van je!