Ancestry and dental development: a geographic and genetic perspective

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ABSTRACT

Objective: In this study, we investigated the influence of ancestry on dental development in the Generation R Study.

Methods: Information on geographic ancestry was available in 3,600 children (1,810 boys and 1,790 girls, mean age 9.81±0.35 years) and information about genetic ancestry was available in 2,786 children (1,387 boys and 1,399 girls, mean age 9.82±0.34 years). Dental development was assessed in all children using the Demirjian method. The associations of geographic ancestry (Cape Verdean, Moroccan, Turkish, Dutch Antillean, Surinamese Creole and Surinamese Hindustani vs Dutch as reference group) and genetic content of ancestry (European, African or Asian) with dental development was analyzed using linear regression models.

Results: In a geographic perspective of ancestry, Moroccan (β = 0.18; 95% CI: 0.07, 0.28), Turkish (β = 0.22; 95% CI: 0.12, 0.32), Dutch Antillean (β = 0.27; 95% CI: 0.12, 0.41) and Surinamese Creole (β = 0.16; 95% CI: 0.03, 0.30) exceeded Dutch children in dental development. Moreover, in a genetic perspective of ancestry, a higher proportion of European ancestry was associated with decelerated dental development (β = -0.32; 95% CI: -0.44, -0.20). In contrast, a higher proportion of African ancestry (β = 0.29; 95% CI: 0.16, 0.43) and a higher proportion of Asian ancestry (β = 0.28; 95% CI: 0.09, 0.48) were associated with accelerated dental development. When investigating only European children (genetically determined), these effect estimates increased to twice as large in absolute value.

Conclusion: Based on a geographic and genetic perspective, differences in dental development exist in a population of heterogeneous ancestry and should be considered when describing the physiological growth in children.

2.1.1 INTRODUCTION

Dental development is a progressive and continuous process determined by interactions of genetic, epigenetic and environmental factors over time [1].

In different geographical areas, populations have shown variations in dental development including different morphology of teeth and other dental anomalies [2-4]. Characteristics in shape, size and structure of teeth are recognized as indicators of dental differences in populations. For example, Africans have bigger teeth with thicker enamel, whereas Europeans have smaller teeth and a reduction in tooth mass [5-7]. Aside from variations in dental morphology and anomalies, variations in the rate (e.g., accelerations or decelerations) of dental development have been noted across populations. The literature confirms that Africans exceed Europeans in the timing of tooth formation [8, 9], by achieving the stages of dental development about 5% significantly earlier in time [6]. Among the studied populations, Australians have the fastest dental development and Koreans have the slowest, addressing this difference to the ecological and genetic factors [10]. Furthermore, decelerated dental development is recognized in northern populations, whereas accelerated dental development is shown in tropical populations [11].

Genes are known to play a predominant role in dental development [1]. However, due to geographical diversity in climate and latitude, physical factors such as temperature, sun exposure and humidity can be related with ethnic variations in growth and also dental development [11-14].

Thus, a geographic and genetic approach of ancestry is necessary to explain the variations in timing of dental development. In addition, the recognition of differences in dental development within a population is important to understand the environmental influence and genetic implication [9, 15-17].

Beyond the above mentioned facts, due to limited data available on dental development, the literature provides little evidence on the influence of ancestry in dental development within populations [11, 18, 19]. Therefore, in a large number of subjects as part of a multi-ethnic population-based prospective cohort study, we aimed to investigate the influence of ancestry on dental development, based on a geographic and genetic perspective.

2.1.2 MATERIALS AND METHODS

2.1.2.1 Study design

This study was embedded in the Generation R Study, a multi-ethnic population-based prospective cohort study from fetal life onwards, which was initiated to identify early environmental and genetic determinants of growth, development and health [20, 21]. All children were born between April 2002 and January 2006. Enrollment in the study was aimed at early pregnancy but was allowed until the birth of the child. Data collection in children and their parents include questionnaires, interviews, detailed physical and ultrasound examinations, behavioral observations, magnetic resonance imagining and biological samples. The Generation R Study has been conducted in accordance with the World Medical Association Declara-

tion of Helsinki and all study phases have been approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam, the Netherlands (MEC-2012-165) [21].

2.1.2.2 Study population

In total, 4447 dental panoramic radiographs (DPRs) taken in 4447 children at the age-10 assessment, were used to assess dental development. Information about geographic ancestry was available in 3600 children (1810 boys and 1790 girls) with a mean age of 9.81 years (SD; 0.35), and information about genetic content of ancestry was available in 2786 children (1387 boys and 1399 girls) with a mean age of 9.82 years (SD; 0.34).

2.1.2.3 The assessment of ancestry

The ancestry of children was defined in two ways:

1- Geographic ancestry: Information about countries of birth of the parents was obtained by questionnaires. Children of whom both parents were born in the Netherlands were classified as Dutch (N = 2603). The child was of non-Dutch geographic origin if one or both of the parents were born abroad. If the parents were born in different countries, the country of birth of the mother determined the ethnic background [22]. This approach has been previously described in detail [21]. We defined the following non-Dutch groups: Cape Verdean (N = 132), Moroccan (N = 232), Turkish (N = 275), Dutch Antillean (N = 113) and Surinamese (N = 245). The Surinamese population consists of persons who originate from Africa (Creoles) and India (Hindustani), therefore we further classified the child as: Surinamese-Creole (N = 120) or Surinamese-Hindustani (N = 125) based on the origin of the Surinamese parent [23].

2- Genetic ancestry: Blood samples of the children were collected from the umbilical cord at birth. Where an umbilical cord blood sample could not be collected at birth, a blood sample was obtained by venipuncture during the child's visit to the research center at a mean age of 6 years [20]. Genotyping was performed in the Genetic Laboratory of the Erasmus MC, Department of Internal Medicine, Rotterdam, the Netherlands using Illumina HumanHap 610 or 660 Quad chips depending on collection time following manufacturer protocols, and intensities were obtained from the BeadArray Reader [24]. Genetic ancestry was identified by admixture analysis applied in participants of the Generation R Study [25]. This program models the probability of observed genotypes using ancestry proportions and ancestral population allele frequencies. The clustering method was set to group individuals in three ancestral populations (K = 3), corresponding to the expected main Sub-Saharan African, European and East Asian ancestry components [26, 27]. Children were assigned to one of the three ancestry groups, labeled after the HapMap Phase II populations, based on their highest fraction of estimated ancestry (i.e., 40.50) proportions. We defined 2473 children of European origin, 204 children of African origin and 109 children of Asian origin. Cases that didn't reach any significant proportion of the three ancestral populations, were excluded from further analyses (N = 48).

2.1.2.4 Dental development

Dental development was defined using the Demirjian [28]. One experienced examiner (B.D) determined the eight stages of development (1 to 8) for each of the seven permanent teeth

located in the lower left quadrant excluding the third molar. In case any permanent tooth in the left mandible was congenitally missing, the stage of development was assessed from the corresponding tooth in the right mandible; and if the corresponding tooth was missing as well, regression equations which take into account the development of the remaining teeth in the lower left quadrant and age of a child, were applied to assess the stage of development for the missing tooth [29]. The obtained stages of development were weighted using three different dental age standards (Dutch standard, French-Canadian standard and International Demirjian standard) and subsequently for each standard separately were summed to calculate the gender specific maturity scores [10, 28, 30]. Finally, standard tables were used to convert the dental maturity score into dental age. Dental age calculated by the Dutch standard presented consistently the best approximation with chronological age in our study population, hence it was used as a proxy of dental development in the further statistical analysis.

2.1.2.5 Covariates

Age of a child was calculated as the interval between the date when the DPR was taken and the date of birth. Information on child's sex and day of birth were available from medical records and hospital registries. As sex is taken in consideration when dental age is calculated, we used sex as a potential confounder only to study the influence of ancestry on the developmental stages of each left mandibular tooth. Hypodontia was ascertained from the DPRs. Children were classified with hypodontia if no sign of tooth formation or calcification was shown in DPR. Most of children who revealed hypodontia had 1-2 absent teeth. Hence, they were not excluded from the study population as the Demirjian method covers the problem of few missing teeth. BMI (kg/m^2) was calculated using the weight and height measured during the age-10 assessment. The decayed, missing and filled teeth index (dmft) was used to assess dental caries when children were 6 years old, a high-risk age for dental caries in deciduous dentition. The dmft-score of each child was obtained from intraoral photographs [31]. Covariates were included in the regression models based on previous literature or a change of >10% in effect estimates.

2.1.2.6 Statistical analysis

We calculated the Intra-Class Correlation (ICC) to test the agreement between two independent examiners who assessed stages of development (1 to 8) for each of the seven left mandibular teeth in a random subsample of 100 DPRs from the study population. The ICC for the scored teeth ranged between 0.65-0.80 which is considered to be a substantial agreement according to the conventional criteria [32]. First incisors were not taken into account due to the absence of variation in the stage of tooth development fitting with age of the children.

The association between geographic ancestry and dental development (dental age calculated by the Dutch standard) was analyzed using two generalized linear regression models. In Model 1, we adjusted the association for chronological age. In Model 2, we additionally adjusted for hypodontia, BMI, height and dmft. This analysis was performed for Cape Verdean, Moroccan, Turkish, Dutch Antillean, Surinamese Creole and Surinamese Hindustani children with the Dutch children as reference group. The association of each genetic content of an-

cestry (European, African, Asian) with dental age was analyzed using two multivariate linear regression models adjusted for the same potential confounders. This analysis was performed in the total study population and for specificity, only in European children because they represented the majority (88.8%) of our study population.

The association between genetic ancestry and development of each mandibular tooth in the left lower quadrant (the reference quadrant) was analyzed using two ordinal regression models. In Model 1 we adjusted the association for age and sex. In Model 2, we additionally adjusted for hypodontia, BMI, height and dmft. This analysis was performed for African and Asian origin with the European origin as reference group.

We tested for interaction terms of sex and hypodontia with geographic and genetic ancestry in relation to dental age. Since no significant interaction terms were found, we did not stratify our analysis. To check for selection bias, we performed a non-response analysis to test the differences between subjects that were included and subjects that were eligible to be included but were left out due to lack of available data on dental development. The Markov Chain Monte Carlo imputation method [33] was used to reduce potential bias associated with missing data on dmft at the age of 5 years in 1106 children (25%). Five imputed datasets were generated from which the pooled effect estimates are presented in this study (β; 95% CI). All results were considered statistically significant for a p-value ≤0.05. All statistical analyses in this study were performed using statistical software Statistical Package for Social Sciences version 21.0 (SPSS Inc. Chicago, IL, USA).

2.1.3 RESULTS

2.1.3.1 General characteristics

Geographic ancestry (Table 2.1.1a): Hypodontia was significantly more frequent in Cape Verdean children than in Dutch children (p = 0.022). Cape Verdean, Moroccan, Turkish, Dutch Antillean and Surinamese Creole children had a higher BMI than reference group (p<0.001). Moroccan and Turkish children were shorter than reference group (p<0.001) while Surinamese Creole children were taller than reference group (p<0.001). The dmft was significantly higher in Cape Verdean, Moroccan, Turkish, and Surinamese-Hindustani children than in Dutch children (p< 0.001). The calculated dental age by the Dutch standard was significantly higher in children of Cape Verdean (mean:10.46 years), Moroccan (mean: 10.53 years), Turkish (mean: 10.61 years), Dutch Antillean (mean: 10.68 years), Surinamese Creole (mean: 10.54 years) descent compared to Dutch children (mean: 10.25 years). In contrast, there was no statistically significant difference in dental age between Surinamese Hindustani children (mean: 10.36 years) and Dutch children (mean: 10.25 years).

Genetic ancestry (Table 2.1.1b): When compared to children of European origin, no significant difference in the frequency of hypodontia was present in children of African (p = 0.143) and Asian origin (p = 0.072).

BMI was statistically significantly higher in children of African origin than in children of European origin (p<0.001). African children were taller than European children (p = 0.001)

while Asian children were shorter than European children (p<0.001). The dmft was statistically significantly higher in children of Asian origin than in children of European origin (p = 0.013). The dental age estimated by the Dutch standard was significantly higher in children of Afri-

Table 2.1.1a. General characteristics of the study population

	Geographic ancestries							
	Total (N = 3600)	**Dutch** (N = 2603)	**Cape Verdean** (N = 132)	**p-value**	**Moroccan** (N = 232)	**p-value**	**Turkish** (N = 275)	**p-value**
Age	9.81 (0.35)	9.78 (0.32)	9.92 (0.48)	*<0.001*	9.90 (0.41)	*<0.001*	9.90 (0.45)	*<0.001*
Sex				0.459		0.123		0.160
Boys	1810 (50.3)	1304 (50.1)	65 (49.2)		126 (54.3)		147 (53.5)	
Girls	1790 (49.7)	1299 (49.9)	67 (50.8)		106 (45.7)		128 (46.5)	
Maternal age	31.04 (4.87)	31.77 (4.46)	29.98 (5.27)	*<0.001*	29.21 (5.13)	*<0.001*	28.30 (5.00)	*<0.001*
Height	141.77 (6.62)	141.98 (6.36)	142.40 (7.91)	0.461	140.14 (6.53)	*<0.001*	140.29 (6.81)	*<0.001*
Weight	35.51 (7.36)	34.66 (6.39)	39.51 (10.33)	*<0.001*	36.59 (8.17)	*<0.001*	38.35 (8.88)	*<0.001*
BMI	17.56 (2.76)	17.11 (2.34)	19.24 (3.48)	*<0.001*	18.51 (3.16)	*<0.001*	19.33 (3.38)	*<0.001*
dmft	0.0 (0.0-6.0)	0.0 (0.0-3.0)	0.0 (0.0-6.0)	*<0.001*	2.0 (0.0-9.0)	*<0.001*	1.5 (0.0-11.0)	*<0.001*
Dental age [1]	10.33 (0.84)	10.25 (0.78)	10.46 (0.93)	*0.003*	10.53 (0.95)	*<0.001*	10.61 (1.03)	*<0.001*
Dental age [2]	11.21 (1.13)	11.10 (1.07)	11.28 (1.11)	*<0.001*	11.46 (1.18)	*<0.001*	11.59 (1.29)	*<0.001*
Dental age [3]	10.59 (0.93)	10.49 (0.86)	10.78 (1.11)	*<0.001*	10.83 (1.03)	*<0.001*	10.95 (1.14)	*<0.001*
Hypodontia	184 (5.1)	137 (5.3)	2 (1.5)	*0.022*	12 (5.2)	0.438	17 (6.2)	0.388
Dental anomalies of position	91 (2.5)	68 (2.6)	5 (3.8)	0.275	2 (0.9)	0.065	4 (1.5)	0.167
	Total (N = 3600)	**Dutch** (N = 2603)	**Dutch Antillean** (N = 113)	**p-value**	**Surinamese Creole** (N = 120)	**p-value**	**Surinamese Hindustani** (N = 125)	**p-value**
Age	9.81 (0.35)	9.78 (0.32)	9.89 (0.47)	*0.001*	9.85 (0.36)	*0.033*	9.79 (0.31)	0.741
Sex				0.174		0.458		0.237
Boys	1810 (50.3)	1304 (50.1)	51 (45.1)		59 (49.2)		58 (46.4)	
Girls	1790 (49.7)	1299 (49.9)	62 (54.9)		61 (50.8)		67 (53.6)	
Maternal age	31.04 (4.87)	31.77 (4.46)	28.09 (6.36)	*<0.001*	30.83 (5.87)	*0.027*	29.26 (4.63)	*<0.001*
Height	141.77 (6.62)	141.98 (6.36)	142.53 (7.27)	0.370	143.36 (7.52)	*0.021*	140.83 (7.53)	0.052
Weight	35.51 (7.36)	34.66 (6.39)	39.30 (10.50)	*<0.001*	38.19 (8.87)	*<0.001*	34.66 (7.54)	0.996
BMI	17.56 (2.76)	17.11 (2.34)	19.13 (3.68)	*<0.001*	18.41 (3.13)	*<0.001*	17.37 (2.99)	0.226
dmft	0.0 (0.0-6.0)	0.0 (0.0-3.0)	0.0 (0.0-3.0)	0.766	0.0 (0.0-3.6)	0.600	0.0 (0.0-8.9)	*<0.001*
Dental age [1]	10.33 (0.84)	10.25 (0.78)	10.68 (0.98)	*<0.001*	10.54 (0.66)	*<0.001*	10.36 (0.77)	0.130
Dental age [2]	11.21 (1.13)	11.10 (1.07)	11.74 (1.27)	*<0.001*	11.53 (1.00)	*<0.001*	11.28 (1.11)	0.064
Dental age [3]	10.59 (0.93)	10.49 (0.86)	11.02 (1.12)	*<0.001*	10.84 (0.80)	*<0.001*	10.63 (0.90)	0.096
Hypodontia	184 (5.1)	137 (5.3)	4 (3.5)	0.122	6 (5.0)	0.517	6 (4.8)	0.448
Dental anomalies of position	91 (2.5)	68 (2.6)	4 (3.5)	0.352	2 (1.7)	0.396	6 (4.8)	0.121

Abbreviations: N- number of participants, dmft-decayed-missing-filled teeth Index; Values are numbers (%) for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (95% range) for continuous variables with a skewed distribution; Differences were tested using one way ANOVA and Chi-square tests for variables with a normal distribution and Kruskal-Wallis Non-Parametric test for variables with a skewed distribution; with the Dutch ethnicity as the reference group; Significant p-values are presented in italic font; [1] dental age calculated by the Dutch standard; [2] dental age calculated by the French-Canadian standard; [3] dental age calculated by the International Demirjian standard

Table 2.1.1b. General characteristics of the study population

	Genetic ancestries					
	Total (N = 2786)	**Europeans** (N = 2473)	**Africans** (N = 204)	**p-value**	**Asians** (N = 109)	**p-value**
Age	9.82 (0.34)	9.81 (0.34)	9.92 (0.49)	*<0.001*	9.82 (0.32)	0.794
Sex				0.251		0.086
Boys	1387 (49.8)	1243 (50.3)	97 (47.5)		47 (43.1)	
Girls	1399 (50.2)	1230 (49.7)	107 (52.5)		62 (56.9)	
Maternal age	30.91 (4.81)	31.23 (4.58)	28.06 (6.16)	*<0.001*	28.94 (4.68)	*<0.001*
Height	141.87 (6.75)	141.85 (6.60)	143.45 (7.42)	*0.001*	139.30 (7.92)	*<0.001*
Weight	35.47 (7.17)	35.22 (6.83)	39.22 (9.25)	*<0.001*	33.97 (8.28)	0.063
BMI	17.52 (2.66)	17.41 (2.54)	18.90 (3.33)	*<0.001*	17.32 (3.04)	0.713
dmft	0.0 (0.0-7.0)	0.0 (0.0-7.0)	0.0 (0.0-6.8)	0.958	0.0 (0.0-9.6)	*0.013*
European content of ancestry	1.0 (0.1-1.0)	1.0 (0.5-1.0)	0.3 (0.1-0.5)	*<0.001*	0.4 (0.0-0.5)	*<0.001*
African content of ancestry	0.0 (0.0-0.8)	0.0 (0.1-0.4)	0.7 (0.5-1.0)	*<0.001*	0.0 (0.0-0.4)	*<0.001*
Asian content of ancestry	0.0 (0.0-0.5)	0.0 (0.0-0.3)	0.0 (0.0-0.2)	0.132	0.6 (0.5-1.0)	*<0.001*
Dental age [1]	10.34 (0.82)	10.32 (0.82)	10.65 (0.87)	*<0.001*	10.31 (0.77)	0.900
Dental age [2]	11.23 (1.12)	11.19 (1.11)	11.70 (1.19)	*<0.001*	11.21 (1.11)	0.877
Dental age [3]	10.61 (0.92)	10.58 (0.90)	10.98 (1.06)	*<0.001*	10.57 (0.90)	0.922
Hypodontia	149 (5.3)	134 (5.4)	7 (3.4)	0.143	8 (7.3)	0.072
Dental anomalies of position	77 (2.8)	64 (2.6)	7 (3.4)	0.295	6 (5.5)	0.112

Abbreviations: N – number of participants, dmft – decayed-missing-filled teeth Index; Values are numbers (%) for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (95% range) for continuous variables with a skewed distribution; Differences were tested using one way ANOVA and Chi-squared tests for variables with normal distribution and Kruskal-Wallis Non-Parametric test for variables with a skewed distribution; with the European children as the reference group; Significant p-values are presented in italic font; [1] dental age calculated by the Dutch standard; [2] dental age calculated by the French-Canadian standard; [3] dental age calculated by the International Demirjian standard

can origin (mean: 10.65 years) than in children of European origin (mean: 10.32 years). Dental age in children of Asian origin (mean: 10.31 years) was not statistically significantly different (p = 0.900) compared with children of European origin.

Children who did not participate in the follow-up measurements of dental development differed statistically significantly from the participants in age, height and dmft (Table S2.1.1).

2.1.3.2 The association between geographic ancestry and dental age

In Model 1, Moroccan (β, 0.20; 95% CI: 0.09, 0.30), Turkish (β, 0.27; 95% CI: 0.18, 0.37), Dutch Antillean (β, 0.35; 95% CI: 0.21, 0.50) and Surinamese Creole (β, 0.24; 95% CI: 0.10, 0.38) children exceeded Dutch children in dental development (Table 2.1.2.a). No differences in dental age were found either between Cape Verdean and Dutch children (β, 0.11; 95% CI: -0.03, 0.24), or between Surinamese Hindustani and Dutch children (β, 0.10; 95% CI: -0.04, 0.24). After adjusting for hypodontia, BMI, height and dmft (Model 2) the association remained significant, however the effect estimates decreased. Still, Moroccan (β, 0.18; 95% CI: 0.07, 0.28), Turkish (β, 0.22; 95% CI: 0.12, 0.32), Dutch Antillean (β, 0.27; 95% CI: 0.12, 0.41) and Surinamese Creole (β, 0.16; 95% CI: 0.03, 0.30) children exceeded Dutch children in dental development. Again, no difference on dental age was found either between Cape Verdean and Dutch children

Table 2.1.2. The association between ancestry and dental development (dental age)

	Model 1			Model 2		
a. Geographic ancestry	**β**	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**
Dutch (reference)	-	-	-	-	-	-
Cape Verdean	0.11	-0.03, 0.24	0.122	0.01	-0.12, 0.15	0.845
Moroccan	0.20	0.09, 0.30	*<0.001*	0.18	0.07, 0.28	*0.001*
Turkish	0.27	0.18, 0.37	*<0.001*	0.22	0.12, 0.32	*<0.001*
Dutch Antillean	0.35	0.21, 0.50	*<0.001*	0.27	0.12, 0.41	*<0.001*
Surinamese Creole	0.24	0.10. 0.38	*0.001*	0.16	0.03, 0.30	*0.020*
Surinamese Hindustani	0.10	-0.04, 0.24	0.155	0.10	-0.03, 0.24	0.137
b. Genetic ancestry	**Model 1**			**Model 2**		
1. Total (N = 2786)	**β**	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**
European content of ancestry	-0.37	-0.49, -0.25	*<0.001*	-0.32	-0.44, -0.20	*<0.001*
African content of ancestry	0.41	0.27, 0.55	*<0.001*	0.29	0.16, 0.43	*0.001*
Asian content of ancestry	0.19	-0.01, 0.39	0.066	0.28	0.09, 0.48	*0.005*
2. Europeans (N = 2473)	**β**	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**
European content of ancestry	-0.69	-0.93, -0.45	*<0.001*	-0.63	-0.87, -0.40	*<0.001*
African content of ancestry	0.68	0.38, 0.99	*<0.001*	0.57	0.27, 0.87	*<0.001*
Asian content of ancestry	0.64	0.27, 1.01	*0.001*	0.62	0.26, 0.98	*0.001*

Abbreviations: β –regression coefficients, CI – confidence interval; genetic contents of ancestry are investigated as continuous variables; Significant p-values are presented in italic font
Model 1: adjusted for age
Model 2: was additionally adjusted for hypodontia, BMI, height and dmft (decayed-missing-filled teeth Index)

(β, 0.01; 95% CI: -0.12, 0.15), or between Surinamese Hindustani and Dutch children (β, 0.10; 95% CI: -0.03, 0.24).

2.1.3.3 The association between the genetic content of ancestry and dental age

Total population: In Model 1, the increase in European content of ancestry was associated with lower dental age (β, -0.37; 95% CI: -0.49, -0.25) (Table 2.1.2.b.1). After adjusting for hypodontia, BMI, height and dmft (Model 2) the association remained, however the effect estimate attenuated. Still, the increase in European content of ancestry was associated with lower dental age (β, -0.32; 95% CI: -0.44, -0.20). In contrast, the increase in African content of ancestry was associated with higher dental age (β, 0.41; 95% CI: 0.27, 0.55) in Model 1. After adjusting for hypodontia, BMI, height and dmft (Model 2) the effect estimate decreased (β, 0.29; 95% CI: 0.16, 0.43). No statistically significant association was revealed between Asian content of ancestry and dental age in Model 1 (β, 0.19; 95% CI: -0.01, 0.39) when adjusted only for chronological age. In contrast, after additionally adjusted for hypodontia, BMI, height and dmft in Model 2, the increase in Asian content of ancestry was statistically significantly associated with higher dental age (β, 0.28; 95% CI: 0.09, 0.48).

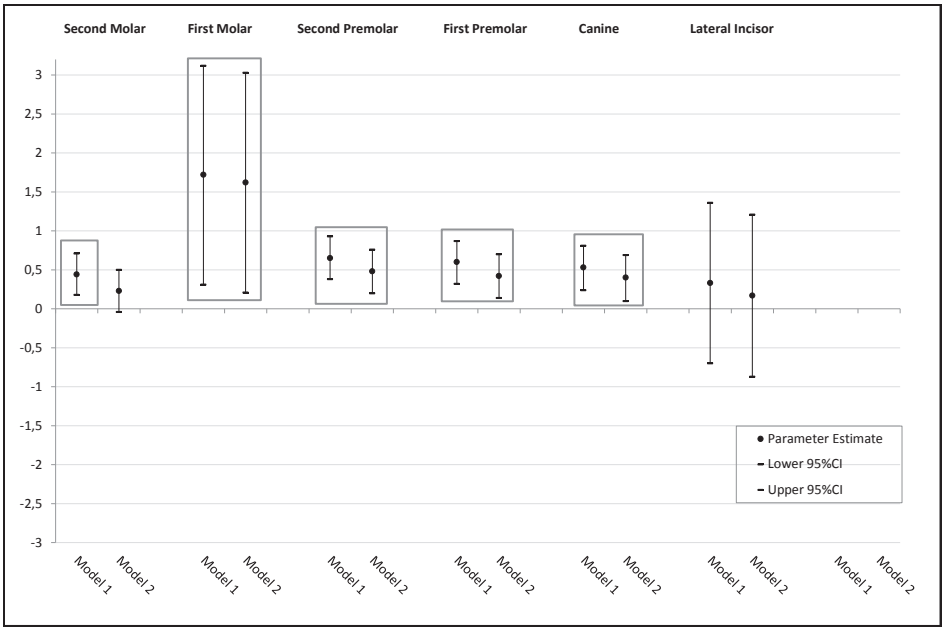
European children: When the same analysis was performed only in European children who presented the majority of our study population and a more homogeneous sample as well, the studied associations remained in the same directions for each genetic content of ancestry (Table 2.1.2.b.2). Considering all the potential confounders, Model 2 revealed a statistical

significant association of European content of ancestry with lower dental age (β, -0.63; 95% CI: -0.87, -0.40). In contrast, the African content of ancestry (β, 0.57; 95% CI: 0.27, 0.87) and Asian content of ancestry (β, 0.62; 95% CI: 0.26, 0.98) were both statistically significantly associated with higher dental age in European children.

2.1.3.4 The association between genetic ancestry and development of each left mandibular tooth

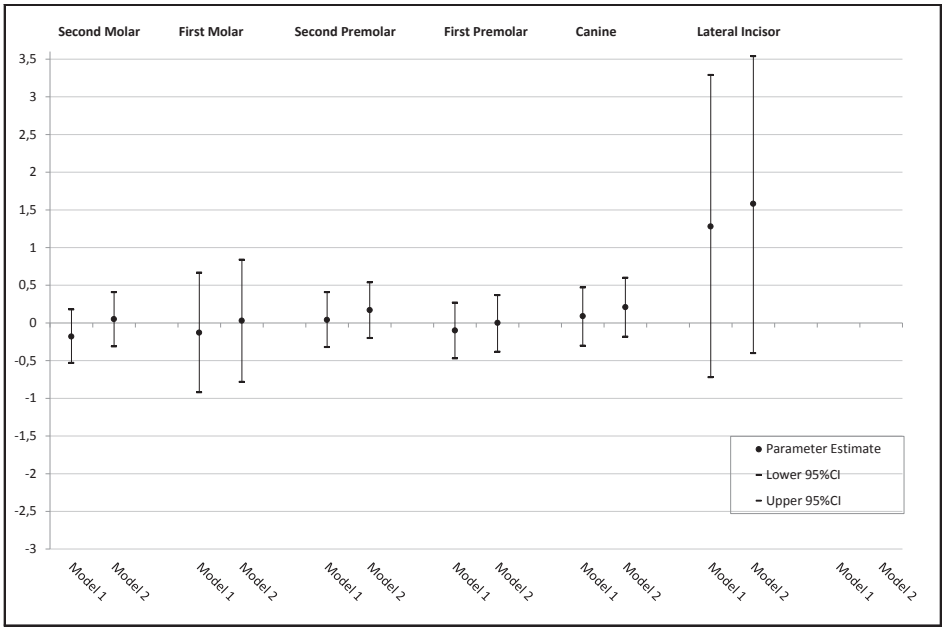
Taking in consideration the potential confounders, Model 2 revealed statistically significant higher developmental stages for the canine (β, 0.40; 95% CI: 0.10, 0.69), first premolar (β, 0.42; 95% CI: 0.14, 0.70), second premolar (β, 0.48; 95% CI: 0.20, 0.76) and first molar (β, 1.62; 95% CI: 0.21, 3.03) in children of African origin than in children of European origin (Figure 2.1.1). Both Model 1 and Model 2 did not reveal any statistically significant difference in developmental stages of each left mandibular tooth in children of Asian origin compared with children of European origin (Figure 2.1.2). The ordinal regression analysis for the lateral and central incisor presented uninterpretable parameter estimates because these teeth were in the final stage of development.

Figure 2.1.1. The association between genetic ancestry (Africans vs Europeans) and the development of each left mandibular tooth



Abbreviations: Model 1 is adjusted for age and sex; Model 2 is additionally adjusted for hypodontia, BMI, height and dmft (decayed-missing-filled teeth Index); the statistically significant parameter estimates are presented inside the squares

Figure 2.1.2. The association between genetic ancestry (Asians vs Europeans) and the development of each left mandibular tooth



Abbreviations: Model 1 is adjusted for age and sex; Model 2 is additionally adjusted for hypodontia, BMI, height and dmft (dental caries in deciduous dentition)

2.1.4 DISCUSSION

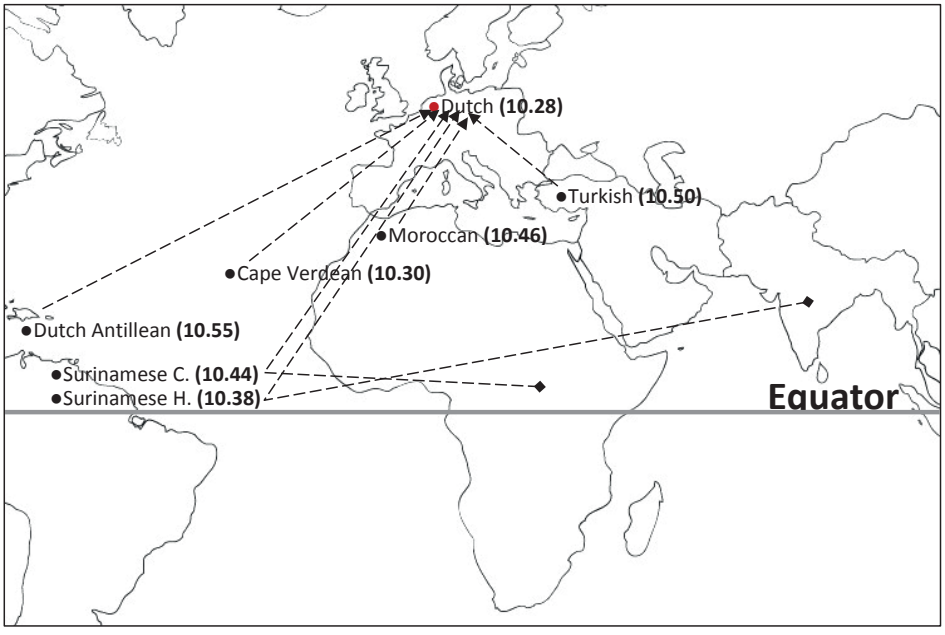
In this multi-ethnic population-based prospective cohort study among 10 year old children born in the Netherlands, Moroccan, Turkish, Dutch Antillean and Surinamese-Creole children exceeded Dutch children with 2-4 months in dental development. Meanwhile, Cape Verdean and Surinamese Hindustani children did not significantly differ in dental development compared with Dutch children. Furthermore, the increase in European content of ancestry was associated with decelerated dental development of approximately 4-5 months. In contrast, the increase in African content of ancestry was associated with accelerated dental development of approximately 3-5 months and the increase in Asian content of ancestry was associated with accelerated dental development of approximately 3 months. The effect estimates of the European, African and Asian proportion of ancestry in dental development increased twice when investigated only in the European children.

The results of the current study are consistent with foundational work of scholars like Stanley Garn and Derek Roberts [9, 16, 17, 34, 35]. Garn and colleagues explored the influence of genetic, nutritional, and economic factors on variation in human dental development. Considering also the findings of our study, the genetic component is an important indicator for the acceleration of dental development. However, factors related to the environment such as

physical factors (sun exposure, temperature, humidity, altitude), cultural habits in nutrition and hormonal levels could be important determinants affecting dental development and decreasing the effect of the genetic component [11, 36]. According to the geographical context, Dutch Antillean revealed the highest dental age (Figure 2.1.3) and according to the genetic perspective this ethnic group reaches high proportion in African ancestral content. African children had the highest dental age (Figure 2.1.4) leading to consistent findings by both geographic and genetic perspective.

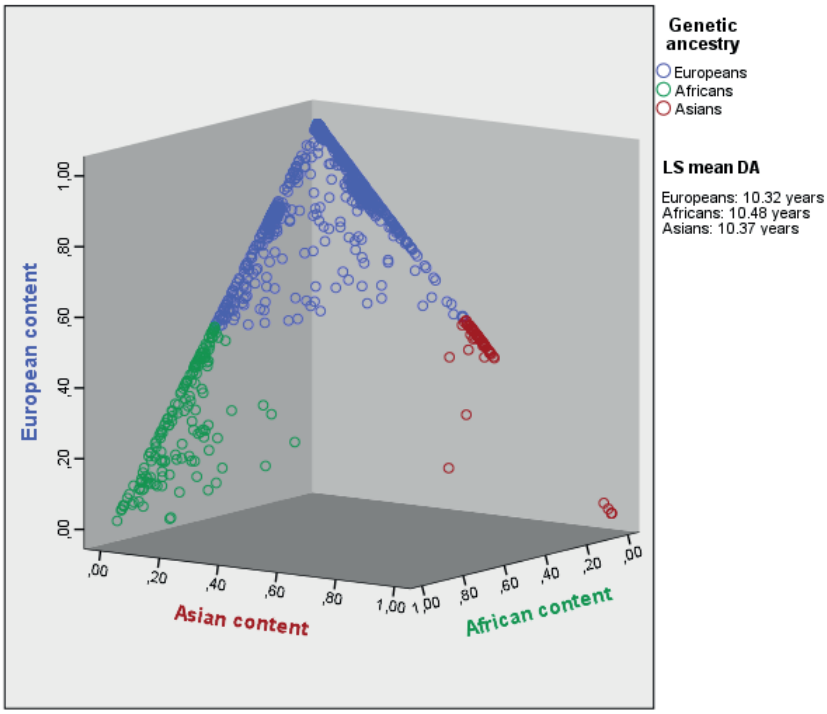
The acceleration of dental maturity is recognized as an indicator of pubertal growth spurt [37]. Based on the geographic ancestry in this study, Dutch Antillean children, followed by Turkish, Moroccan and Surinamese Creole children, were the most advanced in dental development. Previous studies in the Netherlands have shown that children of Turkish and Moroccan ethnicity start puberty later than Dutch children, however they pass through the pubertal stages faster than the Dutch children [38, 39]. Lacking information on sexual maturity, we let the explanation of the association between the spurt of dental development and puberty to the future research in our cohort, when children will be approximately 13 years old. Referring to the current literature, puberty occurs earlier in children of African origin than in children of European origin [40] and especially the completion of the root formation of the mandibular canine (Stage "7" of development), prior to apical closure (Stage "8"of development) may be clinically used as an indicator of the pubertal growth spurt [37]. In our study,

Figure 2.1.3. Schematic presentation of dental age for each geographic ancestry



Abbreviations: The numbers in brackets and bold font represent the LS (least square) mean of dental age for each ethnic group, adjusted for age, hypodontia, BMI, height and dmft (decayed-missing-filled teeth Index); The lines in dashes show the migration of each ethnic group from the place of origin to the Netherlands; Surinamese C. (Creole) and Surinamese H. (Hindustani)

Figure 2.1.4. Graphic presentation of dental age for each genetic ancestry based on proportions (%) of European, African and Asian content



Abbreviations: LS mean- least square mean; DA- dental age; LS mean DA was adjusted for age, hypodontia, BMI, height and dmft

Addition: The highest reached fraction of estimated ancestry proportions such as European content, African content and Asian content, (presented as x, y and z axis in sides of the cub) assigned children to one of the three ancestry groups Europeans, Africans or Asians

African children exceeded European children in development of the mandibular canine, first premolar, second premolar and first molar (0.4-1.6 stages). Whether acceleration in the development of these teeth might be associated with any initial sign of puberty remains a matter of future investigations.

Genetic studies confirm that the majority of the variations exist within a population made of different ethnic groups rather than between large populations [41, 42]. Accordingly, recent studies have demonstrated variations of dental maturity within a population [18, 19, 43]. The prior strength of our study is the inclusion of a large number of subjects from a multi-ethnic population-based prospective cohort design, with ascertained measurements of dental development (Figure S2.1.1). Based on the colonial and working immigration history, the largest ethnic minority groups in the Netherlands are Cape Verdean, Dutch Antillean, Moroccan, Surinamese-Creole, Surinamese-Hindustani and Turkish [22]. Both geographic and genetic transition may play an important role for the differences in dental development [1, 44]. Hence, specifying the ancestry based on geography and genetics in our study adds more

insight to the understanding of dental maturity in a population with heterogeneous ethnic background. The geographic origin distinguishes between more ethnic groups, thus more information for differences in dental development within a population. However, beside the reference group of children, the other ethnic groups were of small sample size. Furthermore, because all children were born in the Netherlands, it is difficult to accurately distinguish between the ethnic groups. We did not distinguish between the first and the second-generation migrants and did not take into account the existence of heterogeneity within ethnic groups, which may have attenuated our results. Therefore, we also used the genetic ancestry in the present study as an objective approach. A limitation counted by applying the genetic ancestry is the simple categorization of the study population in three ancestral groups, when no distinct boundaries are recognized between populations [45]. As the members of each group named as European, African or Asian are highly variable, the genetic analysis might not accurately distinguish separate genetic groups. Thus, in our main analysis we considered genetic ancestry continuously, based on European, African and Asian genetic proportion for each individual. Furthermore, we excluded from the analysis cases that didn't reach any significant proportion of the three ancestral populations. Another limitation to be counted is the small sample size of Asian children present in our study population, which might have affected the non-significant difference in developmental stages of each left mandibular tooth between European and Asian children. To decrease the heterogeneity related to the environmental component between Europeans, Africans and Asians when the study population is investigated as a whole, we further studied the influence of each genetic content of ancestry only in the European children.

A combination of several methods for determining dental development is generally recommended for a better estimation of dental age [46]. We used three different dental age standards (Dutch, French-Canadian and International Demirjian standard) in order to obtain the best approximation of dental age to chronological age. Dental ages calculated by the three standards assembled around one time point and the accordance of the three polynomial functions to the study population resulted to be low to moderate (R^2 = 0.06-0.32), consequently. Longitudinal measurements of dental development would be necessary to overcome the concern about the dental age standard that would best represent dental development of our study population. The Demirjian method to assess dental development is the most applicable method worldwide, making possible the comparisons between the findings obtained in different populations. Few studies in Europe have previously investigated ethnic differences in dental development, applying Demirjian method. Liversidge et al. (1999) reported no difference in dental development between British children of white Caucasian origin and British children of Bangladeshi origin; a non-surprising finding for the authors due to the similar physiological growth of children with these origins [18]. Few years after, Liversidge et al. (2006) reported no difference in stages of development between children coming from eight different countries [43]. In contrast, Nystrom et al. (1988) reported that northeastern Finnish children (rural area in Kuhmo) exceed southeastern Finnish children (Helsinki) in dental development, suggesting that differences in dental development within a homogeneous population should be considered when using the national charts [19]. Our findings add to the

current literature that differences in dental development need to be considered in populations with heterogeneous origin as well, when using the national charts.

All the regression models were adjusted for potential confounders such as hypodontia, BMI, height and dmft, however residual confounding can still be present. Throughout all the statistical analysis, the effect of hypodontia, BMI and height on dental development stood out due to the evident significant results ($p<0.001$). Hypodontia showed a negative effect on dental development whereas the BMI and height showed a positive effect on dental development within our population. The findings of this study were in accordance with what is already reported in the literature since hypodontia is recognized as an indicator of delayed dental development, whereas the BMI and height are recognized as indicators of advanced dental development [47-50]. In our investigation BMI and height explained at the maximum 13% of the variation in dental development between ancestral groups. The limited value of explained variance from BMI and height is addressed to the fact that dental development is predominated by genetic control with a minor role of environmental factors such as nutrition. BMI and height explain more about the physiological growth in children. Hence, whether dental development indicates ancestral differences in the general growth and development of children, needs to be further explored in the future. Lastly, also selection bias cannot be excluded because it is difficult to assess whether the associations of geographic and genetic ancestry with dental development of children were different between those included and those not included in the final analyses (Table S2.1.1).

In conclusion, based on a geographic and genetic perspective, differences in dental development exist in our population, which is heterogeneous with regard to the ancestral background. The approach of this study is appropriate for orthodontists to detect whether dental development of a child happens "faster" or "slower" at a fixed age in comparison with children of the same age but of a different ethnicity.

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SUPPLEMENT

Table S2.1.1. General characteristics of the non-participants in the follow-up measurements of dental development

Available information	Geographic ancestry			Genetic ancestry		
	Participation (N = 3600)	No-Participation (N = 1015)	p-value	Participation (N = 2786)	No-Participation (N = 824)	p-value
Age	9.81 (0.35)	9.76 (0.46)	*<0.001*	9.82 (0.34)	9.77 (0.48)	*0.001*
Sex			0.104			0.098
Boys	1810 (50.3)	487 (48.0)		1387 (49.8)	388 (47.1)	
Girls	1790 (49.7)	528 (52.0)		1399 (50.2)	435 (52.8)	
Missing	-	-	-	-	1 (0.0)	
Maternal age	31.04 (4.87)	30.70 (5.09)	0.054	30.91 (4.81)	30.95 (4.88)	0.839
Missing					1(0.0)	
Height	141.77 (6.62)	141.19 (6.42)	*0.018*	141.87 (6.75)	141.53 (6.22)	0.222
Missing					107 (13.0)	
Weight	35.51 (7.36)	35.09 (7.04)	0.125	35.47 (7.17)	35.25 (6.97)	0.469
Missing					106 (12.9)	
BMI	17.56 (2.76)	17.52 (2.77)	0.670	17.52 (2.66)	17.53 (2.78)	0.961
Missing					107 (13.0)	
dmft	0.0 (0.0-6.0)	0.0 (0.0-6.0)	*<0.001*	0.0 (0.0-7.0)	0.0 (0.0-7.1)	*<0.001*
Missing	878 (24.4)	276 (27.2)		650 (23.3)	227 (27.5)	

Abbreviations: N – number of participants, dmft – decayed-missing-filled teeth Index; Values are numbers (%) for categorical variables, means (SD) for continuous variables with a normal distribution, or medians (95% range) for continuous variables with a skewed distribution; Differences were tested using independent t-test for continuous variables, chi-squared test for categorical variables and Kruskal-Wallis Non-Parametric test for variables with a skewed distribution; using the participation group as the reference; Significant p-values are presented in italic font

Figure S2.1.1. Flowchart of the participants included in the study