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

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\section*{ABSTRACT}

\textbf{Background} Dental agenesis is the most common, often heritable, developmental anomaly in humans. Mutations in \textit{MSX1}, \textit{PAK9}, \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, odontoonychoderma 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{PAK9}, \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{PAK9} 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\%.

\section*{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 odontoonychoderma dysplasia (OODD) and Schöpf-Schulz-Passarge syndrome, both combining classic ectodermal developmental anomalies (eg, hypohidrosis, hypotrichosis, nail dysplasia, lacrimal duct hypoplasia, hypo/hypotrichosis, nail dysplasia, lacrimal duct hypoplasia, hypo/hypotrichosis, nail dysplasia, lacrimal duct hypoplasia, hypo/hypotrichosis, nail dysplasia, lacrimal duct hypoplasia, hypo/hypotrichosis, nail dysplasia, lacrimal duct hypoplasia, hypo/hypotrichosis, nail dysplasia, lacrimal duct hypoplasia, hypo/hypotrichosis, nail dysplasia, lacrimal duct hypoplasia, hypo/hypotrichosis, nail dysplasia, lacrimal duct hypoplasia, hypo/hypotrichosis, nail dysplasia, lacrimal duct hypoplasia, hypo/hypotrichosis, nail dysplasia, lacrimal duct hypoplasia, hypo/hypotrichosis, nail dysplasia, lacrimal duct hypoplasia, hypo/hypotrichosis, nail dysplasia, lacrimal duct hypoplasia, 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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 (MJvdB). 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, 34 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: 9–55). 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 features of an ED or other syndromes, 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).

**Results**

**Genotyping**

Mutation analysis of the exons and their flanking sequences of the genes WNT10A, MSX1, PAX9, IRF6, AXIN2 in the 34 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.

For heterozygosity in a mutation in PAX9 was identified in three patients (p.Y60*, p.Y143C and p.S49L, respectively). In one of the probands, a probably pathogenic MSX1 mutation (p.R225L) was detected. One patient showed a non-sense mutation in AXIN2 (p.R656*).

In comparison, in 15 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 1). One patient showed a WNT10A mutation in addition to a pathogenetic EDAD1 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 OODD, including facial telangiectases, evident palmar plantar 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.\textsuperscript{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.\textsuperscript{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 65% of all non-syndromic 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.
WNT10A mutations. The absence of more than 20 teeth was observed (syndromic patient 5; table 1; online supplementary table 2). The mildest hypodontia, with an agenesis of six teeth, was present in a syndromic patient compound heterozygous for p.C107* and p.F228I (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).

**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

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

<table>
<thead>
<tr>
<th>Proband</th>
<th>Genotype</th>
<th>Gender</th>
<th>Age</th>
<th>Family history*</th>
<th>Number missing teeth</th>
<th>Abnormal shape</th>
<th>Scalp</th>
<th>Eyebrows</th>
<th>Dry skin</th>
<th>Hypohidrosis</th>
<th>Plantar hyperkeratosis</th>
<th>Nails</th>
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<td>1</td>
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<td>2</td>
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| Syndromic |                         |        |     |                 |                      |                  |      |          |          |               |                        |       |
| 1         | p.[C107*]+[=]           | M      | 9   | +               | 12                   | +                |      |          |          | E            |                        |       |
| 2         | p.[C107*]+[=]           | M      | 22  | +               | 13                   | -                |      |          |          | +            |                        |       |
| 3         | p.[F228I]+[=]           | F      | 34  | -               | 11                   | -                |      |          |          |              |                        |       |
| 4         | p.[F228I]+[=]           | M      | 45  | +               | 18                   | +                |      |          |          | +            |                        |       |
| 5         | p.[C107*]+[C107*]       | F      | 7   | -               | 30                   | +                |      |          |          | +            |                        |       |
| 6         | p.[C107*]+[F228I]       | F      | 8   | +               | 18                   | +                |      |          |          | +            |                        |       |
| 7         | p.[C107*]+[F228I]       | F      | 12  | +               | 20                   | +                |      |          |          |              |                        |       |
| 8         | p.[C107*]+[F228I]       | M      | 15  | +               | 6                    | +                |      |          |          | ±            |                        |       |
| 9         | p.[F228I]+[F228I]       | M      | 8   | +               | 16                   | -                |      |          |          |              |                        |       |
| 10        | p.[F228I]+[F228I]       | F      | 45  | -               | 14                   | +                |      |          |          | ±            |                        |       |
| 11        | p.[F228I]+[W277C]       | M      | 11  | +               | 12                   | Ab               |      |          |          | ±            |                        |       |

*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.\(^4\)\(^6\)\(^16\)\(^18\) 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.\(^9\)\(^11\) Standard methods of imaging of the tongue papillae are non-invasive video microscopy, contact endoscopy or digital camera after staining with methylene blue.\(^19\)\(^25\) 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.\(^4\)\(^6\)\(^16\) Bergendal et al (2006) showed that 14.7% of the oligodontia patients had impaired function of hair, nails and/or sweat glands,\(^5\) which is considerably lower than in the studies performed in tertiary centres.\(^4\)\(^6\)

The p.F228I mutation in WNT10A was found in normal controls with an allele frequency of 2.3%. 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.\(^1\)\(^2\) According to the Hardy and Weinberg rules, and considering an allele frequency of the p. F228I of 1/45, nearly 1 out of 2000 individuals might have a severe hypodontia due to heterozygosity 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.\(^23\)

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.\(^24\)\(^26\) WNT10A is strongly expressed in the dental epithelium at the tooth initiation stage.\(^25\)\(^26\) WNT10A, as well as MSX1 and PAX9, are also required for normal tooth development beyond the bud stage.\(^26\) AXIN2 is expressed during tooth development in the dental mesenchyme, enamel knot and odontoblasts.\(^27\)\(^28\)

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.\(^11\) 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.\(^9\)\(^11\)\(^12\) 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.

Acknowledgements The authors wish to thank all the patients and family members who participated in this study. The authors also thank Nine Knoers for critical reading of the manuscript and helpful discussions.

Contributors M-JHvdB, Marijn C, Marco C and JvPA contributed to the conception and design of the study. M-JHvdB, Marijn C, YB, Avdh, Marco C and FAMH collected data. Marijn C and Marco C contributed to the referral of patients. YB and Avdh performed the molecular analysis. M-JHvdB analysed the data and along with all the authors was involved in data interpretation. MJHvdB, Marco C and JvPA drafted the article. Marijn C, Avdh, DL, Marco C and JvPA critically revised the article for intellectual content. All the authors contributed to the final approval of the version to be submitted.

Funding This study is in part funded by the Dutch Association for Prosthetic Dentistry and Orofacial Pain (NVGPT).

Competing interests None.

Patient consent Patients gave their informed consent. The data are the results of daily clinical practice.

Provenance and peer review Not commissioned; externally peer reviewed.

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