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Mutations in \textit{WNT10A} are present in more than half of isolated hypodontia cases

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\textbf{ABSTRACT}

\textbf{Background} Dental agenesis is the most common, often heritable, developmental anomaly in humans. Mutations in \textit{MSX1}, \textit{PAX9}, \textit{AXIN2} and the ectodermal dysplasia genes \textit{EDA}, \textit{EDAR} and \textit{EDARADD} have been detected in familial severe tooth agenesis. However, until recently, in the majority of cases (\sim 90\%) the genetic factor could not be identified, implying that other genes must be involved. Recent insights into the role of \textit{WNT10A} in tooth development, and the finding of hypodontia in carriers of the autosomal recessive disorder, odontoonychodermal dysplasia, due to mutations in \textit{WNT10A} (OMIM 257980; OODD), make \textit{WNT10A} an interesting candidate gene for dental agenesis.

\textbf{Methods} In a panel of 34 patients with isolated hypodontia, the candidate gene \textit{WNT10A} and the genes \textit{MSX1}, \textit{PAX9}, \textit{IRF6} and \textit{AXIN2} have been sequenced. The probands all had isolated agenesis of between six and 28 teeth.

\textbf{Results} \textit{WNT10A} mutations were identified in 56\% of the cases with non-syndromic hypodontia. \textit{MSX1}, \textit{PAX9} and \textit{AXIN2} mutations were present in 3\%, 9\% and 3\% of the cases, respectively.

\textbf{Conclusion} The authors identified \textit{WNT10A} as a major gene in the aetiology of isolated hypodontia. By including \textit{WNT10A} in the DNA diagnostics of isolated tooth agenesis, the yield of molecular testing in this condition was significantly increased from 15\% to 71\%.

\textbf{INTRODUCTION}

Hypodontia, defined as the congenital absence of one or more permanent teeth, is the most common congenital anomaly in man. Excluding the third molar, in Europeans, 5.5\% fail to develop one or more permanent teeth.\textsuperscript{1, 2} Congenital lack of six or more permanent teeth, again excluding the third molar (oligodontia), is observed in approximately 0.14\% of the population and is highly heritable.\textsuperscript{1-4} Congenital dental agenesis can occur as an isolated anomaly or as one of the features in a large variety of syndromes.\textsuperscript{2-4, 6} Hypodontia is also a common feature of ectodermal dysplasia (ED).\textsuperscript{3, 6}

ED involves the abnormal development of at least two of the ectodermal structures regarding teeth, hair, nails and sweat glands and is a clinically and genetically heterogeneous disorder.\textsuperscript{7, 8} Genes associated with ED include \textit{EDA}, \textit{EDAR}, \textit{EDARADD} and \textit{WNT10A}.\textsuperscript{7, 8}

Typically, homozygous mutations in \textit{WNT10A} cause various EDs often corresponding to the odontoonychodermal dysplasia (OODD) and Schöpf-Schulz-Passarge syndrome, both combining classic ectodermal developmental anomalies (eg, hypohidrosis, hypotrichosis, nail dysplasia, lacrimal duct hypo/aplasia, hypo/oligodontia) with additional cutaneous features including facial telangiectases and palmoplantar keratoderma. Schöpf-Schulz-Passarge syndrome (SPSS) is distinguished by the presence of multiple eyelid cysts, histologically corresponding to apocrine hidrocystomas. OODD is apparently characterised by a smooth tongue (ie, hypoplasia of lingual papillae).\textsuperscript{9-12} However, extreme variability of the associated clinical findings, including hypodontia and additional ectodermal features, may be observed in patients homozygous but also heterozygous for mutations in \textit{WNT10A}.\textsuperscript{10, 11}

Interestingly, Bohring et al. (2009) suggested that nearly 50\% of heterozygotes for \textit{WNT10A} mutations might display isolated ectodermal developmental defects such as missing teeth.\textsuperscript{14} According to this original finding, more recently, Kantaputra and Sripathomsawat (2011) demonstrated segregation of a heterozygous \textit{WNT10A} mutation in an American family with autosomal-dominant tooth agenesis without recognisable ectodermal features.\textsuperscript{13}

These observations prompted us to study the contribution of \textit{WNT10A} mutations in comparison with mutations in other genes associated with hypodontia among isolated hypodontia patients who hypodontia status was ascertained in a tertiary dental clinic.

\textbf{METHODS}

\textbf{Participants} Individuals with apparent isolated dental agenesis of six or more permanent teeth 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, were referred to the Department of Medical Genetics of the UMC Utrecht for syndrome diagnostics and genetic counselling. Tooth agenesis in the patients was assessed by clinical examination by the dentist and on panoramic radiographs (figure 1).

In total, 58 patients were referred. Thirteen of these patients were related. These patients were from six unrelated families and included three sib pairs (n=7), one parent-child pair, one pair of first cousins, and one uncle-niece pair. From each family,
the oldest patient (n=6) referred was included in the patient cohort (n=51 patients), taking into account a potential age-related expression of additional features. In order to identify possible additional features of an ED or other syndromes, all patients were physically examined by a single clinical geneticist (MvdB). In addition, patients were asked about possible symptoms of sweat glands, skin, hair and nails using a standardized form.

The patients were classified as displaying syndromic or non-syndromic hypodontia, based on the presence or absence of dysmorphic features or evident additional features (skin, hair, nails, sweat glands) suggestive of ED. Patients with one major additional ectodermal feature, more than two very mild additional ectodermal features, or with specific dysmorphic features, were classified as syndromic. Patients without additional symptoms, or with a very mild additional ectodermal feature of the skin and hair regarded as part of the normal spectrum in the general population, were classified as non-syndromic.

In total, 54 patients (14 men (41%) and 20 women (59%)) were classified as non-syndromic and included in this study. A mean of 14.6 (range: 6–28) teeth were missing. The mean age of these patients was 19.7 years (range: 9–63 years). In 25 patients (73.5%), there was a positive family history (third degree or more closely related) for tooth agenesis.

In 17 patients (10 men (59%) and seven women (41%)), the hypodontia was classified as suspect for ED or syndromic hypodontia due to their additional features (eg, sparse hair, nail abnormalities, cleft). The mean age of these patients was 20.5 years (range: 7–63 years).

Blood samples were obtained and DNA analyses of the genes WNT10A, MSX1, PAX9, IRF6 and AXIN2 were performed in both non-syndromic and syndromic cases. In the syndromic cases, additional DNA analysis was performed when a specific ED or syndrome was suspected.

When a mutation was detected, family members were asked to participate in this study. Data on tooth agenesis and possible additional ectodermal features were obtained from all participating family members. In total, 34 family members of WNT10A probands were available for DNA analysis.

Mutation analysis
High molecular weight genomic DNA was extracted from blood samples using standard procedures. PCR amplification of all exons and their splice site consensus sequences was performed with AmpliTaq Gold 360 Master Mix (Applied Biosystems, Bedford, Massachusetts, USA). Sequencing of the MSX1 (NM_002448.3), PAX9 (NM_006194.2), IRF6 (NM_006147.2), AXIN2 (NM_004655.3) and WNT10A (NM_025216.2) genes was performed using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). An ABI 3150, or 3730 sequencer (Applied Biosystems), was used for analysis. 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.

RESULTS
Genotyping
Mutation analysis of the exons and their flanking sequences of the genes WNT10A, MSX1, PAX9, IRF6, AXIN2 in the 54 patients with non-syndromic hypodontia revealed mutations in 24 probands (71%). In 19 cases (56%), a mutation in WNT10A was identified: eight probands were homozygous, four probands were compound heterozygous and seven probands were heterozygous for a single WNT10A mutation (table 1; also see online supplementary table 1). All mutations identified were interpreted as potentially damaging. Genealogy showed that the probands carrying an identical WNT10A mutation were not related. No consanguinity was found in patients homozygous for an identified WNT10A mutation.

Heterozygosity for a mutation in PAX9 was identified in three patients (p.Y60*, p.Y145C and p.S49L, respectively). In one of the probands, a probably pathogenic MSX1 mutation (p.R223L) was detected. One patient showed a non-sense mutation in AXIN2 (p.R656*).

In comparison, in 13 syndromic hypodontia cases (76%), mutations were identified of which a WNT10A mutation was present in 12 cases (71%) (table 1; also see online supplementary table 2). One patient showed a WNT10A mutation in addition to a pathogenetic EDAR mutation that was previously reported in X-linked hypohidrotic ED (OMIM 305100).

The most frequent mutation, F228I, represents 62% of the identified WNT10A mutations in the non-syndromic hypodontia cohort. This frequency is significantly (OR 17.9, p<0.05) higher than the frequency (2.3%) observed in the control population. The hypodontia status of these anonymous controls is not known.

Phenotype of WNT10A probands
In six non-syndromic hypodontia patients showing a WNT10A mutation, extra-oral symptoms were present. These were considered to be very mild, being part of the normal variation in the population (table 1; online supplementary table 1).

Characteristic features of ODDD, including facial telangiectases, evident palmoplantar keratoderma and smooth tongue were not observed. In the syndromic WNT10A cases, the most frequent additional features were sparse hair, sparse eyebrows, short eyelashes and abnormalities of the toenails. A dry skin was present in several cases (table 1 and online supplementary table 2).

Dental characteristics in WNT10A mutation cases
The dental numerical characteristics are presented and the tooth agenesis code (TAC) is calculated (see online supplementary tables 3 and 4). The TAC is a unique number that is consistent with a specific pattern of tooth agenesis.14 15 No specific TAC could be observed for WNT10A mutation carriers. Third molars are seldom present in the current panel. The percentages of tooth agenesis per tooth type are quite similar to those from a larger population of non-syndromic oligodontia patients.15 The symmetry in agenesis patterns between the left and right sides of the jaw was in line with the population of non-syndromic hypodontia and seen in 58% and 63% of all non-syndromic
WNT10A cases for the maxilla and the mandible, respectively. In the syndromic WNT10A cases, this symmetry for the maxilla and mandible was observed in 46% and 64% of the cases, respectively. Neither the patterns of missing teeth that would distinguish the current population from a general population of non-syndromic hypodontia patients, nor the peculiarities of tooth morphology were observed. The third molar and the upper second premolar were most frequently absent.

### Genotype-phenotype

The mean number of missing teeth for the non-syndromic and syndromic WNT10A probands was similar, at 15.6 (range 10–28) and 15.4 (range 6–30), respectively (table 1 and online supplementary tables 1 and 2). The highest number of missing teeth (30) was present in a p.C107* homozygous girl; an almost complete absence of the permanent dentition was seen and furthermore, also nail dysplasia and mild, sparse curly hair was observed (syndromic patient 5; table 1; online supplementary table 1). A patient with the same genotype did not show additional ectodermal features (non-syndromic patient 10; table 1; online supplementary table 1). A patient carrying the p.C107* mutation had an orofacial cleft (syndromic patient 2; table 1; online supplementary table 2).

Variability of extra-oral features is observed in carriers of a WNT10A mutation. Patients with and without additional ectodermal features could be either heterozygous for p.C107*, heterozygous or homozygous for p.F228I. A patient compound heterozygous for p.C107* and p.F228I showed significant features suggestive for an ED (syndromic patient 8; table 1; online supplementary table 2). A patient with the same genotype did not show additional ectodermal features (non-syndromic patient 10; table 1; online supplementary table 1). A patient carrying the p.C107* mutation had an orofacial cleft (syndromic patient 2; table 1; online supplementary table 2).

### Family members

To gain more insight into the phenotypic variability of the WNT10A mutation within families, family members of patients with a WNT10A mutation were studied (online supplementary tables 5 and 6). Tooth agenesis was frequently observed in family members of non-syndromic and syndromic WNT10A cases. Sparse hair was most frequently reported in family members of syndromic WNT10A cases.

### DISCUSSION

This study shows a surprisingly high frequency of WNT10A mutations in isolated hypodontia. In 19 out of 34 patients with apparent isolated hypodontia (56%), a mutation in WNT10A could be identified. In five probands, a mutation was identified in

**Table 1 Clinical symptoms and genotype in 19 non-syndromic and 11 syndromic hypodontia patients with WNT10A mutations**

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*Family members with tooth agenesis.
Am, male alopecia; Ab, abnormal hair structure, E, Eczema; +, present; −, absent; ±, very mild.
the candidate genes MSX1 (one proband), PAX9 (three probands), AXIN2 (one proband), respectively. No mutations were found in the IRF6 gene.

A diagnosis of isolated hypodontia is not easily made. Individuals with ED show variations in phenotypic expression that may range from prominent to very subtle ectodermal symptoms. The latter can be difficult to classify and might hint at features of ED or normal variations. Moreover, hypoplasia of lingual papillae, considered as a characteristic feature in WNT10A mutation carriers is difficult to identify. Standard methods of imaging of the tongue papillae are non-invasive video microscopy, contact endoscopy or digital camera after staining with methylene blue. However, these are not routinely performed or available in daily clinical practice, and so, were not applied in this study.

After careful examination of our patients, 67% of them were finally classified as non-syndromic. This percentage corresponds with previous studies. Bergendal et al (2006) showed that 14.7% of the oligodontia patients had impaired function of hair, nails and/or sweat glands, which is considerably lower than in the studies performed in tertiary centres. The p.F228I mutation in WNT10A was found in normal controls with an allele frequency of 2.5%. This frequency corresponds with the high prevalence of tooth agenesis in the general population. Based on the assumption that heterozygosity for WNT10A is involved in 50% of less severe dental agenesis, the expected prevalence of dental agenesis in the Dutch population is approximately 5%. This is in line with the observation that in the European population, 5.5% fail to develop one or more permanent teeth, excluding the third molar. According to the Hardy and Weinberg rules, and considering an allele frequency of the p.F228I, nearly 1 out of 2000 individuals might have a severe hypodontia due to homozygosity for p.F228I. This is approximately half the prevalence (0.14%) of severe hypodontia reported in the European population.

A mutation screen of MSX1, AXIN2, PAX9 and the ED genes EDA, EDAR and EDARADD in a population with severe isolated hypodontia revealed a mutation in approximately 11% of the probands. By including the WNT10A gene in the DNA testing, the detection rate of the genetic cause of apparently isolated hypodontia increases to approximately 70% (this study). Data obtained in mice support the involvement of WNT10A like MSX1, PAX9 and AXIN2 in tooth development. WNT10A is strongly expressed in the dental epithelium at the tooth initiation stage. WNT10A, as well as MSX1 and PAX9, are also required for normal tooth development beyond the bud stage. AXIN2 is expressed during tooth development in the dental mesenchyme, enamel knot and odontoblasts.

**Genotype–phenotype correlations WNT10A**

Heterozygosity, compound heterozygosity and homozygosity can be responsible for severe hypodontia. Homozygosity, for a non-sense mutation, seems to be associated with an almost complete absence of the permanent dentition. We did not observe a specific pattern of missing teeth in the population carrying a WNT10A mutation. A sex-influenced expression of hypodontia in heterozygotes for a WNT10A mutation, as previously suggested by Bohring et al., could also not be confirmed in our study.

Because heterozygosity and compound heterozygosity or homozygosity for WNT10A mutations are associated with tooth agenesis, pseudodominant or multigenic patterns of inheritance cannot be excluded.

No relation between the presence or absence of ectodermal features and the specific type of mutation and/or the heterozygous or homozygous state has been detected. In our patient panel, there were less additional ectodermal features compared with previously reported patients. This may reflect a selection bias, but also indicates that other factors, for example, additional genetic factors, may play a role in the phenotypic expression of WNT10A mutations. Further study is needed to determine involvement of other factors.

Therefore, we conclude that there is no unambiguous relationship between WNT10A genotype and the number of missing teeth, pattern of tooth agenesis and the presence of additional features.

**DNA diagnostics in hypodontia patients**

To identify the genetic cause in probands with an agenesis of more than six teeth, excluding the third molar, and in probands with a lower number of agenesis with a positive family history, we recommend to test for mutations in WNT10A, and if negative to continue with testing for MSX1, PAX9 and AXIN2. In case of AXIN2 mutation analysis, one should specifically ask for possible hereditary colon cancer in the family. Physical examination with focus on additional ectodermal features is of importance. Analysis of EDA, EDAR and EDARADD should be considered in all cases with non-syndromic tooth agenesis of more than six teeth. This approach will improve counselling of patients with hypodontia and their family members.

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**Data sharing statement** The authors have additional clinical and dental data on the patients without a WNT10A mutation and the patients with a MSX1 and PAX9 mutation available in three additional tables.

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Developmental defects

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