Tooth agenesis patterns and phenotype variation in a cohort of Belgian patients with hypodontia and oligodontia clustered in 79 families with their pedigrees

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SUMMARY

BACKGROUND: Even though tooth agenesis is the most common developmental anomaly of the human dentition, its genetic background and pathogenetic mechanism(s) still remain poorly understood. Syndromic and isolated forms of hypodontia have been described and can occur sporadically or in families.

OBJECTIVES: We describe and analyse the hypo-/oligodontia phenotype variations in families. The index patient suffers from severe or mild hypodontia; case-parents/sib records are available. Furthermore, we aim to evaluate whether the different agenesis patterns in the pedigrees are predictive of mutations in specific genes based on reported genotype-phenotype associations.

MATERIALS AND METHODS: Dental records and pedigrees were collected from 79 families. In 67 families, the index patient presented with oligodontia while in 12 families with hypodontia. The phenotype data of 66 oligodontia index patients were analysed with the Tooth Agenesis Code software.

RESULTS: Nine families counted two members; one family counted three members affected with oligodontia. Twenty-four oligodontia families respectively had one (n = 17), two (n = 4), three (n = 2) or four (n = 1) additional family members presenting with hypodontia. Of the 77 oligodontia cases, two showed the same tooth agenesis pattern, while 75 patients showed unique tooth agenesis patterns.

CONCLUSIONS: Despite familial aggregation and expected Mendelian segregation, the number of missing teeth in the familial hypo-/oligodontia phenotypes and the tooth agenesis patterns are highly variable between the affected family members. Therefore, we hypothesize that tooth agenesis is not (always) a simple monogenic condition, but additional genetic or environmental factors can modify the expression of the phenotype.

Introduction

Agenesis of one or more permanent teeth is the most common dental developmental anomaly in humans. Severe hypodontia, or oligodontia refers to the absence of six teeth or more, excluding the third molar. Approximately 1% (0.08–1.1%) of the population suffers from oligodontia (Schalk-van der Weide, 1992; Schalk-van der Weide et al., 1992; Stockton et al., 2000). Despite its high prevalence (Polder et al., 2004), the aetiology and pathogenetic mechanism(s) underlying hypodontia in humans still are poorly understood. Oligodontia can occur in association with many genetic syndromes, as a sporadic finding or as a non-syndromic isolated familial form (Gorlin et al., 2001). Familial occurrence is often present and Mendelian (monogenic) segregation has been demonstrated in families with syndromic as well as isolated hypodontia. So far causal mutations for isolated forms have been evidenced in MSXI, PAX9, EDAI, WNT10A, and EDARADD genes (Vastardis, 2000; Stockton et al., 2000; Lidral and Reising, 2002; De Muynck et al., 2004; Lammi et al., 2004; Han et al., 2008; Bohring et al., 2009; Kantaputra and Sripathomsawat, 2011; van den Boogaard et al., 2012; Bergendal et al., 2011).

A combination of hypodontia/oligodontia and cleft lip and/or palate was found to be caused by an MSXI mutation in a large Dutch family (van den Boogaard et al., 2000), and an association of hypodontia with increased risk for colorectal cancer was shown to be linked to a mutation in AXIN2 in a Finnish family (Lammi et al., 2004). As four genes (EDAI, EDAR, EDARADD, and WNT10A) account for 90% of hypohidrotic/anhidrotic ectodermal dysplasia (ED) cases (Cluzeau et al., 2011), it can be expected that specific mutations in genes along these pathways or in these gene networks can also account for isolated hypodontia/oligodontia (Mues et al., 2010; van den Boogaard et al., 2012).

Despite the advances in unraveling the genetic aetiology of isolated and syndromic hypodontia, the vast majority of the cases still remain to be solved. In families with a
probable dominant or recessive Mendelian inheritance there seems to be a variable hypodontia phenotype (due to incomplete penetrance and variable expression; Burzynski and Escobar, 1983), indicative of additional modifying influences in which Brook (2009), De Coster et al. (2009), and Townsend et al. (2012) propose a multifactorial aetiological model, with possibly many genes, and also environmental and epigenetic factors contributing to tooth development (Suarez and Spence, 1974; Brook, 1984; Peck et al., 1993). In their twin study, Townsend et al. (2005) found a high discordance rate of hypodontia sub-phenotypes in monozygotic (MZ) twins, and the authors claim epigenetic differences in spacio-temporal gene regulation to be responsible for the differences in hypodontia patterns in MZ twins. Lauwerijns et al. (1992) found a left-right mirror image for the absence of a lower second premolar in mono-chorionic MZ-twins.

In his review and based on the genetic network involved during tooth formation, and several mutations that give rise to dental defects, Michon (2011) discusses the possible impact of fine-tuning and network regulation by miRNAs on tooth development and the formation of dental defects.

In this study we aim to describe and analyse hypodontia/oligodontia phenotype variation in families of the Leuven Hypo/ Oligodontia Cohort (LHOC) in which the index patient suffers from either severe (agenesis of six or more permanent teeth, called oligodontia) or mild hypodontia (agenesis of five or less permanent teeth), with exclusion of third molars and of which at least case-parents records or sib records are available for evaluation. For each family, we will propose a most likely inheritance pattern, and we compare the tooth agenesis patterns among the affected members within each family. From these data we will discuss the best possible approach for the genetic analysis of the LHOC.

**Material and methods**

**Patients and their families**

Patients with hypodontia (including oligodontia) were mainly referred by Belgian colleague orthodontists and general dentists to the Hypodontia–Oligodontia Consultation supervised by the second and last authors, Department of Orthodontics of the KU Leuven and University Hospitals of the KU Leuven, (UZ-KU Leuven), Belgium. Some patients were recorded in the cohort for their oligodontia before being treated in the Departments of Orthodontics and/or Restorative Dentistry of the UZ-KU Leuven. Other patients were referred by the Centre of Human Genetics of the UZ-KU Leuven, Belgium.

**Methods**

A medical and dental history as well as familial anamnesis was taken from all index patients and their families. To this end we also used a short questionnaire, set up together with the Clinical Geneticist.

Standard orthodontic diagnostic records (set of extra-oral and intra-oral photographs, dental casts, orthopantomogram (OPG), and eventually, a lateral headplate if necessary for the orthodontic treatment) were collected from the index patients for their orthodontic and restorative treatment planning, unless they were available from the referring colleagues’ records. All the first-degree relatives of the index patients were invited for an oral and extra-oral clinical inspection and were asked for their cooperation to the study. The patients and their family dentists were contacted concerning the availability of earlier dental records in the patient’s files. An OPG was a minimal requirement for the diagnosis of the dental phenotype and assessment of the severity of the hypodontia/oligodontia. In case of doubt on the dental phenotype and no radiograph was available from the index patients’ first-degree relatives, informed consent was asked to make an OPG. Pedigrees of each family were drawn to evaluate the inheritance patterns of the hypo-/oligodontia phenotypes. Based on the familial anamnesis of the parents, no consanguineous marriages were known in the present status of LHOC.

We have been collecting medical history, dental records, and DNA samples isolated from mainly peripheral blood, sometimes from saliva of index patients with hypodontia/oligodontia and a variable number of their family members, since 2002 to date (dd April 2012).

The LHOC now comprises medical and dental records from 295 individuals. These are affected and non-affected members of 79 hypodontia/oligodontia families (Supplementary Table 1, available online). The phenotype data of the 66 index patients with oligodontia were analysed with the Tooth Agenesis Code (TAC) software described earlier by van Wijk and Tan (2006) (http://www.toothagenesiscode.com). Out of these 79 families, six index patients were diagnosed clinically with syndromic oligodontia; including one patient with Asperger syndrome, one patient with Klinefelter syndrome, two patients with ED, and two patients with Incontinentia Pigmenti (IP). Furthermore, one patient with hypodontia presented with IP; she is the mother of one of the affected girls with IP. From 36 oligodontia families we sampled case-parent trios, while broader pedigrees could be collected from the other 31 oligodontia families.

All patients and their parents gave informed consent in accordance with the regulations of the Biomedical Ethics Committee (UZ KULeuven) to participate in this study (reference number of approval letter: ML3711).

**Results**

From the 79 hypodontia/oligodontia families collected in this study, 67 families have at least one family member affected with oligodontia (six or more permanent teeth missing, excluding the third molars) and 12 families have at least one member with hypodontia (less than six permanent teeth missing, excluding the third molars). In both groups we analysed the data with
exclusion of the third molars for two reasons: (1) third molars are the most commonly missing teeth in the dentition, with at least one being absent in 20–30% of the population (Matalova et al., 2008) and (2) detection of agenesis was mostly done by children at young age while the initial calcification of the third molars starts at the age around 9–12 years (Demirjian et al., 1973; Sisman et al., 2007). None of the patients in the 12 hypodontia families showed other associated features, while in six patients of the 67 oligodontia families, syndromic oligodontia was diagnosed clinically, and some of them at the molecular level (unpublished data). From 36 oligodontia families case-parent trios were collected, and from the 31 other oligodontia families we collected broader pedigrees (Supplementary Figure 1, available online). In the 67 oligodontia families, 11 family members (other than the index patients) were found to be affected with oligodontia and 35 family members were (also) affected with hypodontia (Figure 1A–C).

For description of the tooth agenesis patterns with the TAC method, only the 60 non-syndromic index patients with oligodontia were used. We excluded the six syndromic oligodontia index patients when analysing the tooth agenesis patterns because of the possibility of other genetic factors that can contribute to a different clinical phenotype. Furthermore, the index patient of oligodontia family, 76 was excluded for the TAC calculation, because the exact number of missing teeth could not be determined (Supplementary Table I, available online, Family O-76).

From the 67 oligodontia index patients, 61 presented with non-syndromic oligodontia (91.04%) and the other six oligodontia patients (8.96%) presented with four different syndromes. These associated syndromes were as follows: (1) Klinefelter syndrome, (2) Asperger syndrome, (3) ED, and (4) IP. In the patient with Klinefelter syndrome (O10-II-1), 10 permanent teeth did not develop; the patient with Asperger syndrome (O40-II-1) missed 18 teeth; the first patient with ED (ED1—O44-II-1) missed 26 teeth, while ED2 (O79-II-1) missed 12 teeth. The three patients with IP syndrome suffered from a variable degree of hypodontia, ranging from 2 agenesis of permanent teeth in one patient (IP1—O12-I-2), 10 agenesis in IP2 (who is the daughter of IP1—O12-II-1) and 7 in IP3 (O72-II-1) (Supplementary Table I, available online). So far the clinical syndromic diagnosis was confirmed with karyotyping analysis for the patient with Klinefelter syndrome and with molecular genetic analysis for one patient with ED and for two patients (a mother and her daughter) with IP.

After uploading the oligodontia phenotype data onto the web based TAC software tool (http://www.toothagenesiscode.com), frequency results were generated for individual teeth per quadrant, per jaw (maxilla and mandible) and in total. With the TAC tool, frequencies of the individual agenesis patterns for the 60 non-syndromic oligodontia index patients were generated. The number of missing teeth in the 60 non-syndromic oligodontia index patients ranged between 6 and 24 teeth. On average 10.63 (SD = 4.46) teeth were missing per individual, with a slightly higher average incidence in the maxilla (5.62; SD = 2.27) than in the mandible (5.02; SD = 2.71). In Table 1, 16 different groups are shown according to the number of missing teeth for the non-syndromic oligodontia patients (n = 60). In this group, the highest frequency is for agenesis of seven teeth, which occurs 11 times (18.3%). There were no non-syndromic index patients missing 18, 20, 23, 25, 26, 27 or 28 teeth. The Supplementary Table II (available online) shows the frequency distribution of the 66 oligodontia patterns for the full dentition.

In the total group of 77 syndromic and non-syndromic oligodontia cases, only two cases (O34-II-1 and O54-II-1) showed the same tooth agenesis pattern (Supplementary Table I, available online and Figure 2) both missing the same eight teeth, i.e. the 2 premolars in each quadrant of the maxilla and of the mandible (q1 = 24, q2 = 24, q3 = 24, q4 = 24). Both patients belonged to different families (O-34 and O-54) and showed a different number of ageneses if the third molars would be taken into account. All other 75 cases show unique tooth agenesis patterns (Supplementary Table II, available online).

Second premolars and upper lateral incisors are the most frequently missing teeth (Arte, 2001). These teeth are also most often lacking in the more severe phenotype, oligodontia of our index patients of the LHOC. For the separate patterns in the maxilla and the mandible, the most frequent patterns for the 60 non-syndromic patients were symmetric agenesis of the two second premolars and lateral incisors for the maxilla (q1 = 18, q2 = 18) (Figure 3A); this pattern was present in 6 (10%) patients. For the mandible, there were two most frequent patterns, which occurred respectively five (8.33%) and four (6.66%) times: (1) agenesis of both second premolars (q3 = 16, q4 = 16); and (2) agenesis of the first and second premolars in the mandible (q3 = 24, q4 = 24) (Figure 3B) (both patterns can also be seen in Supplementary Table II, available online). Forty-five different patterns were represented in the mandible and 44 in the maxilla. For the frequency patterns of the separate quadrants, the upper right quadrant of the maxilla (q1) showed two most common patterns: q1 = 18 (18 indicates agenesis of PM2 + LI) and q1 = 30 (ageneis of LI + C + PM1 + PM2), both with a frequency of eight times (13.3%), while in the upper left quadrant (q2) also two most common patterns were shown: q2 = 18 and q2 = 24 (ageneis of PM1 + PM2), both occurring also eight times (13.3%). Frequency patterns for the separate quadrants of the mandible showed different results; the most common pattern for the lower left quadrant (q3) was q3 = 24 (ageneis of PM1 + PM2) and q3 = 16 (ageneis of PM2), both occurring eight times (13.3%), while for the lower right quadrant (q4) the most common pattern occurred 10 times (16.7%) and so was for q4 = 16 (ageneis of PM2). Table 2 shows the relative symmetry in presence/agenesis of every single tooth left versus right in the upper and lower jaw for the 60 index patients.

Within families with several affected hypodontia/oligodontia members, the n-fold change ranged from 1-fold (no difference, in three cases) up to 10-, 13-, and 15-fold
differences in number of missing teeth within one family (Figure 4). Although in three instances no differences (1-fold changes) in the number of missing teeth were found within one family, still the oligodontia patterns differed among the affected members of this family.

The 79 documented families of the LHOC database include one four-generation, two three-generation, and 76 two-generation families (Supplementary Figure 1, available online). A selection of pedigrees of the present cohort is depicted in Supplementary Figure 1 (available online), and with each pedigree a prediction of possible segregation patterns is included. Although the families nowadays do not have suitable sizes to provide evidence on a confident/reliable determination of an eventual Mendelian inheritance
Table 1  Frequency table, distribution of total number of missing teeth for the 60 non-syndromic oligodontia index patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of missing teeth</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>11</td>
<td>18.3</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>4</td>
<td>6.7</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>5</td>
<td>8.3</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>5</td>
<td>8.3</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>5</td>
<td>8.3</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>11</td>
<td>16</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>12</td>
<td>17</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>13</td>
<td>19</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>14</td>
<td>21</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>15</td>
<td>22</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>16</td>
<td>24</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>Total</td>
<td>638</td>
<td>60</td>
<td>100%</td>
</tr>
</tbody>
</table>

Number of cases = 60; Total number of missing teeth = 638; Maximum number of missing teeth = 24; Minimum number of missing teeth = 6; Average number of missing teeth = 10.63 (SD = 4.46); Average number of missing in teeth upper jaw = 5.62 (SD = 2.27); Average number of missing in teeth lower jaw = 5.02 (SD = 2.71)

pattern, we estimated the inheritance pattern of 48 of the 79 families (61%), assuming the following:

1. Autosomal dominance, if affected individuals are present in two or more generations: e.g. family O-7 and family O-10 and O-21.
2. Autosomal recessivity, if two or more siblings are affected (female or of both genders) with normal parents: e.g. family O-53.
3. X-linked inheritance, if affected males related by unaffected (or mildly affected) females, or multiple affected males: e.g. family O-34 (could also be recessive).
4. Sporadic cases cannot be classified reliably, since they may represent a de novo dominant mutation, autosomal recessive or even X-linked disorder, or autosomal dominant mutation with variable expressivity.

Based on these criteria, we suspected an autosomal recessive inheritance pattern or a de novo genetic mutation in 23 pedigrees, a dominant model in 19 pedigrees and an X-linked pattern in 6 cases (including the IP2 patient). From 32 pedigrees (39%) we decided not to be able to predict any Mendelian inheritance pattern at all.

Discussion

In our LHC of cases selected on basis of the willingness of trio’s and families to cooperate or for their familial occurrence, we observed a marked intrafamilial variation in expressivity. This led us to the suggestion that transmission of these phenotypes probably is not strictly monogenic but that additional modifying factors might contribute to the phenotype. In our LHC Cohort, we found differences not only in tooth number and in tooth agenesis patterns in family members with non-syndromic hypodontia and oligodontia but also in affected family members with syndromic oligodontia. In one family with non-syndromic hypodontia/oligodontia the phenotype of the proband differed 15-fold from the phenotype of an affected first-degree relative (see family O-34). In a family with three members affected with non-syndromic oligodontia—a father and his two children—that were previously diagnosed with the same non-sense mutation (559 C→T, resulting in Gln187Stop) in the homeodomain of the MSX1-gene (De Muynck et al., 2004), a different number of teeth is missing, giving rise to different tooth agenesis patterns (Table 1, family O-3). Furthermore, eight patients of the LHC with severe oligodontia were screened for PAX9 and AXIN2 mutations by Gerits et al. (2006). No mutations were discovered, but a unique nucleotide change in a conserved 5' flanking region of PAX9 was revealed. Whereas earlier screening of the same patients for MSX1 mutations had a negative outcome. Swinnen et al. (2008) investigated the aetiology of multiple tooth agenesis in a family of the LHC with three sisters suffering from severe oligodontia (Table 1, family O-45). After mutational screening, it could be concluded that the oligodontia phenotype of the three sisters was not caused by mutations in the coding regions of MSX1, PAX9, AXIN2, DLX1, or DLX2 genes. In a mother and her daughter (IP1 and IP2, O-12, see Table 1), affected with the same X-linked gene mutation in the NEMO-gene, causing IP syndrome (Dreessen et al., submitted for publication), a major difference in number of tooth agenesis is present. In these cases it consists of a 5-fold difference in missing teeth (2 versus 10 tooth agenesis), classifying the mother as a mild hypodontia case (with two agenesis) and her daughter as a severe oligodontia case. This difference could be due to differential X-chromosome inactivation (mosaicism) in the specific cell lines, giving rise to tooth development in the mother compared with the daughter. In tooth precursor cells originating from migrating craniofacial neural crest stem
cells, the X-chromosome with the mutated allele eventually is not inactivated in the most severely affected individual. In our data, it is shown that this dental phenotype variability also applies to different families affected with the same syndrome (evidenced with clinical diagnosis only), like in the patients affected with IP from two different families in this study: patients IP2 and IP3 were respectively missing 10 and 7 teeth and thus also show different TACs. In these cases the dental phenotype variability present in the IP-patients of different families can also be caused by a different mutation in \( \text{NEMO} \) at the molecular level (different type of nucleotide change), a different location of the mutation in the gene (affecting the function of the protein differently) or eventually even a mutation in another gene (genetic heterogeneity). It is peculiar, however, that the interfamilial difference in phenotype (number of agenesis) is smaller than the intrafamilial difference between IP1 and IP2.

In two patients diagnosed clinically and molecularly (our unpublished data) with \textit{de novo} ED (ED1) syndrome with a mutation in \textit{WNT10A} and (ED2) in \textit{EDAR}-gene, the difference in the oligodontia phenotypes was also striking, with more than a 2-fold difference in the number of agenesis as ED1 misses 26 teeth and ED2 misses 12 teeth. Although, from this example, it is tempting to conclude that mutations in the \textit{WNT10A}-gene cause more severe oligodontia phenotypes, many more variables could explain these differences. As there are several genes that could possibly cause ED (Cluzeau et al., 2011), also in these cases there might be different mutations either in one gene or in different genes. However, even if the ED would be caused by a mutation in the same gene phenotype variability can be present. Mues et al. (2010) showed that mutations of the Ectodysplasin-A (EDA) gene that are generally associated with the hypohidrotic ED syndrome can also manifest as selective, non-syndromic tooth agenesis. Recently, van den Boogaard et al. (2012) also found considerable phenotype variability in syndromic as well as non-syndromic oligodontia cases with the same \textit{WNT10A}-mutations at the molecular level. They reported a phenotype difference between individuals of as many as 12 agenesis (14 versus 26) in patients with non-syndromic oligodontia carrying the same \textit{WNT10A}-mutation. In syndromic cases they reported differences of 14 agenesis (6 versus 20) between individuals with the same \textit{WNT10A} mutation.

**Table 2** Single tooth symmetry statistics.

<table>
<thead>
<tr>
<th>Tooth</th>
<th>Present bilaterally</th>
<th>Missing right side (q1)</th>
<th>Missing left side (q2)</th>
<th>Missing bilaterally</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper jaw</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>59 (98.3%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (1.7%)</td>
</tr>
<tr>
<td>L1</td>
<td>13 (21.7%)</td>
<td>7 (11.7%)</td>
<td>3 (5%)</td>
<td>37 (61.7%)</td>
</tr>
<tr>
<td>C</td>
<td>37 (61.7%)</td>
<td>1 (1.7%)</td>
<td>3 (5%)</td>
<td>19 (31.7%)</td>
</tr>
<tr>
<td>PM1</td>
<td>24 (40%)</td>
<td>5 (8.3%)</td>
<td>6 (10%)</td>
<td>25 (41.7%)</td>
</tr>
<tr>
<td>PM2</td>
<td>10 (16.7%)</td>
<td>5 (8.3%)</td>
<td>2 (3.3%)</td>
<td>43 (71.7%)</td>
</tr>
<tr>
<td>M1</td>
<td>52 (86.7%)</td>
<td>1 (1.7%)</td>
<td>0 (0%)</td>
<td>7 (11.7%)</td>
</tr>
<tr>
<td>M2</td>
<td>37 (61.7%)</td>
<td>2 (3.3%)</td>
<td>4 (6.7%)</td>
<td>17 (28.3%)</td>
</tr>
<tr>
<td>Lower jaw</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>33 (55%)</td>
<td>1 (1.7%)</td>
<td>3 (5%)</td>
<td>23 (38.3%)</td>
</tr>
<tr>
<td>L1</td>
<td>35 (58.3%)</td>
<td>6 (10%)</td>
<td>4 (6.7%)</td>
<td>15 (25%)</td>
</tr>
<tr>
<td>C</td>
<td>48 (80%)</td>
<td>4 (6.7%)</td>
<td>2 (3.3%)</td>
<td>6 (10%)</td>
</tr>
<tr>
<td>PM1</td>
<td>33 (55%)</td>
<td>2 (3.3%)</td>
<td>5 (8.3%)</td>
<td>20 (33.3%)</td>
</tr>
<tr>
<td>PM2</td>
<td>9 (15%)</td>
<td>5 (8.3%)</td>
<td>1 (1.7%)</td>
<td>6 (10%)</td>
</tr>
<tr>
<td>M1</td>
<td>52 (86.7%)</td>
<td>1 (1.7%)</td>
<td>1 (1.7%)</td>
<td>6 (10%)</td>
</tr>
<tr>
<td>M2</td>
<td>37 (61.7%)</td>
<td>2 (3.3%)</td>
<td>4 (6.7%)</td>
<td>17 (28.3%)</td>
</tr>
</tbody>
</table>

Frequency table on presence/absence of each single tooth unilaterally left or right, in symmetry left and right, in upper and lower jaw. Number of non-syndromic index cases = 60.

**Figure 3** The most frequent patterns in the maxilla and mandible for 60 non-syndromic index patients.

**Figure 4** Pedigree of the 15-fold difference in number of missing teeth in family O-34.
There are several possible genetic mechanisms to explain these major differences in expressivity of the phenotype with the same molecular cause. First of all the phenomenon of incomplete penetrance should be mentioned. This was already suggested before by Mostowska et al. (2006) as they found a novel mutation of MSX1, which they thought responsible for the lack of 14 permanent teeth in their proband. However, they identified the same c.581C→T transition, localized in a highly conserved homeobox sequence of MSX1, which was also present in two healthy individuals from the proband’s family. Although this could have been the first described mutation of MSX1 responsible for oligodontia and showing incomplete penetrance, the authors state that their finding may also support the view that this common anomaly of human dentition might be an oligogenic trait caused by mutations/variants of several genes. Candidate genes include not only the known oligodontia/hypodontia genes, but also other genes playing a role in odontogenesis. Testing of this digenic or oligogenic aetiology has now become possible by systematically resequencing all known genes and additional candidate genes of families carrying mutations in known genes. One could question whether this could be interpreted as a mild form of pleiotropy, i.e. mutations in one gene giving rise to different phenotype severities.

Interfamilial variability could possibly be explained by variable effects of mutations in different genes (see earlier for ED) or different mutations in the same gene. For instance Vieira (2008) summarized different mutations to date (2008) located in the MSX1 gene, giving rise to different hypodontia phenotypes as well as orofacial clefting. Examples of hypodontia/oligodontia phenotype variability between unrelated individuals with mutations in the same gene can be due to the homozygous, heterozygous or compound heterozygous states, the type of the mutation, or to its location in the gene and its functional protein. Mutations located in different protein domains can have implications for the severity of the symptoms and switch the hypodontia/oligodontia phenotype from a syndromic to a non-syndromic type. This could be due to interactions at the protein level between proteins in different pathways. It was recently demonstrated in transgenic mice that genetic interactions between Pax9 and Msx1 regulate lip development and several stages of tooth morphogenesis (Nakatomi et al., 2010). In case of such causal relationships, variants in genes might also interact with partially redundant function, eventually securing (part of) the gene activities in case of mutations in genes crucial for survival.

As our results point to extremely variable expressivity of the hypodontia/oligodontia within families, where a clearly dominant phenotype segregates in the pedigree, the hypothesis is fed that there probably is one—or two?—major gene(s) causing hypodontia/oligodontia but that the rest of the genetic constitution of the individual probably contributes significantly to the expressivity of the phenotype, which can range from mild—or even absent—to extremely severe among family members carrying the same major gene mutation. This broad range in expressivity in different individuals within one family could be due to neighbouring genetic variants in coding genes and also in non-coding cis-regulatory elements that can act on different gene promoters. The latter phenomena can give rise to interaction between genes encoding two transcription factors (Nakatomi et al., 2010). Functional roles in tooth development have recently also been evidenced for miRNAs (Michon, 2011); miRNAs can upregulate or downregulate the expression of genes crucial for tooth development through translational regulation of mRNA.

As in tooth development, six major pathways were evidenced (Thesleff and Sharpe, 1997; Miletich and Sharpe, 2003) to contribute to the early epithelial-mesenchymal signalling, ultimately leading to tooth development, differentiation, and morphogenesis; common variants in the interacting partners in these pathways could also contribute to the variance of the phenotype.

Besides genetic factors, general epigenetic factors influencing gene expression have also to be taken into account, which even need other approaches, like the MZ co-twin design as proposed by Townsend et al., (2012).

Whether the hypothesized mechanisms above indeed occur or not can only be verified by performing carefully designed transgenic experiments in animal models. Therefore, each hypothesis generated from these observations should lead the clinician to the lab to scrutinize the mechanisms underlying these important biological questions. Only in this way, clinical problems can be solved in the lab, and only in this way, findings and discoveries can be disclosed and translated into clinically relevant increase of knowledge.

In view of the above, we can conclude for the future aetiological analysis of our LHOc of patients with syndromic and non-syndromic hypodontia/oligodontia, not only that candidate gene mutation analysis and exome sequencing will carry us forward by disclosing the pathogenic gene variants but that Genome Wide Association Studies and functional experiments might be necessary to unravel the mechanisms and complex (epi-) genotype–phenotype relation for tooth agenesis in humans.

Supplementary material
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