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The association between *WNT10A* variants and dental development in patients with isolated oligodontia

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ABSTRACT

Objective: In this study we aimed to determine the effect of *WNT10A* variants on dental development in patients with oligodontia.
Methods: Forty-three (25 boys and 18 girls) individuals were eligible for this study. Stage of development for each present tooth was assessed using the Demirjian method. In case no corresponding tooth was present, regression equations were applied for dental age to be calculated. The ratio between length of root and length of crown was ascertained for each present tooth in all quadrants. All patients were physically examined by a clinical geneticist and DNA analysis of the *WNT10A* gene was performed. Linear regression models were applied to analyze the association between *WNT10A* variants and dental age. The same analysis was applied to study the association between *WNT10A* variants and root elongation for each present tooth. One ordinal regression model was applied to analyze the association between WNT10A variants and development of present maxillary and mandibular teeth.
Results: Thirty-six (84%) patients were detected with *WNT10A* variants of which six patients displayed evident ectodermal features. Dental age was 1.50 (95% CI: -2.59, -0.42) to 1.96 (95% CI: -3.76, -0.17) years lower in patients with *WNT10A* variants compared with patients without variants. The development of maxillary canine, maxillary second molar and mandibular second molar was statistically significantly delayed in patients with *WNT10A* variants compared with patients without variants.
Conclusion: The impact of *WNT10A* variants on dental development increases with presence of the nonsense c. (321C4A p.(C107*)) variant and the number of missing teeth.

4.1.1 INTRODUCTION

Dental agenesis is defined as the congenital absence of one or more primary or secondary teeth, excluding the third molars [1,2]. As a frequent dental anomaly [3], dental agenesis in the permanent dentition is studied for its prevalence in different populations [4-6]. The prevalence of dental agenesis in the Dutch population is estimated to be 5%, similar to the prevalence in Europe [4,7]. Dental agenesis can be classified as hypodontia (1-5 missing teeth), oligodontia (≥6 missing teeth) or anodontia (no tooth present) [2, 8,9]. Oligodontia is observed in approximately 0.14% of the population 10 and specifically for the Dutch population the prevalence of isolated oligodontia is reported to be 0.08% [11]. The numeric distinction between hypodontia, oligodontia and anodontia seems an arbitrary one, since the location of missing teeth is important as well [12]. Due to the lack of teeth, oligodontia has an impact on quality of life [13]. Furthermore, dental agenesis is associated with a delay of dental development, abnormal size and abnormal shape of teeth [14-19]. Dental agenesis is a hereditary condition with both environmental and genetic factors as part of etiology [10,20]. Genetic evidence of dental agenesis has been previously presented [21-26]. *MSX1*, *PAX9*, *AXIN2*, *EDA*, *EDAR*, *EDARADD*, *LRP6* and *WNT10A* variants in mice are associated with dental agenesis and an aberrant development of the dentition [27-36]. Recent findings associate compound heterozygosity/homozygosity or heterozygosity for *WNT10A* variants with isolated dental agenesis in humans [7,37]. In addition, *WNT10A* variants are associated with various ectodermal dysplasias (EDs; OMIM, Entry 305100) often corresponding to the odontoonychodermal dysplasia (OODD; OMIM, Entry 257980) and Schöpf–Schulz–Passarge syndrome (SSPS; OMIM, Entry 224750), which combine abnormal development of ectodermal structures including teeth [38-41]. Van den Boogaard et al. showed that variants of *WNT10A* were present in more than half cases of isolated oligodontia vs less than 10% variants of *MSX1*, *PAX9*, *AXIN2*, concluding that the normal expression of the *WNT10A* gene is important for the formation of the tooth germ [7]. However less information is available on the association between *WNT10A* variants and dental development. Since *WNT10A* is strongly expressed in the dental epithelium at the initiation stage and plays a role in tooth development beyond the bud stage, one may hypothesize that delayed dental development is part of the phenotype *WNT10A*-dental agenesis [32,33]. More insight in genotype-phenotype associations facilitates the recognition of a possible genetic etiology in patients with dental agenesis, which may have consequences for the treatment approach. The aim of this study was to determine the effect of *WNT10A* variants on dental development in patients with oligodontia.

4.1.2 MATERIALS AND METHODS

4.1.2.1 Study population

Individuals with oligodontia visiting the Departments of Oral and Maxillofacial Surgery, Prosthodontics and Special Dental Care of the University Medical Center Utrecht (UMC Utrecht) and the St. Antonius Hospital, Nieuwegein, The Netherlands were referred to the

Department of Medical Genetics of the UMC Utrecht for syndrome diagnostics and genetic counseling. The data of this study including variants and phenotypes was submitted into LOVD funded database (http://databases.lovd.nl/shared/genes/WNT10A). Informed consents were obtained from the patients or parents. Forty-three (25 boys and 18 girls) patients with a mean age of 10.77 (SD 2.4) years were eligible for this study according to the following inclusion criteria: oligodontia (6 or more missing teeth, excluding third molars), age between 6 to 16 years old and presence of a dental panoramic radiograph (DPR). Mean age of the boys was 11.05 (SD 2.59) years and mean age of the girls was 10.38 years (SD 2.16).

4.1.2.2 Oligodontia assessment

Oligodontia was assessed by clinical examination from the dentist and also by DPRs. A tooth was classified as missing when no sign of formation or calcification showed in the DPR. Patterns of oligodontia were identified using the tooth agenesis code (TAC). The TAC is a unique number that is consistent with a specific pattern of dental agenesis [42,43].

4.1.2.3 Dental development assessment

Dental development was defined using the Demirjian method [44]. One experienced examiner (B.D) determined one of the eight developmental stages (A, B, C, D, E, F, G and H) for each present tooth in all quadrants.

Dental age was calculated for each patient referring to the stages of development of teeth in the left quadrant as follows. In order to estimate the stage of development for the missing teeth, a combined method was applied. This method consists of assessing the stage of development for a missing tooth in the lower left quadrant from the corresponding right mandibular tooth or from a corresponding maxillary tooth if the tooth was missing in both sides of the mandible. In case no corresponding tooth was present, regression equations developed by Nystrom et al. [45] were applied. These equations take into account the development of the remaining teeth in the lower left quadrant, age and sex of the patient to calculate dental age. Obtained stages of dental development were used to calculate the dental maturity score by summing up the weighted scores given to every tooth of the lower left quadrant [46]. Finally, the Dutch dental age standard tables for boys and girls were used to convert the dental maturity score into dental age [46].

To obtain a better approach of dental development in our patients, additional measurements were performed using DPRs. In order to estimate the root elongation, the examiner ascertained the ratio between length of root and length of crown for each present tooth in all quadrants. Abnormal shape or size of teeth was signed when detected.

4.1.2.4 Physical examination

All patients were physically examined by a single clinical geneticist, in order to identify possible features of ED (skin, hair, nails, sweat glands) or other syndromes. In addition, deviations in function of sweat glands, skin, hair and nails were assessed using a standardized form. The patients were classified as displaying evident ectodermal features or not.

4.1.2.5 Detection and analysis of *WNT10A* variants

Blood samples were obtained and DNA analysis of the *WNT10A* and the *MSX1, PAX9*, *AXIN2* genes were performed essentially as described in a previous study [7]. High molecular weight genomic DNA was extracted from blood samples using standard procedures. PCR amplification of all exons of *WNT10A* and their splice site consensus sequences was applied. Mutation analysis was performed using the genetic analysis software Sequence Pilot V. 3.4.4 (JSI Medical Systems GmbH, Kippenheim, Germany), and mutation interpretation software Alamut (Interactive Biosoftware, Rouen, France) was used for further interpretation. Nomenclature is according HGVS guidelines. In case ectodermal dysplasia was suspected, additional DNA analysis was performed for *ED1*, *EDAR* and *EDARADD*. To evaluate the effect of the most frequent nonsense variant p. (C107*) (without gene product) and missense p. (F228I) variant, the *WNT10A* variants were sub classified as without any variant, heterozygous/homozygous p. (F228I) missense variant, heterozygous/homozygous miscellaneous variant or heterozygous/homozygous nonsense variant p. (C107*). The detailed description of the *WNT10A* variants is presented using the accession number NM_025216.2 of the reference sequence in supplementary Table S4.1.2.

4.1.2.6 Statistical analysis

The association between *WNT10A* and dental age in patients with isolated oligodontia was studied using two linear regression analysis. The first linear regression analysis tested the association between presence of any *WNT10A* variant and dental age. The second linear regression analysis tested the association between the presence of a nonsense *WNT10A* variant and dental age. Both linear regression analysis were performed in two consecutive models. The first model was adjusted for age and sex. The second model was additionally adjusted for number of missing teeth, abnormal size or shape, number of filled teeth and presence of ectodermal features.

The association between *WNT10A* and root elongation (length of root/ length of crown ratio) of each present tooth in dentition was analyzed using two linear regression models. The first model was adjusted for age and sex. The second model was additionally adjusted for the number of missing teeth, abnormal size or shape, number of filled teeth and presence of ectodermal features.

The association between *WNT10A* and development of the present maxillary and mandibular teeth was analyzed using one ordinal regression model adjusted for age, sex, number of missing teeth, abnormal size or shape, number of filled teeth and presence of ectodermal features.

Interaction terms between sex, number of missing teeth, abnormal shape or size, presence of evident ectodermal features and *WNT10A* in relation to dental development were tested. Since no significant interaction terms were found, no stratification for these interaction terms was applied during analysis. All statistical analysis were performed using statistical software Statistical Package for Social Sciences version 21.0 (SPSS Inc. Chicago, IL, USA).

4.1.3 RESULTS

Thirty-six patients (84%) were detected with *WNT10A* variants (Table 4.1.1). Of these, 11 had a p.(F228I) missense variant (one patient showed ectodermal features), 10 had a miscellaneous variant (one patient showed ectodermal features), and 15 had a nonsense variant (four patients showed ectodermal features). Additional DNA analysis of the patients with WNT10A variants, revealed no variants in the *MSX1*, *PAX9*, *AXIN2* or *ED1*, *EDAR* and *EDARADD* genes. Of the 7 patients without *WNT10A* variants only one showed evident ectodermal features. Additional DNA analysis of the patients without *WNT10A* variants, revealed a *PAX9* variant for one patient. There was no age and gender difference between the group of patients with *WNT10A* variants and the group without *WNT10A* variants. There was no difference for abnormal tooth size and shape, number of missing teeth or presence of evident ectodermal features between both groups.

Table 4.1.1. The descriptive characteristics of patients included in the study (N = 43)

	Without *WNT10A* variants (N = 7)	**With *WNT10A* variants** (N = 36)	**p-value**
Age (mean, SD)	11.27 (1.81)	10.67 (2.53)	0.55
Dental Age (mean, SD)	10.26 (1.87)	7.63 (2.57)	0.01*
Sex (N, %)			0.10
Girls	5 (11.6%)	13(30.2%)	
Boys	2 (4.6%)	23 (53.5%)	
Number of missing teeth (median, 70% range)	9 (6;19)	13 (6;22)	0.35
Abnormal size or shape (N, %)			0.54
Yes	1 (2.3%)	8 (18.6%)	
No	6 (14.0%)	28 (65.1%)	
Filled Teeth (mean, SD)	0.29 (0.76)	0.56 (0.99)	0.50
Ectodermal Features (N, %)			0.69
Yes	1 (2.3%)	6 (14.0%)	
No	5 (11.6%)	30 (69.8%)	

Abbreviations: N- number of participants, SD- standard deviation. Differences were tested using independent t-test for continuous variables and chi-squared test for categorical variables. Significant values: * p<0.05, ** p<0.01. Dental age was calculated if both matching mandibular teeth were missing by scoring them as a developmental stage of the left or right matching maxillary tooth. If corresponding maxillary teeth were missing the developmental stage was calculated from regression equations developed from Nyström et al. (2000)

4.1.3.1 Patterns of oligodontia

The percentages of missing teeth per tooth type were similar to those from other studies [4,43]. The lower second premolar (80.3%), the upper lateral incisor (64.0%), the lower central incisor (64.0%) and the lower second molar (64%) were most frequently absent. The upper central incisor was present in all the patients. The upper first molar (10.5%) and the lower first molar (9.3%) were less frequently absent. Patients with *WNT10A* variants showed a higher number of missing teeth. Furthermore, the number of missing teeth increased with the presence of a

nonsense *WNT10A* variant (p 0.001). The tooth agenesis codes were calculated per quadrant (Table S4.1.1). The most severe oligodontia patterns were more prevalent in boys while the mildest patterns were more prevalent in girls. The most frequent oligodontia patterns were more prevalent in patients with *WNT10A* variants than in patients without *WNT10A* variants.

4.1.3.2 *WNT10A* variants and dental development

WNT10A variants and dental age: First linear regression analysis revealed a statistically significant lower dental age of 1.50-1.96 years for patients with *WNT10A* variants compared to patients without *WNT10A* variants (Table 4.1.2). Second linear regression analysis revealed that dental age decreased statistically significantly 0.68-1.03 years with the presence of a nonsense *WNT10A* variant.

Table 4.1.2. The association between *WNT10A* variants and dental age

	Model 1			Model 2		
1.	**β**	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**
***WNT10A* variants** (without WNT10A variants; ref.)	-1.96	-3.76, -0.17	0.03*	-1.50	-2.59, -0.42	0.01*
Age	0.61	0.34, 0.89	0.00	0.65	0.49, 0.82	<0.01**
Sex (Females; ref.)	0.85	-0.51, 2.20	0.21	0.55	-0.24, 1.34	0.16
Number of missing teeth				-0.30	-0.37, -0.23	<0.01**
Abnormal size or shape (Yes; ref.)				1.15	0.18, 2.13	0.02*
Filled teeth				-0.55	-0.95, -0.14	0.01*
Ectodermal features (Yes; ref)				-0.01	-1.03, 1.00	0.98
2.	**β**	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**
Presence of nonsense *WNT10A* variant (without WNT10A variants; ref)	-1.03	-2.01, -0.06	0.04*	-0.68	-1.30, -0.06	0.03*
Age	0.56	0.28, 0.84	0.00**	0.64	0.47, 0.82	<0.01**
Sex (Females; ref.)	0.97	-0.36, 2.30	0.15	0.64	-0.17, 1.45	0.12
Number of missing teeth				-0.29	-0.36, -0.21	<0.01**
Abnormal size or shape (Yes; ref.)				1.23	0.22, 2.24	0.02*
Filled teeth				-0.65	-1.08, -0.22	<0.01**
Ectodermal features (Yes; ref.)				0.16	-0.90, 1.22	0.77

Abbreviations: β –regression coefficients, CI – confidence interval, ref.-reference; Significant values: * p<0.05, ** p<0.01
Dental age was calculated if both matching mandibular teeth were missing by scoring them: a as a developmental stage calculated from regression equations developed by Nyström et al. (2000); b as a developmental stage of the (left) matching maxillary tooth.
Model 1: the association between *WNT10A* variants and dental age is adjusted for age and sex
Model 2: was additionally adjusted for number of missing teeth, abnormal size or shape, number of filled teeth and presence of ectodermal features
Presence of nonsense *WNT10A* variants was classified as 0-without *WNT10A* variants; 1- missense and miscellaneous *WNT10A* variants; 2- nonsense *WNT10A* variants

4.1.3.3 *WNT10A* variants and root elongation (length of root/length of crown ratio)

Model 1 in the linear regression analysis showed that the roots of the left mandibular second molar were statistically significantly shorter (β = -0.72; 95% CI: -1.23, -0.21) in patients with

WNT10A variants compared to patients without *WNT10A* variants (Table 4.1.3). The effect estimates obtained from Model 1 for all the other present teeth in the dentition were not statistically significant. Model 2 in the linear regression analysis showed that the root of the left maxillary canine was statistically significantly shorter (β = -0.99; 95% CI: -1.89, -0.08) in patients with *WNT10A* variants compared to patients without *WNT10A* variants. The effect estimates obtained from the fully adjusted model (Model 2) for all the other present teeth in the dentition were not statistically significant.

Table 4.1.3. The association between *WNT10A* variants and root elongation (length of root/ length of crown ratio) for each present tooth

	Model 1			Model 2		
Maxilla	**β**	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**
Right Central Incisor	-0.21	-0.59, 0.17	0.27	-0.16	-0.56, 0.25	0.44
Right Lateral Incisor	-0.40	-0.97, 0.18	0.16	-0.35	-1.16, 0.46	0.34
Right Canine	-0.47	-1.06, 0.12	0.11	-0.54	-1.21, 0.14	0.11
Right First Premolar	-0.15	-1.06, 0.77	0.74	-0.41	-1.38, 0.56	0.37
Right Second Premolar	0.36	-1.93, 2.65	0.73	-0.20	-2.03, 1.62	0.79
Right First Molar	-0.35	-0.83, 0.13	0.15	-0.32	-0.80, 0.15	0.18
Right Second Molar	-0.15	-0.50, 0.20	0.37	-0.18	-0.61, 0.25	0.36
Left Central incisor	0.03	-0.46, 0.53	0.89	0.12	-0.38, 0.63	0.62
Left Lateral Incisor	-0.24	-0.59, 0.12	0.17	-0.20	-0.70, 0.29	0.37
Left Canine	-0.68	-1.70, 0.34	0.18	-0.99	-1.89, -0.08	0.04*
Left First Premolar	-0.26	-1.20, 0.68	0.56	-0.42	-1.28, 0.43	0.29
Left Second Premolar	0.11	-1.60, 1.82	0.88	-0.14	-1.77, 1.50	0.83
Left First Molar	-0.45	-0.96, 0.07	0.09	-0.39	-0.89, 0.11	0.12
Left Second Molar	-0.30	-0.78, 0.18	0.20	-0.33	-0.79, 0.13	0.14
Mandible	**β**	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**
Right Central Incisor	-0.17	-0.87, 0.54	0.61	-0.10	-0.85, 0.65	0.75
Right Lateral Incisor	-0.14	-0.63, 0.35	0.57	-0.20	-0.76, 0.35	0.45
Right Canine	-0.07	-0.85, 0.71	0.85	0.08	-0.64, 0.80	0.81
Right First Premolar	0.15	-0.38, 0.69	0.56	0.05	-0.63, 0.75	0.87
Right Second Premolar	0.82	-0.80, 2.43	0.23	1.02	1.02, 1.02	---
Right First Molar	-0.08	-0.39, 0.23	0.61	-0.07	-0.38, 0.24	0.66
Right Second Molar	-0.36	-1.25, 0.52	0.38	-0.80	-2.79, 1.19	0.36
Left Central incisor	-0.27	-0.83, 0.29	0.31	-0.49	-1.17, 0.20	0.14
Left Lateral Incisor	-0.05	-0.73, 0.64	0.89	-0.11	-0.95, 0.73	0.79
Left Canine	-0.53	-1.48, 0.43	0.26	-0.82	-1.91, 0.28	0.13
Left First Premolar	0.22	-0.58, 1.02	0.57	-0.04	-0.77, 0.68	0.90
Left Second Premolar	-0.02	-0.71, 0.67	0.94	-0.05	-2.08, 1.97	0.80
Left First Molar	-0.07	-0.19, 0.06	0.29	-0.06	-0.18, 0.06	0.32
Left Second Molar	*-0.72*	*-1.23, -0.21*	*0.01**	-0.71	-1.57, 0.16	0.10

Abbreviations: β –regression coefficients, CI – confidence interval, p-values presented from independent t-test for continuous variables and Chi-squared test for categorical variables; Significant values: * p<0.05, ** p<0.01

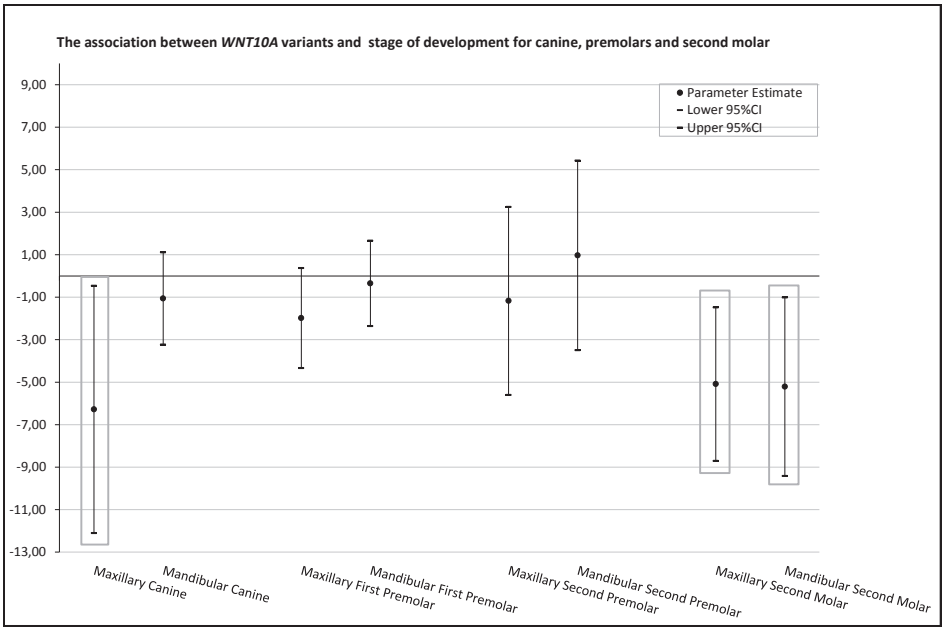
Model 1: the association between *WNT10A* variants and root elongation is adjusted for age and sex

Model 2: was additionally adjusted for number of missing teeth, abnormal size or shape, number of filled teeth and presence of ectodermal features

4.1.3.4 *WNT10A* variants and development of present teeth

Ordinal regression analysis revealed a statistically significant delay of 6.28 (95% CI; -7.58, -1.05) stages of development for the maxillary canine, 5.09 (95% CI; -8.70, -1.48) stages of development for the maxillary second molar and 5.21 (95% CI; -9.41, -1.48) stages of development for the mandibular second molar (Figure 4.1.1). No statistically significant result was obtained for the mandibular canine, maxillary first premolar, mandibular first premolar, maxillary second premolar and mandibular second premolar. The ordinal regression analysis was performed as well for central incisors, lateral incisors and first molars in both jaws but as these teeth were in the final stage of their development and the sample size was small, the values fell in one category and presented uninterpretable parameter estimates.

Figure 4.1.1. Box-plot from ordinal regression analysis



Abbreviations: The ordinal regression model was fully adjusted for age, sex, number of missing teeth, abnormal size or shape, number of filled teeth and presence of ectodermal features; the statistically significant parameter estimates are presented inside the grey squares

4.1.4 DISCUSSION

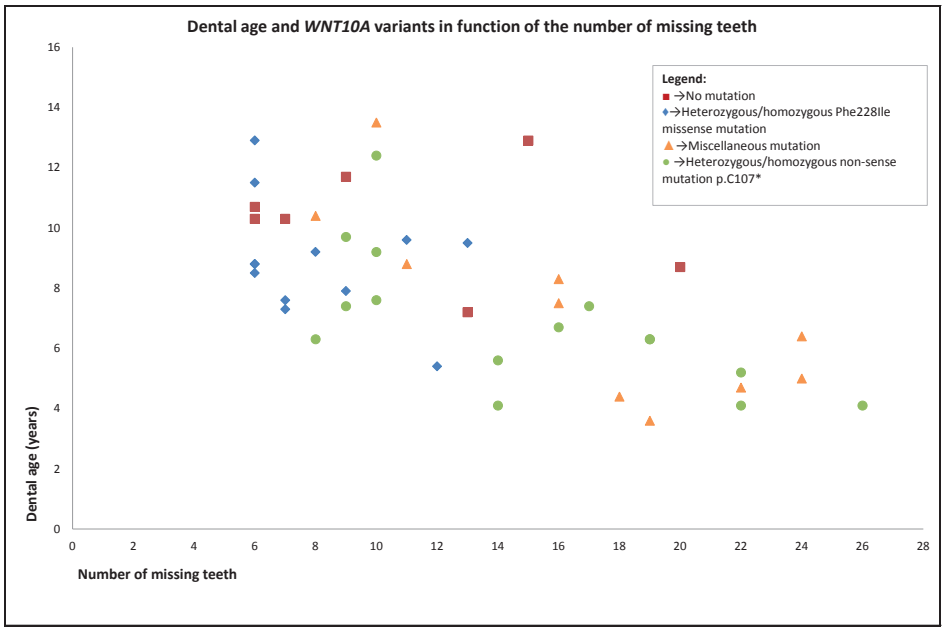
While knowledge on the genetic cause of dental agenesis becomes available, a classification of patients can be made based on genetic characteristics. Before, patients with dental agenesis could only be classified by the number of missing teeth or as a feature of a syndrome [11,47]. With information on the genotype of patients with dental agenesis, information on the phenotype facilitates an individualized patient-centered treatment approach for patients with oligodon-

tia. One of the clinical characteristics can be a delayed dental development. Previous studies on dental agenesis and delayed dental development did not include genetic analysis [15-18]. Possibly, the delayed dental development is related to the genetic etiology of the dental agenesis and caused by *WNT10A*. The population in the present study was relatively small. Oligodontia however, can be considered a rare congenital dental anomaly. Furthermore, *WNT10A* was only recently identified as a major gene in the etiology of dental agenesis [7,48]. To our knowledge a comparable group of patients with information on the dental and genetic characteristics is not available.

The aim of this study was to determine the effect of *WNT10A* variants on dental development in patients with oligodontia. Assessment of dental development and determination of dental age in patients with oligodontia are impaired by the estimation of developmental stages for missing teeth, particularly in the case of oligodontia with a higher number of missing teeth. A specific method for assessing dental age in patients with oligodontia is not available, which may have prevented comparable investigations. Dental age was assessed in patients with cerebral palsy and Down syndrome, a risk group for oligodontia [49] by applying two methods, the one described by Nolla (1960) [50] and the other one described by Demirjian (1973) [44]. The limitation of the method described by Nolla is the impossibility of applying this method in its original form because it tends to underestimate dental age [51,52]. The limitation of the method described by Demirjian is the trend to overestimate dental age due to the acceleration of dental development in the last decades [53,54]. To address the limitations of the existing methods, a combined method was applied in the present study with regression equations for missing teeth in case no corresponding mandibular or maxillary tooth was available [45]. An alternative could have been to skip the estimations from corresponding maxillary teeth and to apply regression equations directly in case a tooth was missing in both sides of the mandible, since the eruption of mandibular teeth precedes maxillary teeth by one year at most. This would be expressed in less than one stage of dental development and not necessarily contribute to a change of dental age. Since the equations are based on a general population, however, it can be questioned whether these can be inferred to patients with oligodontia. For this reason, information from the same patient was preferred for this study.

In general, the complete development of a tooth takes at least 10 years. The findings of this study suggest approximately 3 years delay in dental development for patients with oligodontia and *WNT10A* variants. A previous study revealed an association between a decreased expression of *WNT10A* and inhibition of dentin apposition and root elongation [30]. Furthermore, *WNT10A* defects can lead to molar crown and root dysmorphologies [31]. The present study showed that the left maxillary canine and the left mandibular second molar had statistically significant shorter roots in patients with *WNT10A* variants compared to patients without *WNT10A* variants. This result was confirmed by the ordinal regression analysis, where development of the maxillary canine and second molars was 5-6 stages delayed in patients with *WNT10A* variants compared to patients without *WNT10A* variants. Since in the literature, *WNT10A* variants are associated with both the type and number of missing teeth [36], we expected significant effect of *WNT10A* variants on development of more teeth. *WNT10A* variants

Figure 4.1.2. Schematic presentation of the association between *WNT10A* variants and dental age in relation to the number of missing teeth



in this study were found in cases with more delayed dental development and more missing teeth (Figure 4.1.2). Another recent finding indicates that the association with *WNT10A* is stronger with an increasing number of missing teeth and the presence of the nonsense variant p.(C107*) [48]. These arguments highlight that *WNT10A* is not only involved in tooth germ formation but plays a role in the subsequent stages of tooth development as well. Further investigations on the effect of other genes are necessary for a better understanding of the relation between oligodontia and delayed dental development.

REFERENCES

1. Silva Meza R: Radiographic assessment of congenitally missing teeth in orthodontic patients. Int J Paediatr Dent 2003; 13: 112-116.
2. Goodman JR, Jones SP, Hobkirk JA, King PA: Hypodonta 1: Clinical features and the management of mild to moderate hypodontia. Dental Update 1994; 21: 381-384.
3. Kotecha S, Turner PJ, Dietrich T, Dhopatkar A: The impact of tooth agenesis on oral health-related quality of life in children. J Orthod 2013; 40: 122-129.
4. Polder BJ, Van't Hof MA, Van der Linden FP, Kuijpers-Jagtman AM: A meta-analysis of the prevalence of dental agenesis of permanent teeth. Community Dent Oral Epidemiol 2004; 32: 217-226.
5. Shimizu T, Maeda T: Prevalence and genetic basis of tooth agenesis. Jpn Dent Sci Rev 2009; 45: 52-58.
6. Khalaf K, Miskelly J, Voge E, Macfarlane TV: Prevalence of hypodontia and associated factors: a systematic review and meta-analysis. J Orthod 2014; 41: 299-316.
7. van den Boogaard M-J, Créton M, Bronkhorst Y et al.: Mutations in WNT10A are present in more than half of isolated hypodontia cases. J Med Genet 2012; 49: 327-331.
8. Schalk-van der Weide Y: Oligodontia: a clinical, radiographic and genetic evaluation. Universiteit Utrecht, faculteit Geneeskunde, 1992.
9. Rushmah M: Hypodontia of the primary and permanent dentition. J Clin Pediatr Dent 1992; 16: 121-123.
10. Nieminen P: Genetic basis of tooth agenesis. J Exp Zool B Mol Dev Evol 2009; 312: 320-342.
11. Weide YSV, Beemer FA, Faber JAJ, Bosman F: Symptomatology of patients with oligodontia. J Oral Rehabil 1994; 21: 247-261.
12. Creton M, Cune M, Verhoeven W, Muradin M, Wismeijer D, Meijer G: Implant treatment in patients with severe hypodontia: a retrospective evaluation. J Oral Maxillofac Surg 2010; 68: 530-538.
13. Wong AT, McMillan AS, McGrath C: Oral health-related quality of life and severe hypodontia. J Oral Rehabil 2006; 33: 869-873.
14. Schalk-van der Weide Y, Steen WH, Bosman F: Taurodontism and length of teeth in patients with oligodontia. J Oral Rehabil 1993; 20: 401-412.
15. Tunc ES, Bayrak S, Koyuturk AE: Dental development in children with mild-to-moderate hypodontia. Am J Orthod Dentofacial Orthop 2011; 139: 334-338.
16. Uslenghi S, Liversidge HM, Wong FS: A radiographic study of tooth development in hypodontia. Arch Oral Biol 2006; 51: 129-133.
17. Ruiz-Mealin EV, Parekh S, Jones SP, Moles DR, Gill DS: Radiographic study of delayed tooth development in patients with dental agenesis. Am J Orthod Dentofac Orthoped 2012; 141: 307-314.
18. Ben-Bassat Y, Babadzhanov D, Brin I, Hazan-Molina H, Aizenbud D: Maturation of teeth adjacent to dental agenesis site. Acta Odontol Scand 2014: 1-7.
19. Brook AH, Elcock C, al-Sharood MH, McKeown HF, Khalaf K, Smith RN: Further studies of a model for the etiology of anomalies of tooth number and size in humans. Connect Tissue Res 2002; 43: 289-295.
20. Kennedy DJ: Partial anodontia: A brief review and a case report of multiple familial incidence. Oral Surg Oral Med Oral Pathol 1950; 3: 63-73.
21. Vastardis H: The genetics of human tooth agenesis: new discoveries for understanding dental anomalies. Am J Orthod Dentofac Orthoped 2000; 117: 650-656.
22. Kolenc-Fusé FJ: Tooth agenesis: in search of mutations behind failed dental development. Oral Surg Oral Med Oral Pathol 2003; 9: 390-395; 385-390.
23. Caton J, Tucker AS: Current knowledge of tooth development: patterning and mineralization of the murine dentition. J Anat 2009; 214: 502-515.
24. Cobourne MT, Sharpe PT: Making up the numbers: The molecular control of mammalian dental formula. Semin Cell Dev Biol 2010; 21: 314-324.
25. De Coster PJ, Marks LA, Martens LC, Huysseune A: Dental agenesis: genetic and clinical perspectives. J Oral Pathol Med 2009; 38: 1-17.
26. Mitsiadis TA, Smith MM: How do genes make teeth to order through development? J Exp Zool B Mol Dev Evol 2006; 306: 177-182.
27. Dhanrajani PJ: Hypodontia: etiology, clinical features, and management. Quintessence Int 2002; 33: 294-302.
28. Matalova E, Fleischmannova J, Sharpe PT, Tucker AS: Tooth agenesis: from molecular genetics to molecular dentistry. J Dent Res 2008; 87: 617-623.

29. Cobourne MT: Familial human hypodontia--is it all in the genes? Br Dent J 2007; 203: 203-208.
30. Bae CH, Kim TH, Ko SO, Lee JC, Yang X, Cho ES: Wntless regulates dentin apposition and root elongation in the mandibular molar. J Dent Res 2015: 0022034514567198.
31. Yang J, Wang SK, Choi M et al.: Taurodontism, variations in tooth number, and misshapened crowns in Wnt10a null mice and human kindreds. Mol Genet Genomic Med 2015; 3: 40-58.
32. Chen J, Lan Y, Baek JA, Gao Y, Jiang R: Wnt/beta-catenin signaling plays an essential role in activation of odontogenic mesenchyme during early tooth development. Dev Biol 2009; 334: 174-185.
33. Fujimori S, Novak H, Weissenbock M et al.: Wnt/beta-catenin signaling in the dental mesenchyme regulates incisor development by regulating Bmp4. Dev Biol 2010; 348: 97-106.
34. Massink MP, Creton MA, Spanevello F et al.: Loss-of-Function Mutations in the WNT Co-receptor LRP6 Cause Autosomal-Dominant Oligodontia. Am J Hum Genet 2015; 97: 621-626.
35. Arte S, Parmanen S, Pirinen S, Alaluusua S, Nieminen P: Candidate gene analysis of tooth agenesis identifies novel mutations in six genes and suggests significant role for WNT and EDA signaling and allele combinations. PLoS One 2013; 8: e73705.
36. Arzoo PS, Klar J, Bergendal B, Norderyd J, Dahl N: WNT10A mutations account for (1/4) of population-based isolated oligodontia and show phenotypic correlations. Am J Med Genet A 2014; 164A: 353-359.
37. Kantaputra P, Sripathomsawat W: WNT10A and isolated hypodontia. Am J Med Genet A 2011; 155: 1119-1122.
38. Adaimy L, Chouery E, Mégarbané H et al.: Mutation in WNT10A is associated with an autosomal recessive ectodermal dysplasia: the odonto-onycho-dermal dysplasia. Am J Hum Genet 2007; 81: 821-828.
39. Nawaz S, Klar J, Wajid M et al.: WNT10A missense mutation associated with a complete odonto-onycho-dermal dysplasia syndrome. Eur J Hum Genet 2009; 17: 1600-1605.
40. Bohring A, Stamm T, Spaich C et al.: WNT10A mutations are a frequent cause of a broad spectrum of ectodermal dysplasias with sex-biased manifestation pattern in heterozygotes. Am J Hum Genet 2009; 85: 97-105.
41. Cluzeau C, Hadj-Rabia S, Jambou M et al.: Only four genes (EDA1, EDAR, EDARADD, and WNT10A) account for 90% of hypohidrotic/anhidrotic ectodermal dysplasia cases. Hum Mutat 2011; 32: 70-72.
42. Van Wijk AJ, Tan SPK: A numeric code for identifying patterns of human tooth agenesis: a new approach. Eur J Oral Sci 2006; 114: 97-101.
43. Creton MA, Cune MS, Verhoeven W, Meijer GJ: Patterns of missing teeth in a population of oligodontia patients. Int J Prosthodont 2007; 20: 409-413.
44. Demirjian A, Goldstein H, Tanner JM: A new system of dental age assessment. Hum Biol 1973: 211-227.
45. Nyström M, Aine L, Peck L, Haavikko K, Kataja M: Dental maturity in Finns and the problem of missing teeth. Acta Odontol 2000; 58: 49-56.
46. Leurs IH, Wattel E, Aartman IHA, Etty E, Prahl-Andersen B: Dental age in Dutch children. Eur J Orthod 2005; 27: 309-314.
47. Nordgarden H, Jensen JL, Storhaug K: Oligodontia is associated with extra-oral ectodermal symptoms and low whole salivary flow rates. Oral Dis 2001; 7: 226-232.
48. Song S, Zhao R, He H, Zhang J, Feng H, Lin L: WNT10A variants are associated with non-syndromic tooth agenesis in the general population. Hum Genet 2014; 133: 117-124.
49. Russell BG, Kjær I: Tooth agenesis in Down syndrome. Am J Med Genet 1995; 55: 466-471.
50. Nolla CM: The development of the permanent teeth. J Dent Child 1960; 27: 254-266.
51. Briffa K, Busuttil Dougall N, Galea J, Mifsud D, Camilleri S: Chronologic and dental ages of Maltese schoolchildren: a pilot study 2005.
52. Kumaresan R, Cugati N, Chandrasekaran B, Karthikeyan P: Reliability and validity of five radiographic dental-age estimation methods in a population of Malaysian children. J Investig Clin Dent 2014.
53. Nadler GL: Earlier dental maturation: fact or fiction? Angle Orthod 1998; 68: 535-538.
54. Holtgrave EA, Kretschmer R, Müller R: Acceleration in dental development: fact or fiction. Eur J Orthod 1997; 19: 703-710.

SUPPLEMENT

Table S4.1.1. The most frequent patterns of oligodontia in the study group

	TAC	Missing Teeth (FDI)	Illustration	Percentage (%)	RR (boys vs girls)	RR (Without vs with *WNT10A* variants)
Upper Right	18	12, 15		7.0	1.44	0 vs 3*
	22	12, 13, 15		7.0	3 vs 0	2.57
	30	12, 13, 14, 15		7.0	0.36	0 vs 3*
	64	17		7.0	1.44	0 vs 3*
	90	12, 14, 15, 17		7.0	0.36	2.57
	94	12, 13, 14, 15, 17		7.0	1.44	2.57
Upper Left	30	22, 23, 24, 25		11.6	2.88	0 vs 5*
	94	22, 23, 24, 25, 27		9.3	2.16	1.71
Lower Left	16	35		11.6	0.48	0 vs 5*
	83	31, 32, 35, 37		9.3	0.72	0 vs 4*
	95	31, 32, 33, 34, 35, 37		11.6	1.08	0 vs 5*
Lower Right	16	45		14.0	0.14	1.03
	95	41, 42, 43, 44, 45, 47		16.3	1.80	0 vs 7*
Maxilla	128	17, 27		7.0	1.44	0 vs 3*
	188	17, 15, 14, 13, 12, 22, 23, 24, 25, 27		7.0	1.44	2.57
Mandible	32	35, 45		9.3	0.24	0 vs 4*
	190	37, 35, 34, 33, 32, 31, 41, 42, 43, 44, 45, 47		11.6	1.08	0 vs 5*

Table S4.1.1. The most frequent patterns of oligodontia in the study group (*continued*)

	TAC	Missing Teeth (FDI)	Illustration	Percentage (%)	RR (boys vs girls)	RR (Without vs with *WNT10A* variants)
Overall dentition	378	17, 15, 14, 13, 12, 22, 23, 24, 25, 27 37, 35, 34, 33, 32, 31, 41, 42, 43, 44, 45, 47		4.7	2 vs 0*	0 vs 2*

FDI- World Dental Federation two-digit tooth notation; missing teeth are illustrated in dark gray. The Relative Risk (RR) was calculated by using as a reference group girls in the 6th column and *WNT10A* variants cases in the 7th column.
* When no cases were counted in one group, RR couldn't be calculated so only the number of cases in the other group is reported.

Table S4.1.2. Genotype and clinical symptoms of 36 patients with *WNT10A* variants

		Patient	Genotype**	Sex	Missing teeth
No evident ectodermal features	p.(F228I) missense variants	1	c.[682T>A(;)682T>A] p.[(F228I)(;) (F228I)]	F	6
		2	c.[682T>A(;)682T>A] p.[(F228I)(;)(F228I)]	M	13
		3	c.[682T>A(;)682T>A] p.[(F228I)(;)(F228I)]	F	9
		4	c.[682T>A(;)682T>A] p.[(F228I)(;)(F228I)]	F	6
		5	c.[682T>A(;)682T>A] p.[(F228I)(;)(F228I)]	M	6
		6	c.[682T>A(;)682T>A] p.[(F228I)(;)(F228I)]	M	11
		7	c.[682T>A(;)(=)] p.[(F228I)(;)(=)]	M	6
		8	c.[682T>A(;)682T>A] p.[(F228I)(;)(F228I)]	F	6
		9	c.[682T>A(;)(=)] p.[(F228I)(;)(=)]	M	6
		10	c.[682T>A(;)682T>A] p.[(F228I)(;)(F228I)]	F	8
	Miscellaneous variants	11	c.[383G>A(;)(=)] p.[(R128Q)(;)(=)]	F	16
		12	c.[918C>G(;)(=)] p.[(N306K)(;)(=)]	M	10
		13	c.[487T>A(;)(=)] p.[(R163W)(;)(=)]	F	8
		14	c.[283G>A(;)682T>A] p.[(E95K(;)(F228I)]	M	25
		15	c.[433G>A(;)433G>A] p.[(V145M)(;)(V145M)]	M	22
		16	c.[311G>A(;)682T>A] p.[(R104H)(;)(F228I)]	M	18
		17	c.[1084T>C(;)682T>A] p.[(C362R)(;)(F228I)]	M	24
		18	c.[383G>A(;)682T>A] p.[(R128Q)(;)(F228I)]	M	19
		19	c.[682T>A(;)831G>C] p.[(F228I)(;)(W277C)]	M	16
	p.(C107*) nonsense variants	20	c. [321C>A(;)682T>A] p.[(C107*)(;)(F228I)]	M	10
		21	c.[321C>A(;)321C>A] p.[(C107*)(;)(C107*)]	F	22
		22	c.[321C>A(;)682T>A] p.[(C107*)(;)(F228I)]	F	19
		23	c.[321C>A(;)682T>A] p.[(C107*)(;)(F228I)]	M	19
		24	c. [321C>A(;)682T>A] p.[(C107*)(;)(F228I)]	M	22
		25	c. [321C>A(;)682T>A] p.[(C107*)(;)(F228I)]	M	10
		26	c. [321C>A(;)682T>A] p.[(C107*)(;)(F228I)]	M	10
		27	c. [321C>A(;)682T>A] p.[(C107*)(;)(F228I)]	F	13
		28	c. [321C>A(;)682T>A] p.[(C107*)(;)(F228I)]	F	14
		29	c.[321C>A(;)682T>A] p.[(C107*)(;)(F228I)]	M	17
		30	c.[321C>A(;)682T>A] p.[(C107*)(;)(F228I)]	M	10
Evident ectodermal features	p.(F228I) missense variants	1	c.[682T>A(;)682T>A] p.[(F228I)(;)(F228I)]	M	12
	Miscellaneous variants	2	c.[682T>A(;)831G>C] p.[(F228I)(;)(Trp277C)]	M	9
	p.(C107*) nonsense variants	3	c.[321C>A(;)(=)] p.[(C107*)(;)(=)]	M	8
		4	c.[321C>A(;)682T>A] p.[(C107*)(;)(F228I)]	M	14
		5	c.[321C>A(;)682T>A] p.[(C107*)(;)(F228I)]	F	16
		6	c.[321C>A(;)682T>A] p.[(C107*)(;)(F228I)]	F	22

*nonsense mutation (heterozygous or homozygous)

**The accession number of the reference sequence is: NM_025216.2