SEVERE HYPODONTIA: DENTAL, DENTOFACIAL, OSSEOUS AND GENETIC ASPECTS

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Colofon

ISBN: 978-90-393-5807-8
Cover:
Lay-out: Chris Bor, Medische Fotografie en Illustratie, AMC, Amsterdam
Printed by: Buijten & Schipperheijn, Amsterdam

SEVERE HYPODONTIA: DENTAL, DENTOFACIAL, OSSEOUS AND GENETIC ASPECTS

Ernstige hypodontie: dentale, dentofaciale, osseale en genetische aspecten
(with a summary in English)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op vrijdag 6 juli 2012 des ochtends te 10.30 uur

door

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geboren op 28 september 1973
te Utrecht

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1 General introduction and aims of the study

General introduction

The number of teeth is commonly used as an important indicator for oral health. Already in 1992 the World Health Organization has proclaimed that one of their goals for the year 2000 was that people should have a functional, esthetic dentition of at least 20 teeth throughout life and can go without some sort of dental prosthesis.[1] This is not just a challenge for underdeveloped countries and an ambitious goal, also considering the fact that in the year 1992 an estimated 23% of the Dutch population was fully edentulous in the maxilla and/or mandible. Although this number is rapidly declining, in the years 2000 and 2009 the corresponding numbers were still approximately 16% and 12%, with marked regional and decreasing gender differences (Statline, CBS, 2010).[2]

Most of these patients will have had a full natural dentition at one moment in time and have lost their teeth because of caries and / or periodontal disease. However, this is not as obvious as it may seem. In some cases teeth may not have developed properly and have been absent from birth. The process of tooth development is called odontogenesis.

Odontogenesis is quite a complex process. It consists of a series of events by which teeth develop from embryonic cells that derive from mesenchymal and epidermal tissues and subsequently grow, before erupting into the mouth. Tooth development resembles the development of other organs such as glands and hair, both morphologically and molecularly. Odontogenesis and more specifically tooth morphogenesis (its shape) is regulated through a large number of molecules that are organized in signaling networks.[3] These molecules initiate reciprocal interactions between epithelial and mesenchymal cells that cause the ectoderm to thicken, tooth buds to grow and fold and eventually form the complex shape of a tooth crown.[4] The study of these processes is the domain of expertise of developmental biologists. Their field is growing rapidly and new technologies emerge on a constant basis, helping us understand how dental abnormalities come about, and in the future may even help us to grow teeth.[5, 6]

Because of the many factors involved, odontogenesis is a rather precarious process that can easily be disturbed and disrupted as a result of various endocrine, local, environmental and genetic factors.[3] An endocrine, hormonal cause for hypodontia is for example idiopathic (hypo) parathyroidism.[7] Trauma or cysts in the apical area of the alveolar process are considered local causes.[8] Environmental factors include virus and yeast infections (i.e. Rubella[9] and Candida), exposure to certain toxins (i.e. dioxin)[10], and radio- and chemotherapy during childhood. For example, nearly all patients from a Dutch population of 40 who underwent allogenic hematopoietic stem cell transplantation for hematological malignancies developed dental disturbances, especially those who were younger than 3 years of age when treatment commenced.[11] Also, 50% of surviving children with a rhabdomyosarcoma who had radiation

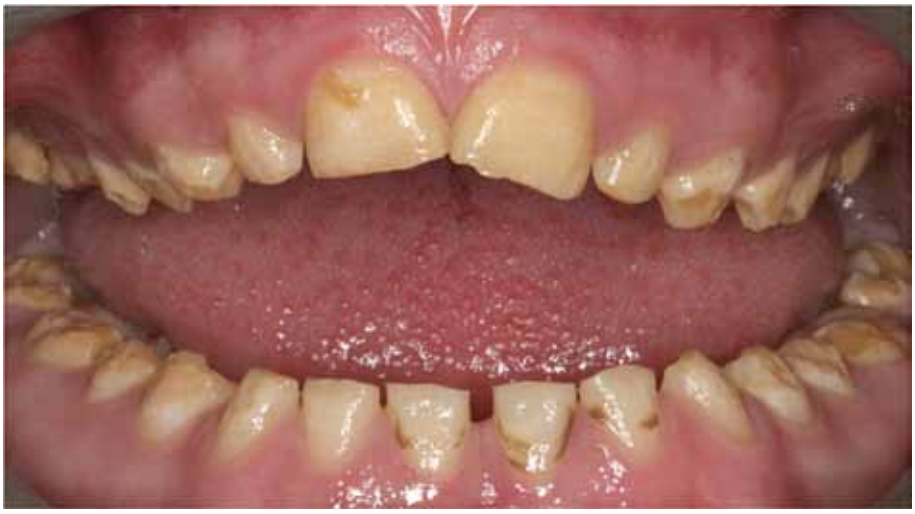

Figure 1 The genetic factors that disturb odontogenesis are comprehensive and mutations in a large and rapidly growing number of genes have been identified as the causes of dental abnormalities such as amelogenesis imperfecta. Amelogenesis imperfecta is a hereditary condition in which the dental enamel does not develop properly in the whole dentition. In this case, the poor condition of the enamel caused chipping with the lower molars and bad esthetics for the front teeth.

therapy to the head or neck during childhood present with dental abnormalities.[12] It is hard to distinguish between the original illness and the therapeutic measures as the actual cause of the dental sequelae.

The genetic factors that disturb odontogenesis are comprehensive and mutations in a large and rapidly growing number of genes have been identified as the causes of dental abnormalities such as amelogenesis and dentinogenesis imperfecta (figure 1), dentin dysplasia's and anomalies of teeth number.[6, 13-15] The latter is the general topic of this thesis and will be discussed in more detail.

Numerical anomalies in tooth development: hyperdontia and hypodontia

With respect to numerical anomalies in tooth development a distinction between hyper- and hypodontia can be made, either in combination with or not in combination with a syndrome. *Hyperdontia* (ICD-10: K00.1) occurs singly, in multiples, unilaterally or bilaterally, in the maxilla, in the mandible or both. It has a prevalence in the permanent dentition that varies from 0.1 – 3.8 %, depending on the population studied.[16] The most common presentation of hyperdontia is the so-called mesiodens; an extra, peg-shaped tooth that is commonly located between the 2 maxillary central incisors. Extra teeth in the molar area are called paramolar teeth, those located posterior to the third molar are referred to as distomolar teeth. Although cases of multiple supernumerary teeth have been reported they are rare and seldom seen without associated diseases or syndromes caused by genetic disturbances such as Gardner's syndrome (*APC* gene), cleidocranial dysostosis (*RUNX2* gene) and tricho-

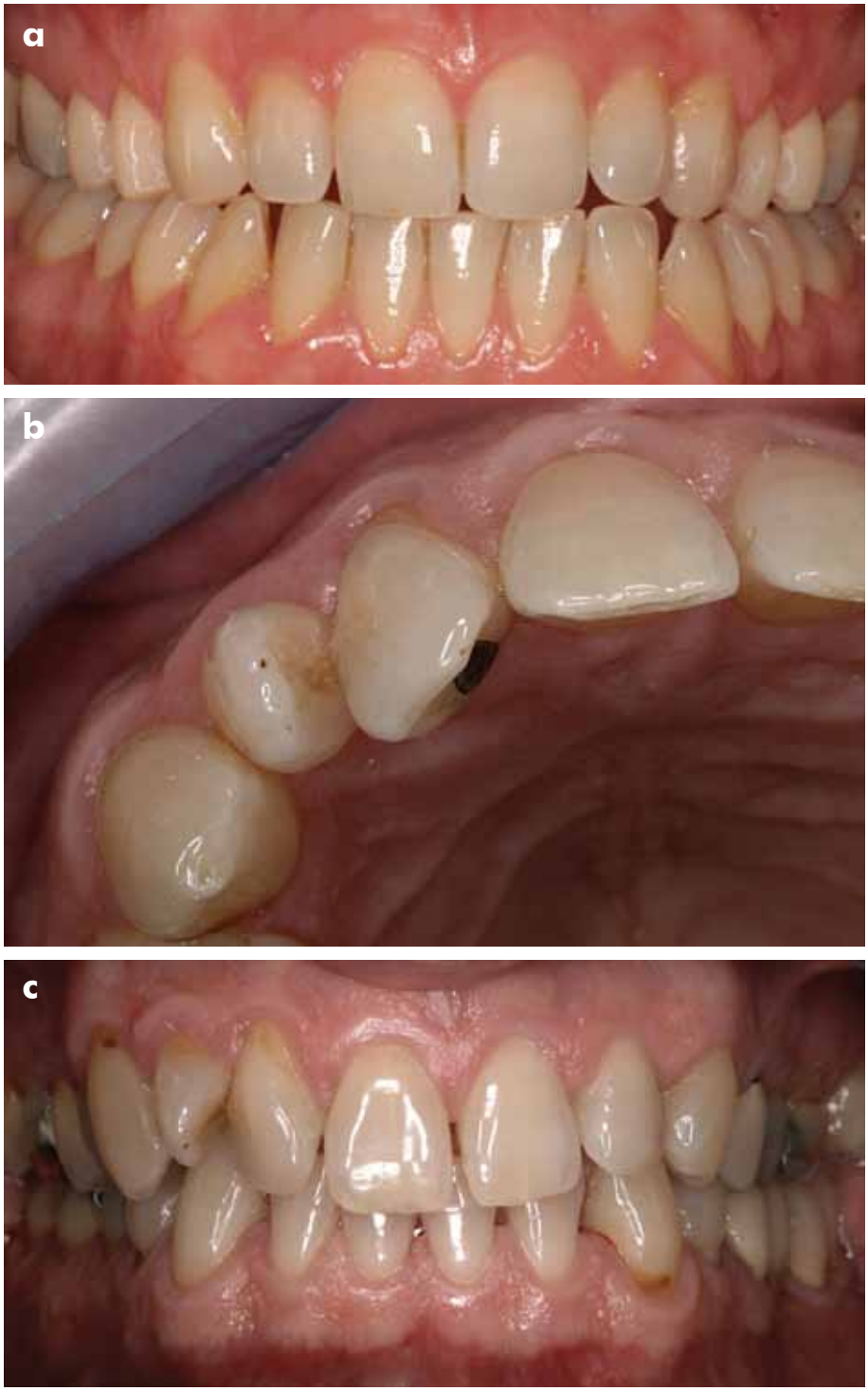

Figure 2a A supernumerary permanent mandibular incisor is a rare finding (2a). **b-c** A supernumerary right lateral incisor is relatively more common.

rhino-phalangeal syndrome (*TRPS1* gene).[8, 16, 17] In uncommon cases 'extra' teeth or tooth like structures can develop outside the mouth (i.e. in the ovaries, lungs or orbit). They are referred to as teratomas.

Subjects with supernumerary teeth generally have larger teeth (macrodontia) than controls.[18-20] Restorative treatment is usually uncomplicated and may consist of extraction of the supernumerary teeth, although on occasions they fit in reasonably well in the dental arch (figure 2a-c). Hyperdontia falls beyond the scope of this thesis.

Hypodontia (ICD-10: K00.0), the congenital absence of one or more permanent teeth, is the most common developmental anomaly in man. It has a prevalence of 5.5% in Europeans, with a preference for women compared to men (1.37:1).[21] There are racial differences with respect to the prevalence of hypodontia. In the Caucasian race the second mandibular bicuspid and maxillary lateral incisor are the most common absent tooth type, whereas in Asians the first mandibular incisor is the most frequently congenitally absent tooth.[22] In case of *anodontia* all permanent teeth are missing, which is extremely rare. By definition, *oligodontia* is the congenital absence of 6 or more permanent teeth, excluding the third molars.[23, 24] The prevalence of oligodontia in Caucasian populations in North America, Australia and in European countries is estimated to be 0.14%, and is also more common in women than in men.[21] Oligodontia can occur as an isolated non-syndromic condition (figure 3) or as part of a syndrome. The more teeth are missing, the greater the chance of systemic involvement.[25] The online database on Mendelian disorders (OMIM) makes reference to 103 syndromes associated with hypodontia (search data 4-3-2012) such as ectodermal dysplasia, incontinentia pigmenti, Down, van der Wouden, Rieger, oto-palato-digital, oro-facial-digital and oculo-facial-cardio-dental syndrome.[26, 27] However, for the studies in this PhD thesis we have excluded syndromal hypodontia patients[1*]. They constitute a broad, separate group with co-pathology. Syndromal hypodontia patients generally miss more teeth than those with hypodontia not associated with a syndrome.[23]

Relevance and reference

The scientific study of any subject requires a justification of the time, attention and money spent on researching it. With respect to the subject of hypodontia several issues can be raised. In the first place, and foremost, hypodontia is a common finding, so it affects a relatively large number of people, imposing complex restorative problems upon the dental profession. In the second place, the consequences of (severe) hypodontia on the daily living

1* In chapter 6 of this PhD thesis a family with a known MSX1 gene mutation is associated with hypodontia and orofacial clefting. For the sake of semantics, orofacial clefting is not considered a syndrome. It is a congenital abnormality that can be part of a syndrome. Hence, this MSX1 family is non-syndromal because it is not a combination of characteristics recognized as a specific syndromal disorder. On the other hand, they present with 2 congenital abnormalities, based on a common cause. In this sense one could argue that in this specific family, it can be considered syndromal.

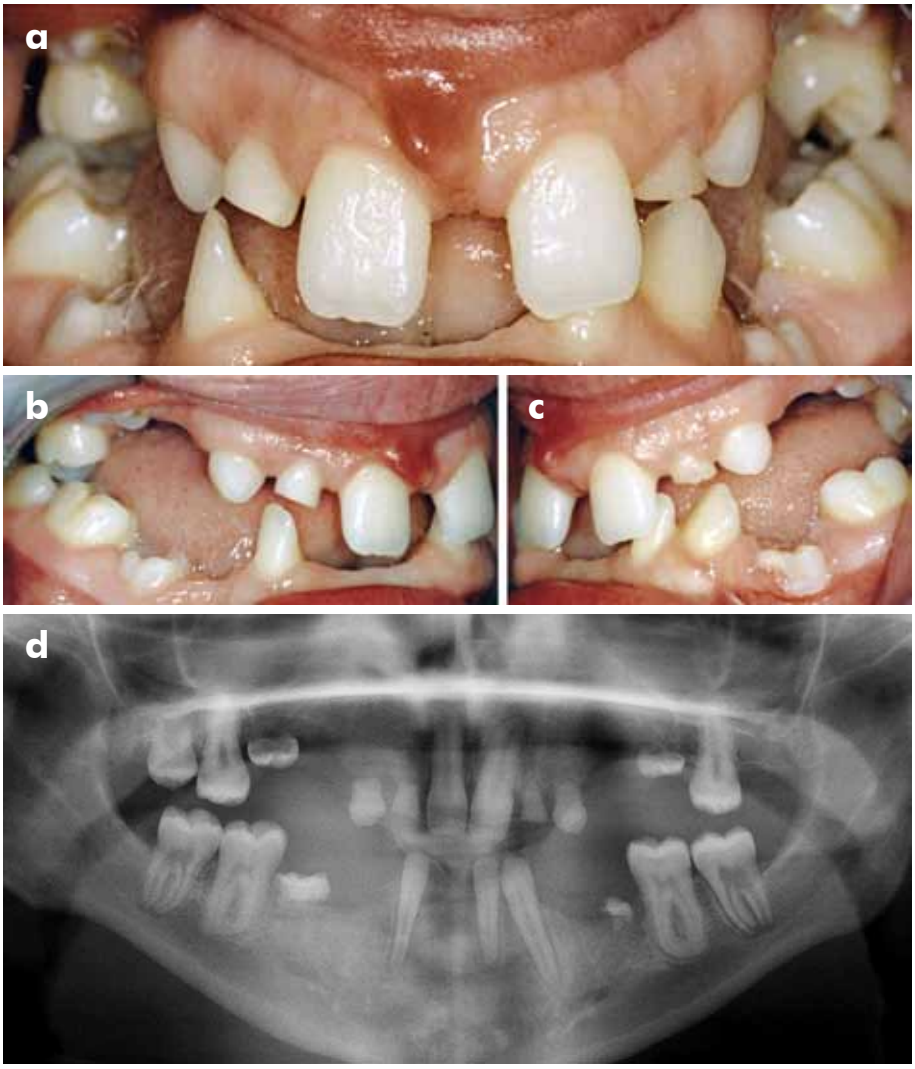

Figure 3 Severe non-syndromic hypodontia with functional and aesthetic consequences during pre-adolescence (TAC overall score 030.094.025.026[66, 67])

of subjects suffering from it can be substantial. Depending on the number and location of the missing teeth, masticatory, speech and aesthetic problems arise. Consequently, psychosocial problems are not uncommon and hypodontia influences quality of life.[28, 29] Finally, the costs that are associated with the restorative treatment of patients with severe hypodontia are substantial[30] and although dental treatment seems to effectively improve quality of life, the cost-effectiveness of various treatment modules has yet to be established.[28, 29, 31-33]

In reference to patients who lack teeth because of caries or periodontal pathology some similar problems are encountered, but there are differences. To name but a few, hypodontia patients have never experienced the benefits of a full set of teeth. From clinical experience

we have noticed that they adapt (more) easily to removable prosthetic appliances. Treatment of hypodontia patients covers a long period of time, running from early childhood to post-adolescence, after which lifelong follow-up and maintenance is indicated. Treatment of tooth loss is generally performed in a more confined period of time. An inter-disciplinary approach is advocated.[34] Dental treatment must in principle not inhibit dentofacial growth and dental implants act as ankylosed teeth.[35] This excludes their application in hypodontia patients until growth and development has arrested. Only in those severe hypodontia casus where esthetics is highly compromised at a young age, growth inhibition can possibly be accepted, for example by the use of an overdenture. Loss of multiple teeth due to caries or periodontitis seldom occurs during childhood these days. Lack of anterior teeth and misalignment of teeth cause unaesthetic malocclusion that stigmatizes people and lowers their self esteem (figure 3). This is especially valid during emotionally vulnerable periods in life such as during puberty in hypodontia patients.

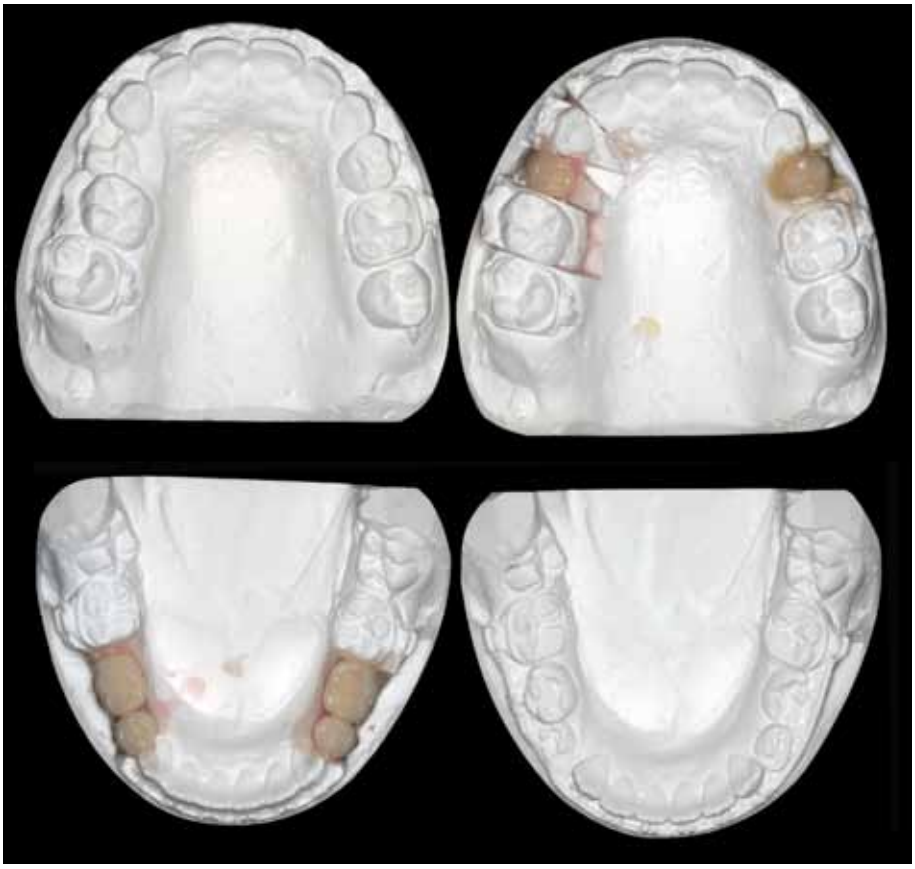

Figure 4a Oligodontia. Congenitally missing first and second bicuspids in the maxilla and in the mandible (TAC overall score 024.024.024.024[66, 67]). Eight missing teeth. Model planning of orthodontic and restorative treatment; not very complex.

Clinical challenges: the management of patients with (severe) hypodontia

People in the Netherlands with a severe developmental or acquired disturbance within the orthognathic system can apply for special dental care when they cannot attain or maintain oral function to the same degree as people in whom these afflictions are absent.[36] Consequently, people with 'oligodontia' may qualify for reimbursement of costs for dental treatment through the National Health Insurance Scheme ('Mondzorgwet') and the term 'oligodontia' is readily used among dentists and legislators. In order to be able to compare the data presented in this thesis to those of others, occasionally we have used 'oligodontia patients' as our subject of study. However, it is important to realize that there is no sound rationale for a cut of point of 6 missing teeth, nor for the exclusion of the third molars. The mere number of missing teeth is insufficient to describe the impairment and restorative challenges of a case, as may be illustrated by figures 4a-b.

We generally prefer the term 'severe hypodontia' to oligodontia in which 'severe' refers to the complexity of the restorative challenge. But what is severe? This is more dependent upon the distribution and location than on the mere number of missing teeth. Dentofacial growth and characteristics, in both vertical and sagittal dimensions, play a role. Hypoplasia

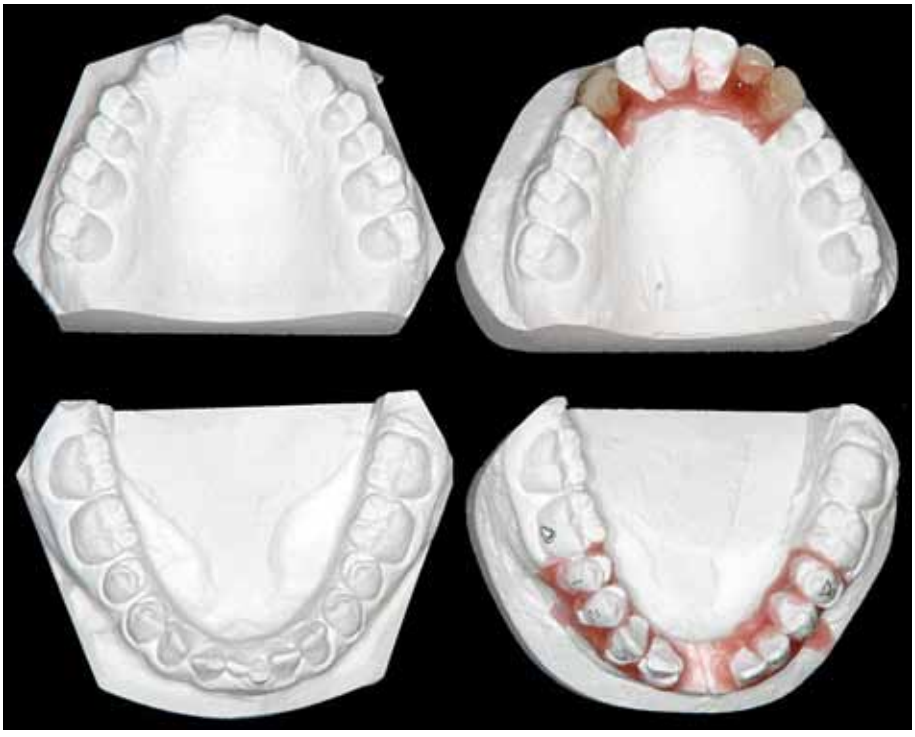

Figure 4b Hypodontia. Congenitally missing left maxillary lateral incisor and bilaterally missing cuspids, as well as two central mandibular incisors (TAC overall score 004.006.001.001[66, 67]. 'Only' five missing teeth. Model planning of orthodontic and restorative treatment; rather challenging because of the aesthetic component involved.

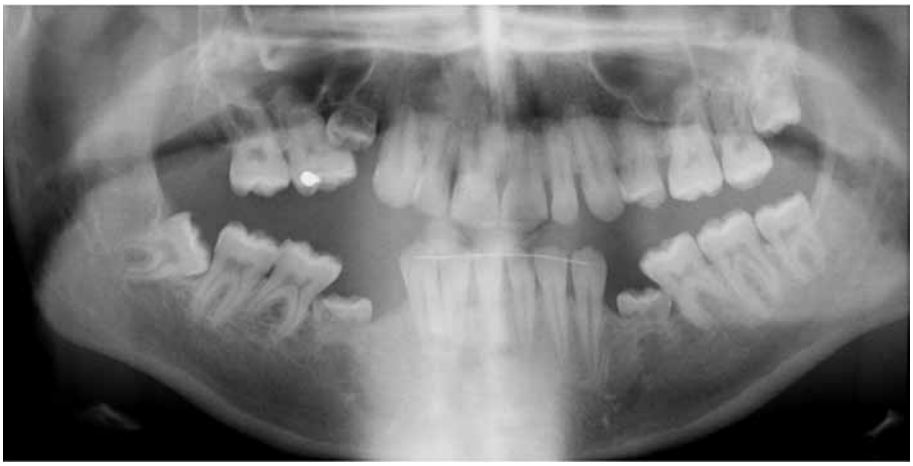

Figure 5 Secondary retained primary molars have inhibited vertical development of the alveolar process (TAC overall score 024.016.024.024)[66, 67]. Removal of the maxillary right primary molar even caused a nasal-oral communication. Vertical bone augmentation is an unreliable surgical procedure that is best avoided when possible and the primary molars should have been extracted in an earlier stage.

of the jaw and underdevelopment of the alveolar process are often seen in case of multiple missing teeth, which may cause specific extra oral features. Variations in tooth morphology occur. Tooth size discrepancy and variations in tooth shape (i.e. microdontia, taurodontism and talon cusp) are common findings in severe hypodontia.[20, 37-39] Also girls and boys with hypodontia show a significant delay in dental development which has to be reckoned with.[40, 41] Hypodontia has been associated with impaction of the maxillary cuspid, especially when the lateral incisor is missing.[42, 43] Maxillary bicuspid-cuspid transposition has been associated with hypodontia as well.[44] The retention of primary teeth is another issue. These may serve a purpose and be functional for a long time. On the other hand, secondary retained primary teeth prevent vertical development of the alveolar process.[45, 46] When to use and when to lose them (figure 5)?

These are all additional factors that may complicate orthodontic, orthognathic / pre-implantologic surgery and restorative treatment, such as their timing, sequence and decision making in general. What armentarium to use, which treatment strategy to choose?

Scientific challenges: comparing treatment strategies in severe hypodontia

Clinical decisions are made on the basis of the best available scientific evidence, taking clinical experience and the patients' unique circumstances and preferences into account (Evidence Based Medicine).[47] The result of this process should be the predictable improvement in patient care. High quality evidence can establish benefits (among which efficacy) and harms of therapies. To be able to choose one treatment strategy over another, randomized controlled clinical trials are needed. For severe hypodontia those studies are lacking and the

level of evidence of the available clinical studies is generally low (i.e. personal observations, retrospective observational studies, case reports and case series). Why is this?

Long treatment span

Treatment of patients with severe hypodontia takes a long time to complete; in fact a reasonable potential endpoint is not reached until the cessation of skeletal growth and the provision of more or less 'permanent' tooth replacement. As a consequence, randomized clinical outcome trials in severe hypodontia will be lengthy, time consuming and very expensive. Problems with compliance and high dropout rates may also arise.

In addition, treatment strategies evolve over the years. One only has to consider the impact of implant treatment, adhesive techniques, improved diagnostic means (i.e. 3-dimensional imaging techniques) on restorative dentistry over the last decades. Patients with severe hypodontia have benefited from these developments as well and it has opened an array of new opportunities for functional and esthetic oral rehabilitation. Today, implants can be utilized to transmit orthodontic forces to teeth and facial bones. They can be applied during suture expansion and distraction osteogenesis or plainly as orthodontic anchorage. The latter can be very helpful in case of many missing teeth, where a lack of anchorage is a common problem.[48, 49] In former times, removable prosthetic appliances were the standard of care in case of severe hypodontia. If ample tooth abutments were present, large, irreversible and somewhat daring fixed partial dentures were made at considerable biological expense.[50] A lot of sound tooth material needed to be sacrificed (figure 6). Applying adhesive techniques can preserve sound tooth substance and implants can often retain fixed partial dentures on the position of the congenitally missing teeth at a lesser biological toll. At least that is what we presume.

New strategies tend to replace old strategies readily, even though their efficiency and even potential harms are not yet well understood, let alone be evaluated over longer periods of time. Personal observations and anecdotal experience come available swiftly, but tend to overestimate efficacy. In this respect, the challenges during the evaluation of treatment strategies in case of severe hypodontia are not unlike those encountered in alveolar cleft treatment studies. New surgical techniques emerge, whereas the final benefits can only be judged after adolescence or later, maybe even through life.

Definition of primary and secondary outcome measures and endpoints is difficult

On what grounds should treatment be evaluated? How do you compare implant- and non-implant strategies? Generic outcome measures such as Quality of Life Measures (i.e. OHIP-49 and OHIP-14[51]) may be useful, but have only recently been validated and become available. An additional problem is that such measures are not well suited for pre-adolescent

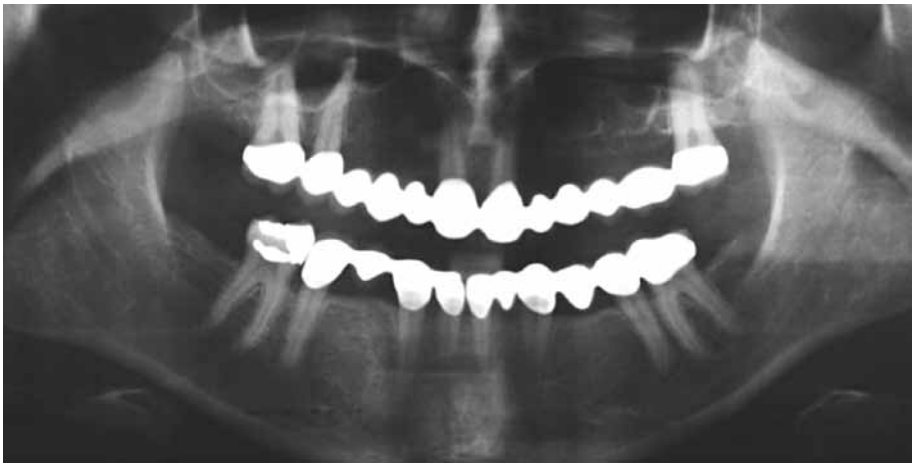

Figure 6 Comprehensive restorative treatment of a patient with severe hypodontia (TAC Overall score 078.094.074.07466, 67) consisting of several porcelain fused to metal fixed partial dentures on few abutment teeth.

subjects and would require adjustment for various age groups. The objective measurement of oral function could be an option.[52] But what biological and financial price and risk of (co-) morbidity is acceptable for a small improvement in oral function?

Blind assessment

Blind assessment while evaluating clinical outcome in treatment of severe hypodontia will be hard to achieve. Patients cannot easily be blinded for the type of treatment that they receive, nor can the assessors for the treatment mode that they are appraising.

Lack of randomization

This is the most important and problematic issue. Effective randomization would minimize the risk of unexpected factors influencing the outcome (confounding). It is however difficult to achieve in cases of severe hypodontia. Although hypodontia is the most common developmental disorder in man, patients who miss a considerable number of permanent teeth are relatively rare. Consequently it is hard to constitute large groups of patients (adequate sample size and statistical power). Most of all, on the basis of the present knowledge, the heterogeneity of (severe) hypodontia patients makes it difficult to produce reasonably comparable subsets of patients at baseline to begin with.

General aims of the study

The research that is presented in this PhD thesis focuses on the latter issue. The general aim has been to characterize patients with (severe) hypodontia on several aspects; dental, dentofacial, skeletal and genetic. Eventually this should aid in the clinical evaluation of former, current and future restorative treatment strategies in severe hypodontia. For this purpose, some new methods of evaluation were developed and tested and already existing, validated tools in other areas were applied on populations of patients with severe hypodontia.

Specific aims of the study: questions of investigation

Dental aspects

In order to classify patients with severe hypodontia counting the number of missing teeth is insufficient, as already stated. The position of the absent teeth is also relevant. A retrospective study was performed to characterize a population of non-syndromic severe hypodontia patients and identify patterns of tooth agenesis. A means of describing the position and number of missing teeth throughout the mouth in a single number is proposed (Chapter 2). It can be used for epidemiological studies and in multicenter studies (Appendix I). It may also serve as a biomarker of genetic disorders.

Dentofacial/Skeletal aspects

One could assume a relationship between numerical aberrations of permanent teeth and craniofacial development and morphology on several theoretical grounds.[53] Some genes are involved in the process of both tooth and craniofacial morphogenesis.[54, 55] Dysfunction of such genes may hamper the development of teeth as well as craniofacial structures. In addition, gene mutations can predispose for specific patterns of agenesis. For example, a reduced lower anterior face height could be the result of decreased posterior occlusal support. Finally, completion of crown formation and root development is important for the development of the alveolar process. Hence, tooth agenesis could result in regions with reduced alveolar ridge dimensions.

A case-control study to compare cephalometric measures between hypodontia patients and patients without congenitally missing teeth was set up. The aim was to identify and characterize groups of hypodontia patients that represent distinctive dentofacial features by means of cluster analysis (Chapter 3).

Osseous aspects

As stated above, genetic defects may disturb both odontogenesis and craniofacial bone formation. The latter is important since contemporary restorative treatment of patients with hypodontia will frequently include implant treatment.[56] The implants are placed in the alveolar bone and serve to support crowns and bridges or removable prosthetic appliances. Therefore the quality of the alveolar bone is of paramount importance . The successful application of dental implants relies heavily on the capability of the host bone to achieve and maintain intimate bone-to-implant contact during initial healing and subsequent implant loading.[57] Whether or not 'bone quality' in hypodontia patients and non-hypodontia patients is similar is not known and conflicting data in the literature are presented with respect to the efficacy of dental implant treatment in severe hypodontia.

A radiographical, case-control study was set up to study parameters of mandibular trabecular bone structure in persons with and without (severe) hypodontia (Chapter 4). In another, retrospective study the result of implant treatment in patients with severe hypodontia was evaluated (Chapter 5).

Genetic and morphological aspects

When tooth development is disrupted a wide variability of clinical features may occur, for example in patterns of tooth agenesis, variation in tooth morphology and tooth size dimensions.

Familial hypodontia may show an autosomal dominant, autosomal recessive or an X-linked mode of inheritance.[6, 15, 58, 59] Increasing evidence accumulates that genes play an important role in the etiology of isolated, non-syndromic hypodontia, such as the homeo-domain protein MSX1 and the paired-domain transcription factor PAX9.[15] A number of other genes have been associated with hypodontia as well, among which are the IRF6, TGFA, FGR1, AXIN2, EDA, EDAR and quite recently the EDARADD and WNT10A genes.[8, 14, 15, 60-64] However, there appears to be incomplete penetrance: some patients with a gene mutation have hypodontia / oligodontia while others with the same gene mutation don't. Furthermore, the phenotypes appear very heterogeneous, in the number of teeth that are missing, and in the tooth types that are absent. Also, carriers in the same family exhibit significant variability with regard to the number of teeth, their location and symmetry within the dental arch. A better understanding of the genetics may contribute to a better diagnosis of some multiple congenital (anomaly) syndromes involving teeth and improved insight in the genotype – phenotype relationship.[13] Morphological tooth traits, parameters of tooth dimension and agenesis patterns may serve as biomarkers of genetic disorders.[65]

Therefore, a novel technique to geometrically evaluate teeth in 3 dimensions has been developed to study genotype-phenotype associations in hypodontia. It was applied to compare features of tooth dimension in a series of patients with an established nonsense MSX1 mutation with non-affected controls (Chapter 6).

To get insight in the genetic heterogeneity of isolated hypodontia in the Dutch population. For this purpose a cohort of hypodontia patients were tested for mutations in the MSX1, PAX9, AXIN2, IRF6 and WNT10A genes (Chapter 7).

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Published in: Int J Prosthodont 2007; 20: 409-413.

2

Patterns of missing teeth in a population of oligodontia patients

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Abstract

The purpose of this study was to characterize a population of oligodontia patients and identify patterns of tooth agenesis.

A total of 116 patients with non-syndromic oligodontia were studied, and the Tooth Agenesis Code (TAC) per quadrant was calculated. Oligodontia was defined as the congenital absence of 6 or more permanent teeth, excluding the third molars. The TAC is a unique number, consistent with a specific pattern of tooth agenesis. The authors suggest the use of an overall Tooth Agenesis Code by means of which the dentition throughout the mouth can be presented in a single number. The pattern of agenesis of each quadrant remains recognizable. Frequency analysis was used to study the prevalence of various patterns.

There was a great diversity of TACs. In the maxilla, agenesis of both premolars and the lateral incisor or mere presence of the central incisor and first molar were the most common patterns. In the mandible, agenesis of the second premolar or both premolars occurred most frequently.

No single pattern of agenesis occured more than twice when the full mouth is viewed. Hence, the presentation of the dentition in oligodontia is very heterogeneous. Evaluation of treatment strategies in oligodontia patients is a methodologic challenge because homogenous, comparable subgroups of patients are not available.

Introduction

Oligodontia is generally defined as the congenital absence of 6 or more permanent teeth, excluding the third molars.[1, 2] The prevalence of oligodontia in Caucasian populations in North America, Australia and in European countries is estimated to be 0.14 %, with a higher prevalence in women than men. It is more common in women than in men.[3] Oligodontia can occur as an isolated non-syndromic condition or as part of a syndrome, such as ectodermal dysplasia, incontinentia pigmenti, Down syndrome, and Rieger syndrome.[4] In the last decade, more light has been shed on the multi-factorial etiology of oligodontia. Endocrine, local, environmental and hereditary factors of congenitally missing teeth have been suggested and identified, the latter through molecular genetics. Recently, mutations in the genes MSX1 and PAX9 that encode transcription factors were demonstrated to be associated with isolated, non-syndromic oligodontia.[5-9]

Oligodontia has a wide diversity of manifestations.[10] Depending on the number and location of the missing teeth, masticatory, speech, and esthetic problems may arise. Van Wijk and Tan recently proposed a practical procedure for assigning unique values for all possible combinations of absent teeth: the Tooth Agenesis Code (TAC), which can be used to describe patterns of missing teeth.[11]

The aim of this study was to characterize a population of non-syndromic oligodontia patients and use the TAC to identify patterns of tooth agenesis.

Materials and methods

Patients

The Utrecht Medical Center is an academic, teaching hospital that with a center for prosthodontics and special dental care. All patients referred to the center by general practioners in the Netherlands between 1990 and 2006 who were classified at their first visit as having oligodontia were selected from the hospitals' database (n=224). Oligodontia was defined as the congenital absence of 6 or more permanent teeth, excluding the third molars.[1] The patients' charts were reviewed and the diagnosis of oligodontia was verified from a panoramic radiograph. When no panoramic radiograph was available (eg, data on microfilm) or when the quality of the radiograph did not allow adequate interpretation as to the presence or absence of permanent teeth, the patient was discarded from the study population (n=50). Patients were originally misclassified (eg, hypodontia, tooth extraction as opposed to congenital absence) in 36 cases. Patients with oligodontia as part of a syndrome are usually missing more teeth than patients with an isolated type of oligodontia.[2] Hence, patients with oligodontia as part of a syndrome were excluded from the study also (n=22).

Consequently, from the 225 patients on the original list, 116 remained for data analysis (66 women, 50 men).

Permanent teeth that were hypoplastic and/or radiographically apparent, but not (yet) erupted permanent teeth were considered as "present". Tooth determination was performed by 2 clinicians. Cases were re-evaluated when there was initial disagreement as to which tooth was present or absent. Agreement was obtained through discussion. Absent teeth were registered by tooth number. The FDI tooth numbering system was used.[12]

Data analysis

Patient and clinical information were entered into a database application, which was designed for the study and was used to obtain a uniform data set (Access 2000, Microsoft). The TAC was calculated. The procedure and rationale for the TAC was previously described by van Wijk and Tan11, so is only summarized here:

- each missing tooth type is assigned a specific value.
- for each quadrant, the values are summed. In this manner, a unique value per pattern of tooth agenesis is calculated: the TAC. Reversibly, from each TAC, the unique combination of missing teeth can be deducted (Table 1).

In addition, the authors of the present study constructed a new variable (TACoverall) that was used to identify similar patterns of tooth agenesis throughout the mouth among different patients. This variable is composed of the TAC of each quadrant, as follows:

TACoverall = (TAC first quadrant x 109) +
(TAC second quadrant x 106) +
(TAC third quadrant x 103) +
(TAC fourth quadrant)

The returned value is a unique number in which, when displayed with thousands separators, the 4 underlying TAC scores remain recognizable (ie, 123.100.038.005; TAC first quadrant = 123, TAC second quadrant = 100, TAC third quadrant = 38, and TAC fourth quadrant = 5).*

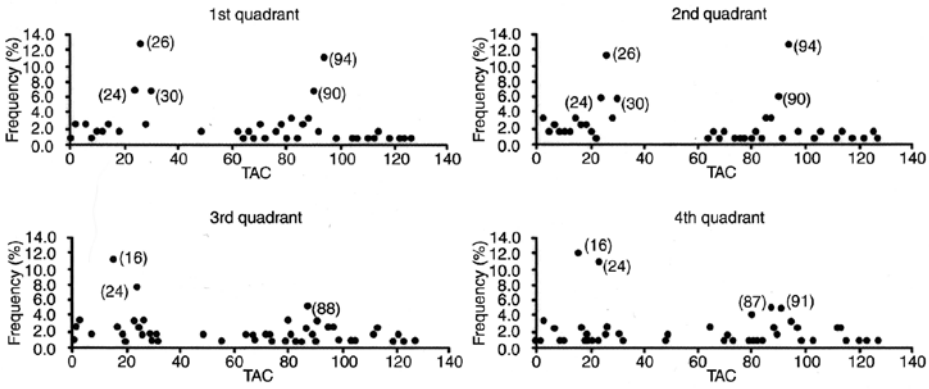

Figure 1 Tooth Agenesis Code per quadrant

Statistical analyses mainly consisted of descriptive procedures. Potential differences in the number of absent teeth among the male and female patients and between quadrants were tested by means of the independent samples t test and paired samples t test, respectively. A standard statistical program was used (SPSS version 11.0, SPSS).

Results

Numeric approach

A mean number of 12.5 teeth were missing in this population of oligodontia patients (range 6 to 26). Table 2 shows the tooth agenesis by tooth type.

The lateral incisors and second premolars in both the maxilla and mandible were the most commonly absent teeth. Agenesis of the maxillary central incisor is quite rare and to a lesser degree, congenital absence of the mandibular canines and maxillary molars was seldom seen.

Table 1 Values for absent teeth*

Tooth	Assigned value when absent
Central incisor	1
Lateral incisor	2
Cuspid	
First premolar	8
Second premolar	16
First molar	32
Second molar	64
Third molar^	128

* For example: absence of the lateral incisor, second premolar and second molar yields the TAC value 82 (2 + 16 + 64) for that particular pattern of agenesis in any quadrant.
^ Not included in this study
Some common TACs
16: agenesis of the second premolar
24: agenesis of the first and second premolar
26: agenesis of the lateral incisor, first and second premolar
94: agenesis of the lateral incisor, cuspid, first and second premolar and second molar

Table 2 Prevalence of absent tooth types (n=116 patients)

FDI tooth number	Frequency
11	0.9%
12	71.6%
13	45.7%
14	72.4%
15	72.4%
16	13.8%
17	52.6%
21	0.9%
22	70.7%
23	47.4%
24	67.2%
25	71.6%
26	11.2%
27	49.1%
31	56.9%
32	38.8%
33	20.7%
34	45.7%
35	74.1%
36	17.2%
37	46.6%
41	54.3%
42	43.1%
43	21.6%
44	39.7%
45	76.7%
45	16,4%
47	48,3&

No statistically significant difference was evident in the number of absent teeth among male and female patients (p=0.38) or between the left and right (p=0.18) or maxillary and mandibular quadrants (p=1.0).

TAC

All TACs and their prevalence are displayed per quadrant in Fig. In the maxilla TAC 26 and 94 were the most common patterns of agenesis. In addition to the functional problems, esthetic aspects play a prominent role in both TACs in the maxilla because of the missing lateral incisor. For the mandible TACs 16 and 24 showed the highest prevalence.

Full-mouth agenesis pattern

The various combinations of missing teeth were calculated as the TACoverall value and is displayed in Appendix II of this PhD thesis. The following TACoveral values occurred twice: 24.024.088.024, 26.026.024.024, and 88.088.088.088. No single pattern occurred more than twice.

Symmetry of agenesis

There was symmetry of agenesis between the right and left side in the maxilla and mandible, in 49.1% of the cases. Symmetry between 2 antagonistic quadrants was relatively rare: 9.5% for the right and 4.3% for the left side. In cases of symmetry in the maxilla, the TACs 26, 94, and 24 were the most common patterns (19.3%, 12.3%, and 8.8% respectively). TACs 26 and 24 represent absence of the premolars with the lateral incisor either absent or present. In TAC 94 only the first molar and central incisor are present. When there was symmetry in the mandible, TACs 16 and 24 showed the highest prevalence (17.5% and 10.5% respectively). TAC 16 represents absence of only the second premolar.

Discussion

There are various ways to categorize numeric anomalies of teeth. A trimodal classification would be to group cases into anodontia, hypodontia, and hyperdontia, with 'syndromic' or 'non-syndromic' as subclasses.[13] According to the common definition, oligodontia is a subpopulation of both the hypo- and anodontia group in which 6 or more teeth are genetically missing, excluding the third molars.[1] In the literature, patients who suffer from oligodontia are usually characterized in terms of number of absent teeth, not the patterns of absent teeth. However, this is not always practical, for various reasons:

Hereditary factors play a role in oligodontia, and the scientific understanding of their significance is increasing. The more specific the cause-effect relationship, the better the evidence. Oligodontia presents in numerous clinical variations (patterns) as a result of

different amounts and locations of missing teeth (see Fig 1 and the appendix II), with tooth size variations and tooth deformaties as coexisting traits.[1] To expand on the knowledge of hereditary factors and their penetration, one needs to pinpoint different presentations of oligodontia (the clinical phenotype) to specific genetic defects. A useful classification of tooth agenesis takes into account both the phenotypes and genetic background. Thus, simply describing oligodontia in terms of the number of congenitally missing teeth is inadequate.
The prosthetic rehabilitation of patients with oligodontia is likely to become more comprehensive with a higher number of absent teeth. However, from a restorative point of view, the distribution of the missing teeth and the missing tooth types are relevant too, if not even more important. When anterior teeth are missing, esthetic features of treatment become more important. When too many adjacent teeth are missing, fixed partial dentures on natural teeth are not a viable treatment option. Thus, the mere number of absent teeth does not necessarily reflect the restorative complexity, lack of function or esthetic consequences of each individual case.
Treatment of oligodontia is typically multidisciplinary. A wide and expanding range of prosthetic, orthodontic, and surgical therapies are currently employed. Consequently, dental treatment for people who suffer from oligodontia can be quite expensive.[14] However, studies addressing the (cost-) effectiveness of different treatment strategies are lacking and are often of a retrospective nature or are simple case series or case reports.[15-17] Treatment strategies can only be compared once the therapies under investigation are specific and well defined. In addition, the clinical situations for which they are employed need to be more or less similar. The number of missing teeth will not suffice for this purpose.

Cluster analysis and principal component analysis to identify clusters of absent teeth in hypodontia patients were used in a previous study.[18] This is a better approach than the numeric classification but cannot be used to classify individual cases. Therefore, in addition to a numeric description, the authors choose the method described by van Wijk and Tan to characterize the population of oligodontia patients,11 which makes use of the TAC and allows individual patterning of absent teeth per quadrant. With a minor modification, it is possible to uniquely characterize tooth agenesis throughout the mouth in a single number. These numbers, in contrast to strings, superiorly facilitate data analysis. The values in Table 2, Fig 1, and Appendix II identify unique combinations of absent teeth, which are now readily available to other groups, for example for meta-analysis and/or for genetic research. The authors encourage other groups to publish data on oligodontia patients in a similar manner. The data of the present authors on oligodontia patients are in agreement with the findings in other studies in hypodontia patients. It is more common in women than in men.[3, 19] However, in contrast to Kirkham et al, no difference was regarding the number of missing

teeth among male and female oligodontia patients, perhaps because Kirkham included hypodontia patients as well.[18]

Congenital absence of the central incisor, mandibular canine and maxillary first upper molar is rare. A relationship has been proposed between tooth formation and innervation of the jaw. The pattern of tooth agenesis seems to follow the different neural fields.[20, 21] The maxillary lateral incisor and first or second premolar were missing in 67.2%-71.6% of all cases, without much difference in prevalence among the 3 tooth types.

TAC 26 in the maxilla, which corresponds with the agenesis of all 3 of these teeth, was the most common pattern of agenesis in the first and second quadrant (12.9% and 11.2% respectively). In TAC 94, the other common pattern in the upper jaw (right: 11.2%, left: 12.9%), only the central incisor and first molar are present. Both patterns of agenesis present functional and esthetic problems. Symmetry in the maxilla occurred in approximately half of the cases, in which again left/right symmetry of TACs 26 and 94 occurred the most often (19.3% and 12.3% respectively). Since all other TACs in the maxilla are relatively rare, therapy evaluation in oligodontia patients should best be focussed on treatment of TAC 26 and 94.

In the mandible, the second premolar was the most frequently absent tooth type in this group of oligodontia patients (right: 76.7%, left: 74.1%). The second premolar is the most common absent tooth in hypodontia in man.[3] TAC 16 which is the agenesis of the second premolar only, was the most frequently seen pattern of agenesis in the mandible (right: 12.1%, left: 11.2%). In contrast to the patterns in the maxilla, the esthetic component of this pattern plays a less prominent role. No frequently occurring patterns of missing teeth in oligodontia patients were identified.

The treatment strategy depends on the specific space requirements, the skeletal relation and the condition of the adjacent teeth. Orthodontic treatment with or without single implants in case of TAC's 16 and 24 are in general viable options when deciduous teeth cannot be maintained. Reports regarding the life span of lower deciduous molars without a permanent successor are not conclusive. An unpredictable life span of deciduous molars in oligodontia patients was described by Haselden et al.[22] In contrast, others conclude that retaining healthy deciduous mandibular second molars is a viable treatment alternative when evaluated radiographically.[23] They saw only little root resorption over a mean observation period exceeding 12 years. Autotransplantation can also be used to reposition permanent teeth to a position where they are most needed. This procedure has a good prognosis.[24, 25]

Conclusions

The presentation of the dentition in oligodontia is very heterogeneous. Thus, the evaluation of treatment strategies in oligodontia patients will be a methodologic challenge because homogenous, comparable subgroups of patients are not available.

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* For the convenience of the reader, an Excel spread sheet [Microsoft] that facilitates the swift back and forth calculations of TAC and the TACoverall scores can be obtained by contacting the author.

Published in: Clin Oral Investig 2010; 14: 467-477.

3 Dentofacial characteristics of patients with hypodontia

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Abstract

This study aims to identify distinctive dentofacial characteristics of hypodontia patients. For this purpose 189 young hypodontia patients (cases) were divided into subgroups, based on criteria from literature. Normalized differences between cases and controls were calculated for various parameters of dentofacial form. Subsequently cluster analysis was applied to disclose subsets of hypodontia patients with distinctive dentofacial features.

The ANB angle, interincisal angle and lower anterior face height were consistently significantly different among the subsets. Four clusters of patients with an increasing number of missing teeth and distinctive dentofacial characteristics could be identified. Patients in cluster 1 display a high angle facial pattern. Patients in cluster 2 and 3 exhibit markable dento-alveolar characteristics (a relatively small and a large interincisal angle, respectively). Patients in cluster 4 exhibited notable sagittal-skeletal discriminative features predominantly because of a retrognathic maxilla. The smallest nasolabial angle and lower anterior face height were seen in this cluster.

It is concluded that the anterior-posterior relationship between the jaws, the interincisal angle and the lower anterior face height are discriminative parameters of dentofacial form in hypodontia patients. Patients with hypodontia can be clustered in 4 groups, each with distinctive vertical-skeletal, dento-alveolar and sagittal-skeletal characteristics. This categorization of patients with hypodontia into meaningful groups may be useful for treatment planning, interdisciplinary communication and as a means of identifying groups of patients that qualify for reimbursement of costs. Other dental factors should be appreciated as well during restorative clinical decision making in patients with hypodontia.

Introduction

Hypodontia, the congenital absence of one or more permanent teeth, is the most common developmental anomaly in man. It has a prevalence of 5.5 percent in Europeans, with a preference for women compared to men (1.37:1).[1] The presentation of severe hypodontia is quite heterogeneous and identical patterns of tooth agenesis are rare when the whole dentition is considered.[2] Tooth size discrepancy and variations in tooth shape are also common findings in severe hypodontia.[3] They constitute additional factors that may complicate orthodontic and restorative decision-making. In addition, dentofacial aspects must be taken into account as well.

One could assume a relation between numerical aberrations of permanent teeth and craniofacial development and morphology on several theoretical grounds.[4] There is increasing understanding with respect to the molecular mechanisms during cell and tissues interactions. Some homeobox genes, among which the *MSX1* gene, bear relevance to the process of both tooth and craniofacial morphogenesis.[5, 6] Recently a new gene has been identified that, when mutated, causes severe hypodontia, short stature and increased bone density when (*LTBP3*).[7] It can be hypothesized that dysfunction of such genes may hamper the development of teeth as well as craniofacial structures. In addition, gene mutations can predispose for specific patterns of agenesis and more or less characteristic patterns of absent teeth have been described in patients with severe hypodontia.[8, 9] For example, mutation of the *PAX9* gene has been associated with agenesis of posterior teeth.[10] Furthermore a reduced lower anterior face height could be the result of decreased posterior occlusal support. Finally, completion of crown formation and root development has been considered to be of importance for development of the alveolar process.[11] Hence, tooth agenesis could result in regions with reduced alveolar ridge dimensions.

Several authors investigated whether or not subgroups of hypodontia patients differ on cephalometric measures of dentofacial form or differ from non-hypodontia patients.[4, 9, 12-23] Grouping of patients was performed on the basis of:

- the location of the missing teeth *e.g.* posterior, anterior or anterior/posterior missing teeth.[4, 15, 20] Groups of anterior missing teeth consisted of missing incisors[4] or missing incisors and cuspids.[15] Subdivision in uni- or bilateral anterior as well as uni- or bilateral posterior missing teeth were made[20];
- the jaw in which the teeth are missing, *e.g.* in the mandible, in the maxilla or both in the mandible and in the maxilla[21];
- the number of missing teeth, *e.g.* mild (2 to 5 missing teeth), moderate (6 to 9 missing teeth) or severe (ten or more missing teeth)[4, 15, 16] or 5 to twelve compared to thirteen to twenty-one missing teeth[22];

- severe hypodontia associated or not associated with a syndrome, *e.g.* severe hypodontia (six or more teeth missing) compared to severe hypodontia associated with hypohidrotic ectodermal dysplasia[12];
- the number of missing tooth types (incisors, canines, premolars and molars)[13];
- on the distinction between hypodontia and oligodontia by observation of typically and atypically missing teeth.[23]

It is hard to compare these, mostly explorative studies with each other in detail. Different anatomical landmarks, reference planes, angles and distances were used. Patient populations from various racial background and inclusion of cases with different degrees of hypodontia add to the problem. Control groups were occasionally rather small, cases and controls were not always age and gender matched or reference values for adolescents were presumed valid for older patients as well because reference values for adults were not available.[4, 15, 20] An additional problem with previous studies is that preconceived assumptions regarding factors that the authors thought to be of influence on dentofacial form were used to define subgroups of patients. In such an explorative approach, determinants of dentofacial form may be overlooked and the importance of co-existing determining factors may never be fully appreciated. In a more objective approach preconceived assumptions for grouping of patients should not form the basis for analysis but grouping should result from statistical analysis. So the question is can groups of patients who share skeletal features be identified by statistical analysis of the data? Cluster analysis is a useful statistical tool to partition data into subsets (called *clusters*) of subjects who share common traits, i.e. dentofacial characteristics, so that subjects from the same cluster are more similar to each other than subjects from different clusters.

For treatment planning, interdisciplinary communication and as a means of identifying groups of patients that qualify for reimbursement of costs a useful characterization of patients with hypodontia is desirable. It should reflect both patterns of absent teeth and skeletal features. The present investigation focuses on the latter.

The purpose of the study is to compare cephalometric measures among hypodontia patients with the aim to identify and characterize groups of hypodontia patients that represent distinctive dentofacial features by means of cluster analysis.

Materials and methods

Subjects

Lateral cephalograms of 189 patients (76 boys, 113 girls), who were classified as having 'hypodontia', 'oligodontia' or 'tooth agenesis' were selected from the databases of the Department of Orthodontics and Oral Biology of the Radboud University Nijmegen Medical

Center, Nijmegen, the Center for Special Dental Care of the University Medical Center Utrecht, various orthodontic practices and other Centers for Special Dental Care in the Netherlands. The mean age at which the cephalograms were taken was 12.1 years (range 7.0-16.9; SD 2,1) with on average 5.1 missing teeth (range 1–22; SD 4.8).

The following inclusion criteria were applied:

- no previous orthodontic treatment;
- maximum 17 years of age;
- Caucasian origin;
- hypodontia is not part of a diagnosed syndrome;
- good quality lateral cephalogram present;
- missing tooth type could be confirmed.

The pre-treatment orthodontic records were examined. The number of missing teeth and the tooth type were verified from panoramic radiographs or intra-oral photographs. Hypoplastic and / or radiographically apparent, but not (yet) erupted permanent teeth were considered as being 'present'. The FDI tooth numbering system was used.[24]

Methods

Lateral cephalometric radiographs were obtained in centric occlusion with the patient positioned in a cephalostat and oriented to the Frankfurt horizontal plane. Analogue radiographs were scanned on a 16-bit scanner (Epson Expression 10000xl, Seiko Epson, Nagano, Japan). All cephalograms were digitized by one observer using a commercially available computer program for digital cephalometric analysis (Viewbox®, dHAL orthodontic software, Athens, Greece). To assess the intra-observer measurement error, cephalograms of 20 randomly selected patients were digitized twice by the same observer with a time interval of one week.

Figure 1 and table 1 show 18 anatomical landmarks that were identified. Hard and soft tissue reference lines were constructed. Because the radiographs originated from different

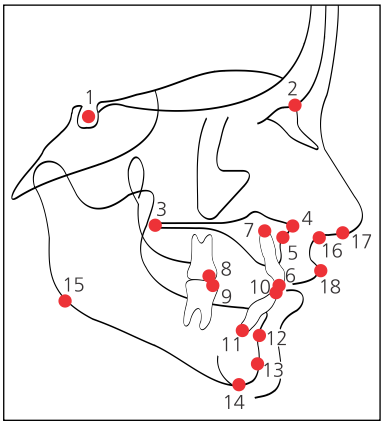
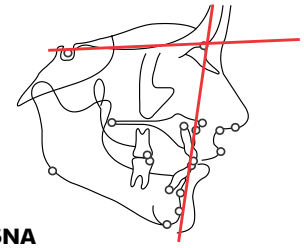
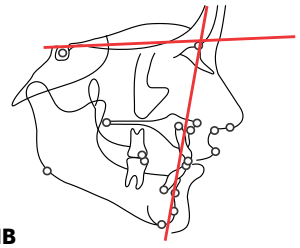


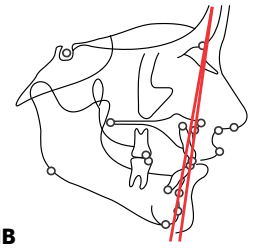
Figure 1 Anatomical landmarks on the profile cephalometric radiographs. The description of the landmarks is given in table 1.



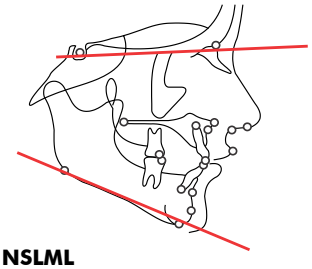
SNA
SNA angle: measures the antero-posterior position of the maxilla in relation to the anterior cranial base.



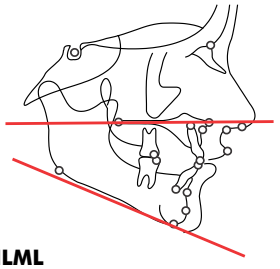
SNB
SNB angle: measures the antero-posterior position of the mandible in relation to the anterior cranial base.



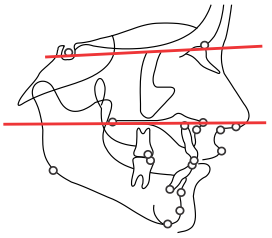
ANB
ANB angle: measures the relative position of the jaws to each other (difference between SNA and SNB).



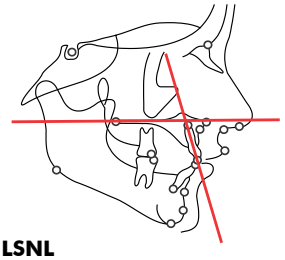
NSLML
Mandibular plane angle: measures the inclination of the mandible relative to the cranial base.



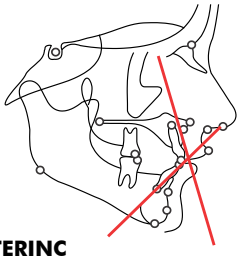
NLML
Palatal to mandibular plane angle: measures the inclination of the maxilla relative to the mandibular plane.



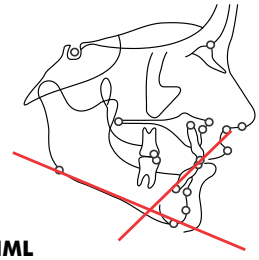
NSLNL
Palatal plane angle: measures the inclination of the maxilla relative to the cranial base.



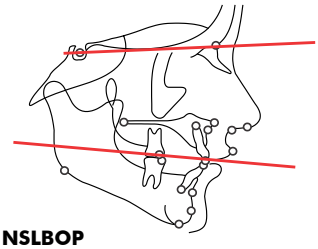
ILSNL
Maxillary incisors angle to palatal plane: measures the relative forward to backward inclination of the upper incisors relative to the palatal plane.



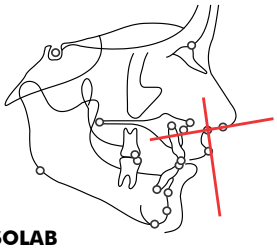
INTERINC
Interincisal angle: measures the inclination of the maxillary incisors and the mandibular incisors relative to each other.



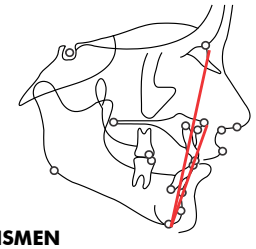
ILIML
Mandibular incisor plane angle: measures the forward or backward inclination of the lower incisors relative to the mandibular plane.



NSLBOP
Cant of the occlusal plane: measures the inclination of the bisected occlusal plane relative to the cranial plane.



NASOLAB
Nasolabial angle: measures the upper lip protrusion relative to the inferior border of the nose. It is formed by two lines, namely a columella tangent and an upper lip tangent.



ANSMEN
Anterior face height ratio: depicts the ratio between the distance from the Anterior Nasal Spine to Menton and Nasion to Menton.

Figure 2 Definitions of the hard and soft tissue lines and ratios used in the cephalometric analysis.[35-38]

Table 1 Anatomical landmarks

Hard tissues (as seen in norma lateralis)
Sella: center of the sella turcica (1) [25]
Nasion: most anterior limit of the frontonasal suture(2) [33]
Posterior nasal spine: most posterior point in the sagittal plane on the bony hard palate (3) [25]
Anterior nasal spine: tip of the anterior nasal spine (4) [34]
A-point: deepest point on the contour of the premaxilla (5) [35]
Incision superius: incisal tip of the most anterior maxillary central incisor (6) [25]
Upper incisor apex: root apex of the most prominent upper incisor (7) [26]
Upper molar mesial cusp tip: anterior cusp tip of the maxillary first molar (8)
Lower molar mesial cusp tip: anterior cusp tip of the mandibular first molar (9)
Incision inferior: incisal tip of the most anterior medial mandibular central incisor (10) [25]
Lower incisor apex: root apex of the most prominent lower incisor (11) [26]
B-point: deepest point on the contour of the mandible (12) [35]
Pogonion: most anterior point on the symphysis of the mandible (13) [34]
Menton: most inferior point on the symphysis of the mandible (14)
Gonion: midpoint of the angle of the mandible (15)
Soft tissues (as seen in norma lateralis)
Subnasale: point located at the junction between the lower border of the nose and the beginning of the upper lip at the midsagittal plane (16) [26]
Subpronasale: point where the columella tangent through subnasale touches the columella (17)
Labrale superius: most prominent point on the vermilion border of the lower lip in the mid-sagittal plane (18) [26]

clinical practices and x-ray devices were replaced over time, reliable magnification factors were not available for all radiographs. Therefore linear measurements were used for the calculation of ratios only. Consequently, 12 cephalometric angles and ratios were calculated, involving sagittal-skeletal, vertical-skeletal, dento-alveolar and soft tissue measurements for dentofacial form (figure 2).

Age and sex-matched reference values were obtained from a non-treated orthodontic population of skeletally normally developed children as described by Riolo *et al.*[25], with the exception of the reference value for the nasolabial angle.[26] Normalized differences per subject per parameter were calculated (the difference between the measured and the reference value, divided by the standard deviation of the reference value). The resulting value indicates how many standard deviations an observation is above or below its reference value.

Identification of subgroups and statistical analysis

- Dental factors thought to be of influence on cephalometric variables were obtained from previous studies. Subgroups were constructed on the basis of:
- the number of missing teeth (1-5, 6-9, >9);
- the number of missing tooth types (incisors, canines, premolars and molars).
- the location of the missing teeth (posterior, anterior, both regions). Anterior teeth were defined as central and lateral incisors, as well as canines);

- the jaw in which the teeth were missing (mandible, maxilla, both jaws)
- left/right symmetry with respect to agenesis of teeth (symmetric, for example agenesis of the 12 and 22, asymmetric, for example agenesis of the 12 and 25);
- symmetry or asymmetry in the upper and opposing lower quadrants, both left and right (symmetric, for example agenesis of the 25 and 35, asymmetric, for example agenesis of the 12 and 45).

Differences in cephalometric normalized values between the groups were tested univariately by means of and one-way Analysis of Variance where appropriate. A significance level of 0.005 was set to compensate for multiple testing. Post-hoc tests (Student-Newman-Keuls procedure) were performed to identify homogeneous subsets. These are presented as contrasts.

Cephalometric measures that proved consistently statistically significant in the univariate analyses were introduced in the SPSS TwoStep cluster method to disclose subpopulations of patients with distinctive dentofacial features. Subsequently, like in the univariate analysis, the cephalometric measures were compared among the identified clusters (table 3). A standard statistical program was used (SPSS version 11.5 , SPSS Inc, Chicago, United States).

Systematic measurement errors were estimated by means of paired Student t-tests and the random error of the method was quantified by means of Dahlberg's formula ($Se2 = \Sigma d2/2n$, where d is the difference between duplicate measurements and n is the number of double measurements.[27, 28]

Table 2 One-way analysis of variance and determination of contrasts between subgroups of hypodontia patients. Mean normalized differences with their standard deviations. All units are in degrees, with exception of the anterior face height ratio (ANSMEN). In each of the contrasts, the clusters are ranked from the highest to the lowest value.

Location of missing teeth	Posterior (a, n=82)		Anterior (b, n=39)		Posterior and Anterior, n=68)		Result of one-way ANOVA and SNK comparison of groups	
	Mean	sd	Mean	sd	Mean	sd	p-value	Contrasts
SNA	-0.10	1.28	-0.06	1.48	-0.66	1.20		
SNB	-0.19	1.23	0.24	1.10	-0.06	1.19		
ANB	0.14	1.44	-0.36	1.58	-0.93	1.45	0.000	a=b>c
NSLML	0.39	1.21	0.36	1.11	0.00	1.27		
NLML	0.62	1.18	0.39	0.98	0.23	1.17		
NSLNL	-0.51	1.44	-0.16	1.10	-0.55	1.35		
ILSNL	-0.26	1.44	-0.41	1.38	-0.66	1.41		
INTERINC	-0.33	1.33	0.18	0.98	0.54	1.21	0.000	c=b>a
ILIML	0.31	1.47	-0.23	1.04	-0.37	1.32		
NSLBOP	-0.13	1.35	0.01	1.29	-0.03	1.60		
NASOLAB	-0.05	1.24	-0.13	1.15	-0.26	1.22		
ANSMEN	0.05	0.58	-0.05	0.49	-0.14	0.59		

Table 2 *(cont.)*

Location of missing teeth	Posterior (a, n=82)		Anterior (b, n=39)		Posterior and Anterior, n=68)				Result of one-way ANOVA and SNK comparison of groups	
Number of missing teeth	1-4 (a, n=119)		6-9 (b, n=37)		>9 (c, n=33)					
	Mean	sd	Mean	sd	Mean	sd			p-value	Contrasts
SNA	-0.15	1.34	-0.37	1.34	-0.74	1.11				
SNB	-0.11	1.19	0.18	1.06	-0.11	1.33				
ANB	-0.02	1.50	-0.76	1.52	-1.05	1.37			0.000	a>b=c
NSLML	0.42	1.19	-0.01	0.98	-0.09	1.47				
NLML	0.62	1.10	0.19	1.03	0.04	1.32				
NSLNL	-0.46	1.35	-0.44	1.39	-0.42	1.33				
ILSNL	-0.36	1.35	-0.57	1.45	-0.57	1.64				
INTERINC	-0.25	1.16	0.62	1.34	0.70	1.20			0.000	c=b>a
ILIML	0.24	1.29	-0.50	1.34	-0.56	1.41			0.001	a>b=c
NSLBOP	-0.04	1.32	-0.31	1.20	0.08	1.96				
NASOLAB	-0.08	1.27	-0.03	1.17	-0.49	0.98				
ANSMEN	0.07	0.53	-0.11	0.55	-0.37	0.61			0.000	a=b>c
Jaw of the missing teeth	Mandible (a, n=57)		Maxilla (b, n=37)		Mandible and Maxilla (c, n=95)					
	Mean	sd	Mean	sd	Mean	sd			p-value	Contrasts
SNA	-0.05	1.42	0.07	1.34	-0.58	1.18				
SNB	-0.24	1.28	0.28	1.25	-0.07	1.09				
ANB	0.31	1.29	-0.22	1.59	-0.79	1.52			0.000	a=b>c
NSLML	0.41	1.15	0.34	1.35	0.11	1.20				
NLML	0.72	0.98	0.45	1.18	0.25	1.20				
NSLNL	-0.60	1.32	-0.31	1.54	-0.42	1.28				
ILSNL	-0.27	1.35	-0.28	1.48	-0.59	1.44				
INTERINC	-0.51	1.25	-0.18	1.04	0.55	1.20			0.000	c>b=a
ILIML	0.50	1.23	0.18	1.12	-0.47	1.40			0.000	a=b>c
NSLBOP	-0.14	1.36	-0.08	1.37	-0.02	1.50				
NASOLAB	0.00	1.15	-0.26	1.53	-0.18	1.10				
ANSMEN	0.11	0.51	0.10	0.54	-0.18	0.59			0.002	a=b>c
Number of missing tooth types*	1 (a, n=104)		2 (b, n=42)		3 (c, n=26)		4 (d, n=17)			
	Mean	sd	Mean	sd	Mean	sd	Mean	sd	p-value	Contrasts
SNA	-0.04	1.34	-0.69	1.33	-0.33	1.18	-0.80	0.99		
SNB	-0.05	1.19	-0.20	1.04	0.33	1.26	-0.31	1.36		
ANB	0.06	1.47	-0.74	1.55	-0.97	1.50	-0.94	1.32	0.001	a>b=d=c
NSLML	0.41	1.20	0.30	0.94	-0.32	1.18	-0.05	1.72		
NLML	0.62	1.11	0.32	1.03	-0.05	1.11	0.34	1.51		
NSLNL	-0.47	1.34	-0.11	1.30	-0.59	1.43	-0.97	1.25		
ILSNL	-0.31	1.37	-0.50	1.39	-0.43	1.36	-1.08	1.84		
INTERINC	-0.27	1.18	0.38	1.26	0.60	1.29	0.78	1.23	0.000	d=b=c>a
ILIML	0.24	1.31	-0.36	1.34	-0.44	1.52	-0.43	1.25		
NSLBOP	-0.10	1.30	0.15	1.21	-0.39	1.53	0.05	2.30		
NASOLAB	-0.12	1.25	0.06	1.24	-0.28	1.16	-0.56	0.87		
ANSMEN	0.06	0.52	-0.16	0.55	-0.13	0.67	-0.24	0.68		

* 4 tooth types are distinguished: incisors. cuspids. bicuspids and molars. Groups are subdivided on the basis of the missing number of tooth types.

Results

Error of the method

The random error of the method varied between 0.3° and 0.9° for angles and 0.3% for the ratio ANS-Me / N-Me (ANSMEN). Paired *t*-tests demonstrated no statistically significant intra-observer differences in any of the measurements.

Cephalometric analysis

The mean normalized differences (and their standard deviations) for cephalometric angles and ratios are presented for the various patient subgroups in table 2. Differences in subpopulations with respect to symmetry between left and right side, as well as upper and lower jaw proved hardly discriminative and are not presented here.

The anterior-posterior relationship between the jaws (ANB), the interincisal angle (INTERINC), the inclination of the mandibular incisor (ILIML) and anterior lower face height ratio (ANSMEN)

Table 3 One-way analysis of variance and determination of contrasts between the 4 clusters resulting from the two-step cluster analysis. Mean normalized differences with their standard deviations are given. All units are in degrees, with exception of the anterior face height ratio (ANSMEN). In each of the contrasts, the clusters are ranked from the highest to the lowest value.

TwoStep cluster analysis	Cluster 1 (a, n=45)		Cluster 2 (b, n=61)		Cluster 3 (c, n=50)		Cluster 4 (d, n=33)		Result of one-way ANOVA and SNK comparison of groups	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd	p-value	Contrasts
SNA	0.51	1.37	-0.19	1.29	-0.45	0.94	-1.32	1.01	0.000	a>b=c>d
SNB	0.09	1.26	-0.31	1.20	-0.21	0.98	0.46	1.23		
ANB	0.72	1.40	0.15	0.84	-0.37	0.96	-2.68	0.92	0.000	a>b>c>d
NSLML	0.54	1.35	0.25	1.35	0.17	1.05	-0.06	0.96		
NLML	1.19	1.00	0.26	1.22	0.42	0.98	-0.26	0.87	0.000	a>c=b>d
NSLNL	-1.26	1.47	-0.14	0.97	-0.59	1.18	0.27	1.44	0.000	d=b>b=c>a#
ILSNL	-0.37	1.27	0.02	1.13	-1.67	1.21	0.49	1.16	0.000	d=b>b=a>c*
INTERINC	-0.65	0.87	-0.63	0.92	1.38	0.71	0.46	1.28	0.000	c>d>b=a
ILIML	0.43	1.04	0.74	1.18	-0.78	1.07	-1.05	1.30	0.000	b=a>c=d
NSLBOP	-0.15	1.49	-0.07	1.35	0.20	1.42	-0.36	1.49		
NASOLAB	0.00	1.38	-0.07	1.18	-0.11	1.11	-0.52	1.14		
ANSMEN	0.66	0.35	-0.30	0.33	-0.01	0.34	-0.55	0.54	0.000	a>c>b>d

Cluster b does not differ significantly from clusters d and c, but d and c are in different subsets.
* Cluster b does not differ significantly from clusters d and a, but d and a are in different subsets.

Table 4 Percentage of patients in whom a certain tooth is missing and the mean number of missing teeth per cluster.

	Percentage of patients with missing teeth per tooth type															
Tooth	11	12	13	14	15	16	17	21	22	23	24	25	26	27	31	32
Cluster 1	0	27	7	7	20	0	7	0	18	4	9	22	0	7	2	4
Cluster 2	2	33	11	20	25	2	10	0	33	10	20	20	2	11	16	8
Cluster 3	0	52	22	28	54	8	22	0	50	28	36	44	8	14	24	14
Cluster 4	0	45	24	27	52	18	36	0	52	27	33	45	18	30	33	12

were frequently discriminative among the various subgroups in the univariate analysis, regardless the way the subgroups were defined (table 2). When a statistically significant difference was present, patients who lacked both anterior and posterior teeth, patients with missing teeth in both jaws and patients with an increasing number of missing teeth and missing tooth types presented with the largest differences from their respective subgroups and from their reference values, suggesting that all differences between subgroups are based on similar grouping of these few patients.

Cluster analysis

Because the interincisal angle and the inclination of the lower incisor are strongly related, it was decided to include only angle ANB, the interincisal angle (INTERINC) and the lower anterior face height (ANSMEN) into the TwoStep cluster analysis. This resulted in 4 clusters of patients with distinctive dentofacial features, all with a substantial number of patients per cluster (Tables 3). Table 4 shows the hypodontia features of the subject in these clusters.

Cluster 1 - High angle facial pattern

Cluster 1 was made up of 45 individuals, with an average of 2.6 missing teeth. Their vertical skeletal dimensions were markedly different from the reference values, as well as from the patients of the other 3 clusters. The group displayed characteristics as seen in high angle patients.

Cluster 2 - Proclined lower incisors

Cluster 2 contained 61 individuals, with an average of 4.3 missing teeth. Of all 4 clusters, this subset most closely mimicked the cephalometric characteristics of the reference group, although there were minor differences with respect to their dentoalveolar features. A more retrusive mandible and a relatively small interincisal angle based upon increasing proclination of the lower incisors were observed.

Cluster 3 - Retroclined upper and lower incisors

Fifty individuals were grouped in cluster 3 with an average of 6.4 absent teeth. Cluster 3 skeletally resembles cluster 2, but from a dental-alveolar perspective there is a major difference: their respective normalized values for the interincisal angle differed approximately

												Number of missing teeth		
33	34	35	36	37	41	42	43	44	45	46	47	mean	sd	range
2	2	51	0	4	2	0	2	0	58	0	4	2.6	2.6	1-15
2	15	61	5	15	21	10	5	10	52	5	16	4.3	4.7	1-22
0	16	62	8	18	20	16	4	12	56	8	20	6.4	4.6	1-18
0	21	52	21	27	27	15	9	21	58	18	27	7.4	5.7	1-20

2 standard deviations. Subjects in cluster 3 exhibit the largest interincisal angle of all 4 clusters. A retrusive mandible and retroclined upper and lower incisors were seen.

Cluster 4 – Retrusive maxillary position

Cluster 4 contains 33 persons with the largest number of missing teeth (7.4 teeth on average). Patients in this group had discriminative sagittal-skeletal characteristics when compared both to reference values and to patients from the other 3 clusters. There was a relative retruded position of the maxilla compared to the mandible. This retrusive pattern was seen in combination with a small mandibular incisor plane angle, anterior face height ratio and nasolabial angle.

Discussion

Previous studies on craniofacial development and morphology in patients with (severe) hypodontia have produced quite conflicting results. Differences between (subgroups of) patients with hypodontia and controls that have been reported are a smaller, more retrognathic maxilla[9, 15, 18, 19, 21, 29] and smaller mandibular length[23], while others specifically state that the mandible has a normal length.[21] Earlier studies showed a decreased gonial angle, both mandibular prognatism[15, 22, 23]. Mandibular retrognathism and a more acute chin angle have also been reported. A more counter clockwise rotated occlusal plane[12, 15] was reported as well as reduced upper and lower face height.[4, 12, 23, 29] The latter was attributed to anterior rotation of the mandible and not the result of skeletal change.[16] Finally, increased frequency in skeletal deep-[30] and normal-bite cases[20, 30], obtuse / blunt naso-labial angle[31], incisor retroinclination[4, 9, 15, 16, 23] causing a larger interincisal angle[15, 23], and in contrast, maxillary protrusion in another study[20] were also mentioned. Confusingly, both anterior and posterior chin positions were observed[16, 23 9] and both bimaxillary protrusion and bimaxillary retrognathia are seen[9, 20] Others found only few and small differences in craniofacial features between cases and controls.[13, 17, 18] Some of the variation among all these findings is probably caused by different inclusion criteria and patient samples as well as different measurement techniques between the various studies.

In the present study the more or less arbitrary sub grouping as proposed by others was applied to a single, large dataset of hypodontia patients. Detailed cephalometric values of non-orthodontic normals for age groups until 16 years are not available for the Dutch population. Therefore cephalometric measures of subjects from a population of Caucasian, skeletally normal developed children, as described by Riolo et al. served as reference values.[25] Age- and sex-matched *normalized* differences between hypodontia patients and controls were calculated. The use of normalized differences has the advantage that it allows

comparison of observations from different normal distributions, despite the fact that there might be differences in skeletal pattern between different populations.

It was observed that patients who lack both anterior and posterior teeth, patients with teeth missing in both jaws and patients with an increasing number of missing teeth and missing tooth types present with the largest cephalometric differences from the other subgroups. With respect to dentofacial form, these subgroups of hypodontia patients can be considered as 'more severe'. The anterior-posterior relationship between the mandible and the maxilla, the interincisal angle and lower face height are the most discriminative in the univariate analysis (Table 2). In general, a smaller ANB angle, a larger interincisal angle and decreasing vertical lower face height are associated with increasing severity of hypodontia. Hence, the population is skeletally different from the reference population but dental compensation is seen as well. The smaller ANB angle seems predominantly determined by a more retrusive maxilla than the reference values, and not so much by the position of the mandible in univariate analysis. It appears that the relatively large interincisal angle in groups with 'severe hypodontia' has to be contributed to a retroclination of the lower incisors, but also to that of the upper incisor, regardless of the sub grouping that is used. This is consistent with many other studies as described above. There is a decrease in lower anterior face height and mandibular plane angle with an increase in severity of hypodontia. However, this was not apparent in the sub grouping that used the number of missing tooth types as criterion. Others could also not confirm the clinical perception that the lower face height decreases with increased severity of hypodontia as reflected by the number of missing tooth types.[13]

The hypodontia patients were subsequently clustered on the basis of the observed discriminative cephalometric measures. This is quite a different and possibly more clinically relevant and objective approach than that adopted by others, who subdivided patients on the basis of preconceived assumptions regarding the influence of dental factors on dentofacial form. The applied statistical clustering procedure is descriptive and can only be used to identify subgroups of patients with different patterns of dentofacial characteristics. However, the recognition of these subgroups opens an alee into further research into relations between different (groups of) dentofacial parameters and to the identification of common variables that affect them. So, the clustering can give novel insights into the development of certain dentofacial features in hypodontia patients. Four groups or clusters of hypodontia patients with distinctively different dento-skeletal features could be distinguished.

Cluster 1 - High angle facial pattern - appears different from the other 3 clusters and their controls with respect to vertical-skeletal characteristics and has the smallest number of missing teeth (2.6 on average). A relatively small angle between the cranial base and the palatal plane and, to a lesser degree, an enlarged mandibular plane angle were observed. The combination of these 2 features accounts for the mildly increased vertical lower face height and the relatively large palatal to mandibular plane angle. A small palatal to mandibular

plane angle was seen by others in a group of hypodontia patients with a large number of missing teeth.[13] The tooth types that are missing in this group are among the ones most commonly missing in case of tooth agenesis. From a restorative point of view, this cluster appears the least complex of all clusters.

Although patients from cluster 2 - Proclined lower incisors - missed an average of 4.3 teeth, their skeletal features did not seem very different from their controls without absent teeth, but there were minor differences with respect to their dental-alveolar features. A relatively small interincisal angle is observed. The latter is caused by an increased proclination of the lower incisors relative to the mandibular plane. The inclination of the upper incisors was not discriminative.

Cluster 3 - Retroclined upper and lower incisors - very much resembled cluster 2 from a skeletal perspective, but in contrast, a large interincisal angle was seen as has been reported frequently in association with hypodontia.[4, 15, 16, 23] This is mainly caused by a retroclination of the upper incisors and, to a lesser degree, to that of the lower incisors. Patients in this group missed, on average 2 more teeth than patients from cluster 2 (6.4 on average). These additional missing teeth were usually located in the maxilla, most commonly the second bicuspids (table 4).

Patients in cluster 4 – Retrusive maxillary position - are the most challenging from a restorative point of view because generally a large number of teeth is missing (7.4 on average). Among these were teeth whose absence is rather uncommon, such as mandibular and maxillary first and second molars (table 4).[1] The most striking dentofacial features of patients in this cluster were their sagittal-skeletal characteristics. Compared to the controls there was a marked retrusive position of the maxilla compared to the mandible. This is mainly because of a retrognathic maxilla. To a lesser degree, the mandible appeared to be prognathic**.** In addition, the smallest nasolabial angle and lower face height and largest retroclination of the lower incisors were frequently seen in patients that were clustered in this group. This population of patients needs complex interdisciplinary treatment, which may involve orthodontic treatment and orthognathic surgery and placement of dental implants as part of a comprehensive treatment plan. From both a skeletal and a dental point of view, patients in this cluster suffer from a severe dento-skeletal disorder.

The resulting clusters differ from each other with respect to the a-priori variables. However, inspection of the 'Contrasts' column of table 3 learns that not all clusters have different values for those variables and that almost all other variables differ between two for more of the resulting clusters. Moreover, the resulting clusters do not overlap with any of the groupings applied in table 2. This shows that the clustering based on variables that were selected because they show differences between groups based on hypodontia features, can identify clusters of patients with different patterns of dentofacial characteristics.

Although the mean number of absent teeth differs among the 4 clusters that were identified in this study, patients with both a large and a small number of missing teeth were represented in all clusters. Hence, the number of missing teeth by itself cannot fully explain the variation is skeletal patterns among hypodontia patients. The preferred dentofacial orthopedic treatment to cope with patients from the various clusters is subject for debate, but this falls outside the scope of this article. Future studies should further elucidate whether or not specific patterns and locations of missing teeth (and not so much absolute numbers) can be associated with the distinguished clusters.

For treatment planning, interdisciplinary communication and as a means of identifying groups of patients that qualify for reimbursement of costs a characterization of patients with hypodontia into meaningful groups is desirable. It should reflect both number and patterns of absent teeth as well as skeletal features. For the former, the full mouth Tooth Agenesis Code could be a useful measure.[2, 32] It constitutes a unique number that identifies the specific pattern of absent teeth for each individual patient (both number of missing teeth and missing tooth types). For the latter, the classification in clusters as described in this article may be practical. The usefulness of combining these dental and skeletal methods of characterization needs to be investigated further, which would require a very large sample size.

Conclusions

The hypodontia population in this study differs from a general orthodontic population on both dental and skeletal aspects. In hypodontia patients, the anterior-posterior relationship between the jaws, the interincisal angle and the lower anterior face height are discriminative parameters of dentofacial form. On the basis of these 3 cephalometric variables patients with hypodontia can be clustered in 4 groups, each with distinctive vertical-skeletal, dento-alveolar and sagittal-skeletal characteristics. Cluster 1 is made up of patients with a high angle facial pattern and patients from cluster 2 exhibit proclined lower incisors. Patients in cluster 3 skeletally resemble those in cluster 2, but are markable because of their retroclined upper and lower incisors. Patients that are grouped in cluster 4 miss the largest number of teeth and stand out because of their retrusive maxillary position. This categorization of patients with hypodontia into meaningful groups may be useful for treatment planning, interdisciplinary communication and as a means of identifying groups of patients that qualify for reimbursement of costs. Other dental factors such as the distribution of the missing teeth as well as the size and shape of the teeth that are present are important factors to consider during restorative clinical decision making in patients with hypodontia as well.

Acknowledgements

The authors are grateful to the following orthodontists J.G. Daggers, L. Kalden, M.T. Wassenaar-te Lintelo, R. A. van Teeseling, S.J.G.A. van 't Veen and the Centers for Special Dental Care of the Isala klinieken Zwolle, The Netherlands (B.J. Polder and P.A.M. Versteegh) and of the Radboud University Nijmegen Medical Center (C. van Heumen) for providing additional patient material. Special thanks to O. van Vlijmen of the Department of Orthodontics and Oral Biology, Radboud University Nijmegen Medical Centre for his help and assistance during the process of data acquisition and radiographic analysis.

Conflict of interest

The authors declare that they have no conflict of interest.

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Clin Implant Dent Relat Res. 2009 Oct 16.
[Epub ahead of print]

4 Radiographic features of mandibular trabecular bone structure in hypodontia

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Abstract

Radiographical parameters of mandibular trabecular bone structure between 67 subjects having hypodontia and those without were studied on digital panoramic radiographs.

Three Regions of Interest (ROI) were defined: the ascending ramus, apical of the mandibular molar and mesial of the first mandibular molar. The effects of the presence of hypodontia and the ROI on the mandibular trabecular bone structure were tested for statistical significance by means of multivariate analysis.

Radiographical parameters of trabecular bone architecture were found to differ between various regions of the mandible *(p=0.000)*, but not between the group of hypodontia subjects and their controls *(p=0.23)*. There was no interaction effect between the ROIs and the two groups *(p=0.79)*. For people having hypodontia, some directional parameters of trabecular bone have a reverse correlation with the number of missing teeth. The fractal dimension and the number and perimeter of white segments in the binarized image correlate positively with the number of congenitally missing teeth.

A limited number of parameters of radiographic mandibular trabecular bone structure correlate with the number of missing teeth. However, a markable difference in radiographic parameters of mandibular trabecular bone structure between hypodontia and non-hypodontia subjects could not be demonstrated.

Introduction

Hypodontia is a condition in which one or more permanent teeth are congenitally missing. It is seen as part of a syndrome (i.e., in ectodermal dysplasia) or as a non-syndromic anomaly. Hypodontia can vary widely in severity, from a single missing tooth to the absence of all permanent teeth (anodontia).[1, 2] In case of an absent tooth, the deciduous tooth may be retained until a high age.[3]

Dental treatment of severe hypodontia can be comprehensive and usually requires an interdisciplinary approach. Current treatment of severe hypodontia includes the use of dental implants as part of the restorative phase of treatment. Information in the literature with respect to the results of dental implant treatment in syndromic and non-syndromic hypodontia subjects is scarce, frequently anecdotic, and sometimes conflicting. Some authors show promising results in non-syndromic hypodontia subjects.[4, 5] Garagiola and colleagues[6] report similar implant survival rates in hypodontia subjects with and without ectodermal dysplasia. Others observed compromised overall success rate in ectodermal dysplasia subjects with hypodontia, especially in the maxilla.[7, 8] Several possible reasons for the latter observation can be hypothesized. Since crown formation and root development of permanent teeth are considered to be important for the development of the alveolar process, their absence and the subsequent lack of growth stimuli of the jawbone will result in impaired alveolar bone volume and, possibly, also compromised bone structure.[9, 10]

In addition, mutations in some homeobox genes, among which is the *MSX1* gene, have been identified as etiological factors in hypodontia.[11] Such genes and molecules bear relevance to the process of both tooth morphogenesis and craniofacial bone formation. Recently, a new gene (LTBP3) has been identified that, when dysfunctional, causes severe hypodontia, short stature, and increased skeletal bone density.[12] Whether or not mandibular trabecular bone structure in hypodontia patients is markedly different from that of non-hypodontia subjects is not known to date.

The present study focuses on differences in radiographic parameters of mandibular trabecular bone structure between persons having hypodontia and those without. Furthermore, it is investigated whether these differences are constant across the mandible. For persons having hypodontia, the relation between the number of missing teeth and the mandibular trabecular bone structure is determined.

Materials and methods

The investigation is set up as a case-control study.

Cases

Sixty-seven subjects who were classified as having 'hypodontia,' 'oligodontia,' or 'tooth agenesis' were selected from the databases of the Center for Special Dental Care of the University Medical Center, Utrecht in the Netherlands (31 males, 36 females). When no panoramic radiograph was present, when the panoramic radiograph was of poor quality, or when the missing tooth type could not be confirmed, the subject was excluded from the study. The number of missing teeth (third molars excluded) was determined from the panoramic radiographs or intraoral photographs when available. Hypoplastic and/or radiographically apparent, but not (yet) erupted, permanent teeth were considered as being 'present.'

Controls

A control group of age-matched and sex-matched non-hypodontia subjects was selected from the radiographic database of the Utrecht University Medical Center. The panoramic radiographs were taken in the same month as that of their corresponding matched cases. Each case was matched with a different control.

Panoramic Radiographs and Regions of Interest

Digital panoramic radiographs were acquired with a Planmeca Promax-2 panoramic x-ray machine (64–66 kV, Planmeca Oy, Helsinki, Finland), as part of the anticipated dental treatment. Bone structure measurements were performed at three rectangular regions of interest (ROI) located in the right-hand side of the mandible on all panoramic radiographs. The manually selected ROIs were located: (1) in the ascending ramus, (2) apical of the mandibular molars, (3) between the mesial root of the first mandibular molar, and the anticipated or actual root of the second mandibular bicuspid (Figure 1).Considering ROIs in three typically different regions enables estimation of the relevance of the location. The mean sizes of the ROIs were 140 x 199 pixels, 206 x 65 pixels, and 29 x 62 pixels for regions 1, 2, and 3, respectively. The ROIs were chosen to reflect the possible changes in the alveolar process, in an area far away from the alveolar process (the ramus), which should not be affected by functional effects but only by systemic influences, and in a region in between these two ROIs.

Radiographic Measurement Procedure

The three ROIs were subjected to a sequence of automatic measurement procedures. The procedure of extracting the quantitative data from the radiographic trabecular texture has been described extensively before and is presented here briefly for completeness.[17,18] First,

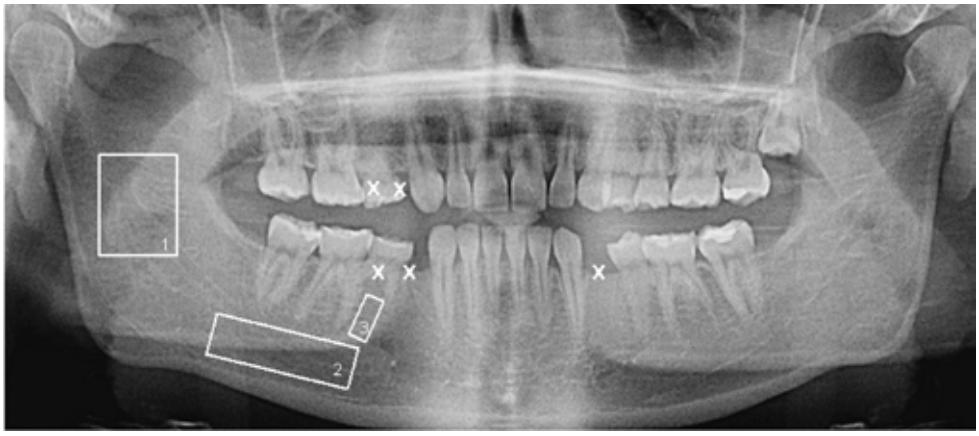

Figure 1. Panoramic radiograph of a hypodontia patient with Regions of Interest (ROI). Congenitally missing teeth are marked with an 'x'. The right upper and lower first and second bicuspid and the left lower first bicuspid are congenitally missing. ROI 1: Ascending ramus. ROI 2: Apically of the mandibular molars and parallel and above the cortical border of the mandible. ROI 3: Parallel to the mesial root of the first molar, stretching from the apex of the mesial root, to half way up the root.

the mean (MEAN) and standard deviation (STDEV) of the gray values were determined in the raw, unfiltered image samples. Subsequently, the image sample was binarized into white and black segments (Figure 2a), and the fractal dimension according to the caliper method (FRACTL), the combined area of white segments (WAREA), and the perimeter of white segments (WCIRC), and the number of white and black segments (WITES and BLAKS) were determined. The measurements WAREA, WCIRC, and WITES and BLAKS were standardized by dividing them by the area of the ROI. Next, the white segments were eroded to a wire frame (Figure 2b) and the total length of the frame (WAXIS), the number of end points (WENDS and WENDS2), and the number of branching points (WFORK and WFORK2) were determined. The black regions were approached in an analogous manner, yielding the parameters BAXIS, BENDS, BENDS2, BFORK, and BFORK2. It is important to mention that the parameters for the white regions and those for the black regions used are not complementary. The measurements on the wire frame were standardized by dividing them by the total surface area of the ROI or by the length of the skeleton (WENDS2, BENDS2, WFORK2, and BFORK2). Finally, the line fraction deviation (LFD) of orientation along 12 directions was measured ranging from 0° (LFD 0) to 165° (LFD 165). The 29 measured geometrical, topological, and directional parameters of the radiographic trabecular bone pattern and the manner of standardization are presented in Table 1. The employed method of measuring spatial orientation of trabecular bone, its validity, and clinical application have been described previously and in more detail.[13-18]

Figure 2a. Binarized version of the region of interest.

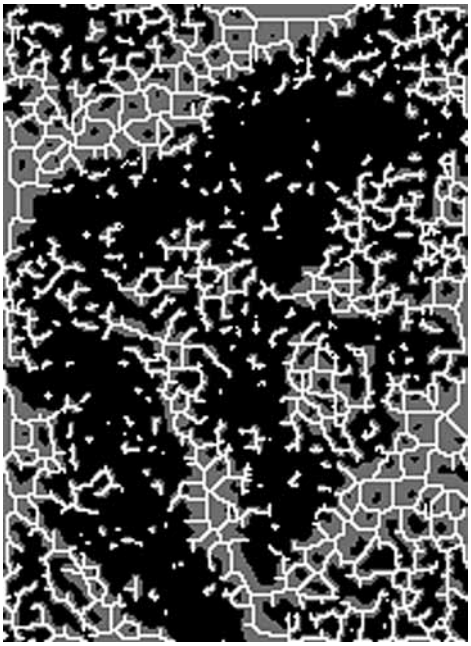

Figure 2b. Wire diagram of the white segments.

Statistical Analysis

The effects of the presence of hypodontia and the ROI on the 29 variables that measure the radiographic mandibular trabecular bone structure were tested for statistical significance using a 2 x 3 repeated measures analysis of variance design, with the presence of hypodontia and the location of the ROI as independent variables. The relation between the number of missing teeth and these 29 parameters was determined using the Pearson correlation. The success of the matching procedure was verified by means of a paired samples *t*-test among cases and their matched controls. All computations were done using the SPSS package version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

The matching procedure was successful, with a perfect match on gender and no statistically significant age difference between cases and controls (respectively, 23.7 1 10.2 years vs. 23.7 1 10.1 years; paired samples *t*-test, $t66 = 0.35$, $p = 0.73$). The cases had 2 to 21 teeth congenitally missing teeth, third molars excluded (mean 9.7, SD 4.5 missing teeth).
Mean values of the radiographic parameters of bone structure among subsets of subjects are presented in Table 2. Multivariate analysis revealed a statistically significant difference in bone

Table 1. Measured radiographical parameters of mandibular trabecular bone architecture.

Simple parameters:			
1.	MEAN	Mean of gray values in the Region Of Interest (ROI)	
2.	STDDEV	Standard deviation of gray values in the region of interest	
Geometrical parameters of the binarized version of the sample:			
3.	FRACTL	Fractal dimension	
4.	WAREA	Area of white segments	divided by the area of the ROI
5.	WCIRC	Perimeter of white segments	divided by the area of the ROI
6.	BLAKS	Number of black segments	divided by the area of the ROI
7.	WITES	Number of white segments	divided by the area of the ROI
Topological parameters of the wire diagram of the white segments:			
8.	WAXIS	Length of struts	divided by the area of the ROI
9.	WENDS	Number of endpoints	divided by the area of the ROI
10.	WENDS2	Number of endpoints	divided by the length of the white skeleton
11.	WFORK	Number of furcations	divided by the area of the ROI
12.	WFORK2	Number of furcations	divided by the length of the white skeleton
Topological parameters of the wire diagram of the black segments:			
13.	BAXIS	Length of struts	divided by the area of the ROI
14.	BENDS	Number of endpoints	divided by the area of the ROI
15.	BENDS2	Number of endpoints	divided by the length of the black skeleton
16.	BFORK	Number of furcations	divided by the area of the ROI
17.	BFORK2	Number of furcations	divided by the length of the black skeleton
Directional parameters of the binarized version as reflected by the Line Fraction Deviation index (LFD):			
18.	LFD 0	orientation along 0 degrees	
19.	LFD 15	orientation along 15 degrees	
20.	LFD 30	orientation along 30 degrees	
21.	LFD 45	orientation along 45 degrees	
22.	LFD 60	orientation along 60 degrees	
23.	LFD 75	orientation along 75 degrees	
24.	LFD 90	orientation along 90 degrees	
25.	LFD 105	orientation along 105 degrees	
26.	LFD 120	orientation along 120 degrees	
27.	LFD 135	orientation along 135 degrees	
28.	LFD 150	orientation along 150 degrees	
29.	LFD 165	orientation along 165 degrees	

parameters among different ROIs ($F58,9 = 79.53$, $p = 0.000$), but no differences between the group of hypodontia cases and the controls ($F29,4 = 1.28$, $p = 0.23$). Hence, a marked difference in radiographic mandibular trabecular bone structure between hypodontia and non-hypodontia subjects could not be confirmed. No evidence was found for an interaction effect between the ROIs and the two groups ($F58,9 = 0.78$, $p = 0.79$).

However, the number of absent teeth correlated statistically significant with some of the geometrical and directional bone parameters (Table 3). There is a positive but weak correlation with the fractal dimension ($r = 0.31$, $p = 0.01$), and both the perimeter and number of white segments in the binarized radiographic image ($r = 0.33$, $p = 0.006$ and $r = 0.29$, $p = 0.02$, respectively). In addition, a negative correlation can be observed with

Table 2. Measured radiographical parameters of mandibular trabecular bone architecture among subsets of subjects.

Parameters	Group		Location		
	Cases (n=67)	Controls	ROI1	ROI2	ROI3
1. MEAN	115.7 (26.8)	124.1 (30.2)	129.5 (31.3)$^{+}$	110.6 (24.0)$^{-}$	119.6 (27.8)
2. STDDEV	19.0 (8.5)	17.0 (8.1)	24.1 (8.7)	15.7 (5.5)	12.6 (5.5)$^{-}$
3. FRACTL	1.496 (0.068)	1.497 (0.070)	1.536 (0.036)$^{+}$	1.512 (0.045)$^{+}$	1.441 (0.070)$^{-}$
4. WAREA	0.469 (0.033)	0.463 (0.035)	0.459 (0.018)$^{-}$	0.459 (0.024)$^{-}$	0.478 (0.049)$^{+}$
5. WCIRC	0.336 (0.021)	0.334 (0.022)	0.322 (0.013)$^{-}$	0.323 (0.016)$^{-}$	0.350 (0.023)$^{+}$
6. BLAKS	0.004 (0.002)	0.004 (0.002)	0.003 (0.001)$^{-}$	0.003 (0.001)$^{-}$	0.005 (0.002)$^{+}$
7. WITES	0.005 (0.002)	0.005 (0.002)	0.005 (0.001)$^{-}$	0.005 (0.001)$^{-}$	0.006 (0.002)$^{+}$
8. WAXIS	0.183 (0.009)	0.181 (0.011)	0.180 (0.064)$^{-}$	0.180 (0.008)$^{-}$	0.185 (0.014)$^{+}$
9. WENDS	0.025 (0.003)	0.025 (0.003)	0.025 (0.019)	0.025 (0.023)	0.025 (0.047)
10. WENDS2	0.136 (0.018)	0.138 (0.020)	0.136 (0.011)	0.136 (0.014)	0.138 (0.028)
11. WFORK	0.018 (0.002)	0.018 (0.003)	0.020 (0.001)$^{+}$	0.018 (0.001)$^{+}$	0.016 (0.003)$^{-}$
12. WFORK2	0.099 (0.012)	0.099 (0.012)	0.108 (0.004)$^{+}$	0.101 (0.005)$^{+}$	0.087 (0.013)$^{-}$
13. BAXIS	0.247 (0.011)	0.248 (0.011)	0.242 (0.006)$^{-}$	0.245 (0.008)$^{-}$	0.254 (0.014)$^{+}$
14. BENDS	0.022 (0.003)	0.022 (0.003)	0.021 (0.002)$^{-}$	0.021 (0.002)$^{-}$	0.024 (0.004)$^{+}$
15. BENDS2	0.089 (0.011)	0.087 (0.012)	0.086 (0.006)$^{-}$	0.084 (0.007)$^{-}$	0.094 (0.016)$^{+}$
16. BFORK	0.022 (0.003)	0.022 (0.003)	0.024 (0.001)$^{+}$	0.023 (0.002)$^{+}$	0.020 (0.004)$^{-}$
17. BFORK2	0.090 (0.011)	0.089 (0.012)	0.098 (0.004)$^{+}$	0.092 (0.006)$^{+}$	0.077 (0.012)$^{-}$
18. LFD 0	0.163 (0.073)	0.161 (0.065)	0.104 (0.025)$^{-}$	0.158 (0.047)	0.223 (0.066)$^{+}$
19. LFD 15	0.144 (0.066)	0.143 (0.063)	0.089 (0.016)$^{-}$	0.132 (0.036)$^{-}$	0.210 (0.060)$^{+}$
20. LFD 30	0.134 (0.059)	0.136 (0.060)	0.091 (0.019)$^{-}$	0.116 (0.026)$^{-}$	0.197 (0.059)$^{+}$
21. LFD 45	0.132 (0.052)	0.130 (0.052)	0.099 (0.026)$^{-}$	0.114 (0.022)$^{-}$	0.181 (0.054)$^{+}$
22. LFD 60	0.131 (0.051)	0.131 (0.054)	0.100 (0.029)$^{-}$	0.116 (0.026)$^{-}$	0.177 (0.058)$^{+}$
23. LFD 75	0.127 (0.050)	0.129 (0.055)	0.096 (0.023)$^{-}$	0.115 (0.024)$^{-}$	0.172 (0.063)$^{+}$
24. LFD 90	0.130 (0.050)	0.135 (0.055)	0.101 (0.025)$^{-}$	0.122 (0.029)$^{-}$	0.174 (0.063)$^{+}$
25. LFD 105	0.136 (0.050)	0.144 (0.052)	0.113 (0.028)$^{-}$	0.127 (0.032)$^{-}$	0.180 (0.061)$^{+}$
26. LFD 120	0.145 (0.052)	0.145 (0.052)	0.109 (0.028)$^{-}$	0.142 (0.042)	0.184 (0.043)$^{+}$
27. LFD 135	0.152 (0.062)	0.148 (0.060)	0.097 (0.022)$^{-}$	0.157 (0.046)$^{-}$	0.197 (0.059)$^{+}$
28. LFD 150	0.156 (0.072)	0.152 (0.071)	0.091 (0.017)$^{-}$	0.157 (0.046)	0.214 (0.072)$^{+}$
29. LFD 165	0.160 (0.078)	0.160 (0.072)	0.094 (0.017)$^{-}$	0.157 (0.046)	0.229 (0.072)$^{+}$

Mean values and standard deviations between brackets. For explanation of the abbreviated parameter names see table 1 and figure 1. There is a statistically significant overall effect of the location of the measurements (Region Of Interest, $p<0.001$). Contrasts are presented as a statistically significant difference from the mean and denoted with a '$^{+}$' or a '$^{-}$'. No statistical overall effect of the group (cases versus controls) was observed and contrasts are not presented.

the directional orientation along 0° and along 165° (r = -0.24, p = 0.05 and r = -0.32, p = 0.008, respectively).

Discussion

On hypothetical grounds, a difference in mandibular trabecular bone structure may be present among subjects with and without numerical aberrations of tooth formation. The presence of permanent teeth plays a role during the development of alveolar bone. Some

Table 3. The relation between radiographical parameters of mandibular trabecular bone architecture and the number of congenitally missing teeth in hypodontia patients (Pearson correlation, n=67 patients). Significant correlations are denoted.

Parameters	Pearson's correlation coefficient	p-value
1. MEAN	-0.06	
2. STDDEV	-0.10	
3. FRACTL	0.31	p=0.01
4. WAREA	-0.19	
5. WCIRC	0.33	p=0.006
6. BLAKS	-0.07	
7. WITES	0.29	p=0.02
8. WAXIS	-0.13	
9. WENDS	-0.21	
10. WENDS2	-0.15	
11. WFORK	-0.08	
12. WFORK2	-0.16	
13. BAXIS	0.24	
14. BENDS	0.07	
15. BENDS2	0.01	
16. BFORK	0.16	
17. BFORK2	0.06	
18. LFD 0	-0.24	p=0.05
19. LFD 15	-0.18	
20. 2LFD 30	-0.13	
21. LFD 45	-0.11	
22. LFD 60	-0.05	
23. LFD 75	0.09	
24. LFD 90	0.02	
25. LFD 105	0.09	
26. LFD 120	0.17	
27. LFD 135	0.06	
28. LFD 150	-0.12	
29. LFD 165	-0.32	p=0.008

genes and molecules that are known to be involved in tooth formation are also relevant to the process of craniofacial development. To our knowledge, no studies have focused on this issue to date, yet it bears clinical relevance, since for the functional and aesthetic oral rehabilitation of subjects with (severe) hypodontia, the placement of oral implants is a common treatment modality. The implants are placed in the alveolar bone and serve to support crowns and bridges, or removable prosthetic appliances; therefore, the quality of the alveolar bone is of paramount importance. The successful application of dental implants relies heavily on the capability of the host bone to achieve and maintain intimate bone-implant contact during initial healing and subsequent implant loading.[19] It remains to be seen whether or not the bone in hypodontia and non-hypodontia subjects is of the same 'bone quality' and will respond to implants and functional loading in a similar and favorable manner, and studies investigating this particular subject are lacking.

The term 'bone quality' is frequently used in implant dentistry and has been identified as a predictor of implant success.[20] It was originally based on the clinical distinction in macro-architecture of bone, expressed as the relative proportion of trabecular to cortical bone, although the accuracy and efficacy of assessing the quality of jawbone on a radiograph in this manner has been questioned.[21, 22] Since then, other aspects of bone, such as its vascularity and mineral density, have gained clinical appreciation in achieving and maintaining osseointegration as well.[19]

Invasive and destructive per surgical diagnostic measures to evaluate aspects of 'bone quality,' such as laser Doppler flowmetry (vascularity), the assessment of implant insertion

resistance torque, and resonance frequency analysis (bone density), have been described.[23-25] Radiographic techniques are used to obtain presurgical structural information about bone in a non-destructive manner. For this purpose, sophisticated radiographic methods are employed in general medicine and implant dentistry alike, including dual x-ray absorptiometry and various forms of computed tomography and magnetic resonance imaging.[26-30] An important advantage of the use of the less sophisticated conventional panoramic radiographs is that they are routinely made in dentistry, and oral and maxillofacial surgery. Therefore, they are easily and widely available.

Measuring trabecular spatial orientation on panoramic radiographs can be performed in a reliable and reproducible manner.[16] The manual selection of ROI does not introduce large amounts of noise. The technique was applied successfully in the past to study the predictive value of trabecular architecture of jawbone on bone mineral density among osteoporotic and non-osteoporotic subjects.[17] The same measurement technique and parameters of bone architecture were used in the present study with regard to potential differences in mandibular trabecular bone structure among subjects with various degrees of hypodontia (cases) and non-hypodontic subjects (controls).

It was decided to choose the ROIs located in the ascending ramus, apical of the mandibular molars, and between the mesial root of the first mandibular molar, and the anticipated or actual root of the second mandibular bicuspid. The latter position was chosen because the first mandibular molar is seldom absent. It would have been ideal to measure at locations where implant placement was actually anticipated or from which bone grafts were going to be obtained instead. However, controls would then have to be non-hypodontia cases requiring implants at comparable sites as their matched case. Since much effort was put in matching cases and controls on age and gender, this would have been practically not achievable. In addition, radiographs of hypodontia subjects and matched controls that were selected were made around the same date. The latter was considered relevant in order to compensate for possible unregistered alterations in the settings in time of the x-ray device. As a consequence, the data presented in this study should be considered exploratory and relate to mandibular trabecular bone in general at the selected ROI (that are not necessarily relevant to implant dentistry). Translation of the findings to, for instance, histological sections would be speculative.

By means of the used technique, apparent differences of many parameters of the radiographic spatial architecture of mandibular jawbone were apparent between regions located in the ascending ramus, below the molars, and mesial of the root of the first molar. This is not surprising for various reasons. One reason is that the regions that were chosen lie far apart, were located in both basal bone and in the alveolar process, and were both in the vicinity of and far away from teeth. No statistically significant differences within the group of hypodontia subjects and the controls could be demonstrated. However, two directional

parameters correlate statistically significantly with the number of absent teeth. It has been shown that the masticatory performance of people with a reduced number of occluding teeth is impaired when compared with subjects with a complete natural dentition.[31] Bone structure functionally adapts to the (muscle) forces exerted upon it, and mandibular bone forms no exception.[32] This may contribute to the correlation between some spatial parameters of mandibular trabecular bone and the number of absent teeth that was noted in the present study.

It was observed that subjects with an increasing number of missing teeth also exhibit a larger fractal dimension, which means a larger perimeter and number of white segments in the binarized radiographic image. The fractal dimension is a measure that reflects the contours of the white areas of the binarized sample. When there are many curves, and each curve by itself has ample twists and turns, the fractal dimension can reach the value of 2, like a flat surface. However, when the contour resembles a straight line, the fractal dimension approaches the value of 1. Hence, a higher value for fractal dimension with an increasing number of congenitally missing teeth suggests a coarser contour of the white areas. It is of interest to mention that fractal dimension and bone mineral density were found to be correlated in mandibular bone in a recent in vitro study using cone beam computed tomography imaging.[30] Since the latter radiographic technique is swiftly becoming more widely available and can provide detailed information regarding both anatomical and structural features of bone, its use for in vivo studies on bone texture in larger populations (i.e., hypodontia vs. non-hypodontia subjects) holds a promise for the near future, but needs further evaluation.

In conclusion, radiographic parameters of trabecular bone architecture differ between various regions of mandibular jawbone. Some directional parameters have a reverse correlation with the number of missing teeth. The fractal dimension, and the number and perimeter of white segments in the binarized image correlate positively with the number of congenitally missing teeth. However, a difference in radiographic parameters of mandibular trabecular bone structure between hypodontia and non-hypodontia subjects could not be demonstrated.

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Published in: Oral Maxillofac Surg 2010; 68: 530-538.

5

Implant treatment in patients with severe hypodontia: a retrospective evaluation

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Abstract

The aim of this retrospective study was to evaluate the result of implant treatment in patients with severe hypodontia and compare some basic characteristics of patients with severe hypodontia who received conventional dental treatment or no treatment at all with those who were treated in combination with endosseous implants.

All patients who had been referred to an academic center of special dental care between 1990 and 2008 and who had been classified at their first visit as having 'oligodontia' or 'severe hypodontia' were selected from the hospital's database. Their charts were reviewed, and surgical treatment details and outcomes of the implants were registered from those patients who received endosseous implants.

Of the 294 patients who met the inclusion criteria, 44 patients were treated in combination with endosseous implants. The cumulative chance of implant survival of the 214 placed implants after 5 years was 89.8% (SE, 2.6%), with a mean observation period of 2.9 years (minimum, 0.1 years; maximum, 18.3 years). No implants failed thereafter. Patients who received implants were missing fewer teeth and were treated more recently compared with those who received conventional restorative treatment or no treatment at all.

Considering the compromised anatomic situation and the complexity of treatment, a 5-year survival rate of 89.8% in patients with severe hypodontia, as seen in this study, is regarded as acceptable.

Introduction

Hypodontia is a condition in which one or more permanent teeth are congenitally missing. It is seen as part of a syndrome, for example, in ectodermal dysplasia (ED), or as a non-syndromic trait. The prevalence of hypodontia in white populations in North America, Australia, and Europe is estimated to be 5.5%, with a higher incidence in women than in men. Hypodontia varies widely in severity, from a single missing tooth to the absence of all permanent teeth (anodontia). [1, 2] Oligodontia is generally defined as the absence of 6 or more permanent teeth, the third molars excluded, [3] and its prevalence is estimated at 0.14% in the white population.[4]

The numeric distinction between oligodontia and non-oligodontia seems an arbitrary one. For restorative treatment planning, the location of the missing teeth (and not merely the number of missing teeth) is an important issue as well. Dentoalveolar features of patients with severe hypodontia include occlusal disturbances, delayed eruption, and alterations of tooth morphology. In addition, skeletal features may differ significantly among patients with and without multiple missing teeth.[5] As a consequence; restorative treatment can be comprehensive, requiring an interdisciplinary approach. [5-9]

In earlier days the use of partial or full removable dentures, crowns, and bridges was the treatment of choice in cases with severe hypodontia.[10] Current and contemporary treatment of severe hypodontia includes the use of dental implants as part of the restorative phase of treatment. Information in the literature with respect to the results of both conventional and dental implant treatment in syndromic and non-syndromic hypodontia patients is scarce and frequently anecdotal. A recent literature search in PubMed using the mesh terms 'hypodontia' and 'implant' (May 1, 2009) found 184 articles. A mere 4 studies describe the clinical outcome of implant treatment in oligodontia patients with a population exceeding 10 patients. Durstberger et al (1999) observed an implant survival rate of 96% after 5 years in 13 patients.[11] All 3 implant losses occurred in a single patient. Finnema et al (2005) reported implant survival rates of 86% and 96% for the mandible and maxilla, respectively, after a mean observation period of 3 years in 13 patients with a total of 87 implants.[12] Both studies did not disclose whether their population consisted of syndromic and/or non-syndromic patients, nor did Krieger et al (2009). They reported implant survival in 22 hypodontia/oligodontia cases, without disclosing the number of congenitally missing teeth.[13] Because a mean number of 2 implants was placed in the latter study, it is presumed that relatively many hypodontia and few oligodontia patients were part of the studied population. Sweeney et al (2005) followed 14 oligodontia patients with ED and reported implant survival in only 67% after 1 year.[14] This is in reasonable agreement with the results in another but smaller group of young ED patients.[8] These disappointing results correspond with unsubstantiated clinical

observations of compromised and unpredictable survival of dental implants in other patient groups with severe hypodontia, which is often contributed to 'poor bone quality.' Small alveolar ridge dimensions may form a surgical challenge and frequently necessitate bone augmentation procedures. Studies comparing the efficacy of various restorative treatment strategies in case of severe hypodontia, that is, with oral implants or with conventional treatment means, have not been performed.

The aim of this retrospective study is to compare some basic characteristics of patients with severe hypodontia who received conventional dental treatment or no treatment at all with those who were treated in combination with endosseous implants. Implant survival is an important measure of the efficacy of dental implant treatment in severe hypodontia, and it was therefore determined.

Materials and methods

Data collection

All patients who had been referred to the center of Special Dental Care of the University Medical Center Utrecht (Utrecht, The Netherlands) between 1990 and 2008 and who had been classified at their first visit as having 'oligodontia' or 'severe hypodontia' were selected from the hospital's database. Only patients with 6 or more congenitally absent teeth (the third molar excluded) were included in the study. Their charts were reviewed, and the number of missing teeth was verified from a panoramic radiograph. When no panoramic radiograph was available (eg, data on microfilm) or when the quality of the radiograph did not allow adequate interpretation as to the presence or absence of permanent teeth, the patient was excluded from the study population. Hypoplastic and/or radiographically recognizable but not (yet) erupted permanent teeth were considered as being 'present.' Tooth determination was performed by 2 of the authors. When there was initial disagreement as to which tooth was actually present or absent, cases were re-evaluated. Agreement was obtained through discussion. Absent teeth were registered by means of the Fédération Dentaire Internationale tooth numbering system.[15] Patient details and specific information about the implant treatment were retrieved from the patients' charts.

Implant survival and statistical analysis

Implant survival was defined as the presence of an implant at the last time of evaluation. Implants that replaced failed implants were considered as new implants in the same patient with a different date of implant placement. An estimation of the time-dependent implant survival according to the Kaplan-Meier method was calculated. Descriptive statistics, analysis

of variance, and survival analysis were performed with standard statistical software (SPSS, version 16.0; SPSS, Chicago, IL).

Results

Of the 294 patients who met the inclusion criteria, 44 patients were treated in combination with dental implants. Conventional restorative treatment without implants or no treatment at all to date was performed in the other 250 patients. Patient details of both the implant and non-implant cases are described and compared in Table 1. There is only little variation in mean age and gender distribution between the 2 populations. However, the mean number

Table 1 Characteristics of implant and non-implant cases (mean values and standard deviations between brackets).

	N	Mean age (not significant)	Male / female distribution (not significant)	Mean number of missing teeth ($p<0.05$)	Mean period between first visit and present study in years ($p<0.05$)
Implant cases	44	21.9 (9.5)	34.1% vs. 65.9%	9.6 (5.9)	6.5 (4.3)
Non-implant cases	250	19.9 (12.4)	39.6% vs. 60.4%	11.5 (5.0)	8.4 (5.4)

of missing teeth is higher in the non-implant group *($P< 0.05$)*. In addition, the mean period between the first visit and the date of this study is shorter for the patients who received implants compared with those who did not *($P< 0.05$)*. In the 44 implant cases, 6 patients had been diagnosed with syndromic hypodontia, among which were 5 patients with ED and 1 patient with cleidocranial dysplasia.

Table 2 shows the distribution of missing teeth in the implant cases and non-implant cases. The most frequently missing teeth in the maxilla were the lateral incisors and the first and

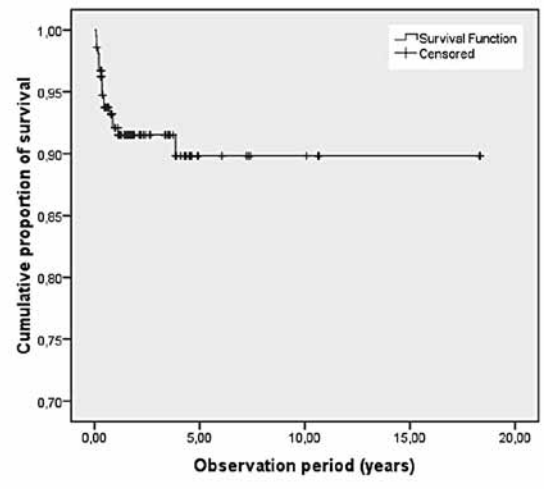


Figure 1. Time-dependent implant survival according to Kaplan-Meier method involoving 214 implants in 44 patients with severe hypodontia. The cumulative proportion of implant survival is estimated at 89.8% (SE, 2.6%) after 5 years and beyond (mean observation period, 2.9 years).

Table 2 Missing teeth in implant and non-implant cases

Missing tooth	Implant cases N = 448 teeth Percent*	Non-implant cases N = 1626 teeth Percent*
11	0.2	0.2
12	6.6	5.7
13	3.9	3.4
14	5.3	5.5
15	5.9	6.3
16	0.8	1.1
17	2.5	4.2
21	0.2	0.1
22	4.7	5.8
23	3.9	3.8
24	4.7	5.0
25	4.9	6.1
26	1.0	1.3
27	2.7	3.8
31	3.7	4.4
32	2.3	3.1
33	1.8	1.4
34	3.9	3.0
35	6.1	6.3
36	1.0	1.7
37	2.7	3.8
41	4.5	4.1
42	3.1	3.3
43	1.6	1.5
44	3.5	3.0
45	7.8	6.5
46	0.8	1.5
47	2.7	4.0
Total	100	100

* Note: approximately due to rounding error.

second bicuspids. In the mandible the second bicuspids were the teeth most frequently absent.

Over the years, surgical procedures were performed by 4 experienced oral and maxillofacial surgeons. Because of insufficient alveolar ridge dimensions for implant placement, various types of bone augmentation procedures before or in conjunction with implant placement were performed in 25 of 44 patients. Among them, the anterior iliac crest was by far the most popular region for harvesting of autologous bone (Table 3). Most implants were placed in the bicuspid and cuspid regions (Table 4). Details of the implants are presented in Tables 5, 6, and 7. All implants placed served to support fixed partial dentures. In total 214 implants were placed in 44 implant patients, which corresponds with a mean number of 4.9 implants (SD, 3.0) per patient (minimum, 1 implant; maximum, 14 implants). The patients' mean age at implant placement was 25.1 years (SD, 8.6) (minimum, 16.6 years; maximum, 48.5 years). The cumulative chance of implant survival of the 214 placed implants after 18 years was 89.8% (SE, 2.6%), with a mean observation period of 2.9 years (minimum, 0.1 years; maximum, 18.3 years) (Fig 1). Of the 214 placed implants, 18 were lost in 6 different patients. Multiple implant loss occurred in 1 patient, who lost 8 implants, and in 2 other patients, who lost 4 and 3 implants, respectively. Implant and treatment details of the failed implants are shown in Table 8. Most implants were lost within the first year after implant placement. One patient with ED (Johanson-Blizzard syndrome) lost a single implant. All others who lost 1 or more implants had non-syndromic hypodontia (at least a syndrome was not diagnosed). Of the 18 implants lost, 12 had been placed in the maxilla. Of the 18 implants that failed, 14 had been placed in patients in need of extensive bone augmentation (patients 1, 2, 4 and 6; Table 8). With respect to the other failed implants, ridge splitting was performed to allow implant placement (patients 3 and 5, Table 8).

Table 3 Origin of autologous bone and allografts as used during the bone augmentation procedures.

Bone augmentation	Percent* N = 44
No augmentation	43.2
Calvarium	6.8
Anterior iliac crest	20.5
Mandibular ramus	2.3
Chin region	4.5
Bio-Gide$	2.3
Bio-Oss$ and Bio-Gide	6.8
Calvarium and Bio-Gide	2.3
Mandibular ramus and chin region	2.3
Mandibular ramus, Bio-Oss and Bio-Gide	4.6
Anterior iliac crest and chin region	2.3
Calvarium and Bio-Gide	2.3
Total	100

* Note: approximately due to rounding error.

Table 4 Implant type

Implant type	Percent* N = 214
Frialit Xive/Synchro#	32.7
Astra OsseoSpeed$	56.5
IMZ ^	0.5
Straumann Standard plus&	8.4
Steri-Oss@	1.9
Total	100

* Note: approximately due to rounding error; # Dentsply Friadent, Mannheim, Germany; $ Astra Zeneca, Mölndal, Sweden; ^ Dentsply Friadent, Mannheim, Germany; & Straumann, Waldenburg, Switzerland; @ Steri-Oss, Yorba Linda, United States

Discussion

There is little information available with respect to restorative treatment outcome in patients with severe hypodontia. Reports in the literature are predominantly case reports or describe rather small and heterogeneous populations. In general, the increased experience with oral implants and several augmentation techniques has created new options for the oral rehabilitation of patients with severe hypodontia. Providing these patients with a fixed partial denture with minimal damage to the natural teeth is frequently feasible (Fig 2).[8, 14, 16]

At the University Medical Center Utrecht, many hypodontia patients have been treated over the years and a total of 294 could be included in this retrospective study. The mean period between the patients' first visit to the clinic and this study was longer for patients who received conventional treatment or no treatment at all when compared with the implant cases. A

Table 5 Implant length

Implant length (mm)	Percent* N = 191
8	1.9
9	0.5
10	1.9
11	16.8
12	2.8
13	31.8
14	1.9
15	31.8
Total	89.3
Missing	10.7

* Note: approximately due to rounding error.

Table 6 Implant diameter

Implant diameter (mm)	Percent* N = 203
3.3	3.7
3.5	25.7
3.8	16.4
4.0	18.7
4.1	4.2
4.5	20.1
4.8	0.5
5.0	5.1
5.5	0.5
Total	94.9
Missing	5.1

* Note: approximately due to rounding error.

Table 7 Implant location

Implant location	Percent* N = 214
11	0.5
12	6.5
13	7.4
14	6.9
15	7.4
16	1.9
21	0.9
22	5.1
23	5.1
24	6.0
25	6.5
26	1.9
31	2.3
32	0.5
33	3.2
34	5.1
35	9.3
36	0.9
41	2.8
42	1.4
43	2.3
44	6.0
45	9.3
46	0.9
Total	100

* Note: approximately due to rounding error.

similar finding was reported by Krieger et al (2009) in a retrospective study on the restorative outcome in a population of 22 hypodontia/oligodontia patients.[13]

This shift in preferred treatment in time is probably a logical consequence of the increased application of implant-supported restorative treatment alternatives in general in recent years. It also reflects the treatment recommendations in the literature over the years when coping with severe hypodontia. As early as 1980, Hobkirk and Brook10 had already proposed an interdisciplinary approach, involving pedodontics, prosthodontics, and orthodontics. Their therapeutic concept was based on the use of partial dentures or overdentures on natural roots and retention of the natural teeth for as long as possible. In the recent literature this interdisciplinary approach is still advocated, but the implementation of implants in the treatment strategy was introduced into the prosthodontic and surgical field.[5, 8-10, 12]

Patients' age and the gender distribution are comparable between the 2 subpopulations. However, the non-implant cases appear to be missing more teeth than the implant cases

Table 8 Patient and implant characteristics in cases with implant failure(s)

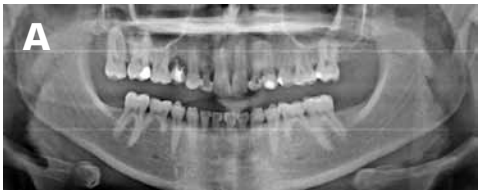
Patient no.	Implant no.	Gender	Location	Augment- ation / details	Length / Diameter (mm)	Implant type	Observ. period (years)
1	1	Male	14	Iliac crest	13/4.5	Frialit Xive$	0.2
	2		15	Iliac crest	13/3.8	Frialit Xive	0.9
	3		16	Iliac crest	13/4.5	Frialit Xive	0.9
	4		25	Iliac crest	13/3.8	Frialit Xive	0.2
	5		26	Iliac crest	13/3.8	Frialit Xive	0.5
	6		35	Iliac crest	13/3.8	Frialit Xive	0.2
	7		44	Iliac crest	13/3.8	Frialit Xive	0.5
	8		44*	Calvarium	11/4.0	Astra OsseoSpeed#	0.3
2	9	Male	44	Calvarium +Bio-Gide^	13/3.5	Astra OsseoSpeed	0.1
3	10	Female	22	Ridge splitting	15/3.8	Frialit Xive	0.2
4	11	Female	12	Chin region	15/3.8	Frialit Xive	0.1
	12		14	Chin region	13/3.8	Frialit Xive	0.1
	13		16	Chin region	13/3.8	Frialit Xive	0.7
	14		26	Chin region	13/3.8	Frialit Xive	1.1
5	15	Female	22	Ridge splitting	13/3.5	Astra OsseoSpeed	0.4
	16		25	Ridge splitting	11/3.5	Astra OsseoSpeed	0.4
	17		35	Ridge splitting	11/4.0	Astra OsseoSpeed	0.4
6	18	Female	45	Iliac crest	11/3.8	Frialit Xive	3.8

* This implant had replaced the original, failed implant (and failed as well). $ Dentsply Friadent, Mannheim, Germany; # Astra Zeneca, Mölndal, Sweden; ^ Geistlich, Wolhusen, Switzerland

(11.5 [SD, 5.0] vs. 9.6 [SD, 5.9]). A comparison between implant and non-implant cases was not made in other studies concerning hypodontia subjects.[7, 8, 11, 12] The trajectory of treatment in combination with implants in the more severe cases can be markedly longer and thus more intensive when compared with conventional prosthodontic treatment. More surgical interventions, among which are bone augmentation procedures, often with extra oral grafting donor sites, are frequently necessary (Table 3). It could be hypothesized that patients in the more severe group tend to opt for a less comprehensive, conventional prosthetic solution, accepting that the prosthetic appliances may be less comfortable. It should be noted, however, that it is hard to reconstruct retrospectively why one treatment option was favored over another.

Because treatment of patients with severe hypodontia is generally comprehensive, invasive, and therefore expensive, there is a need for studies in which the outcome of treatment is documented and evaluated. Implant survival is an important tool in measuring the efficacy of endosseous implant treatment in severe hypodontia.

In this study, a relatively large group of patients with severe hypodontia was evaluated. Although some implants could be followed for up to 18 years, the mean observation period was approximately 3 years. Eighteen implants were lost during the observation period. At 5 years, the cumulative chance of implant survival was 89.8% (SE, 2.6%). No additional



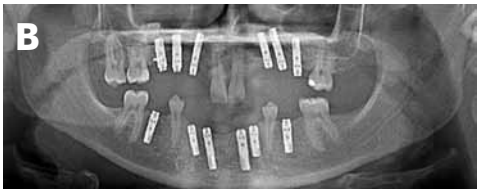
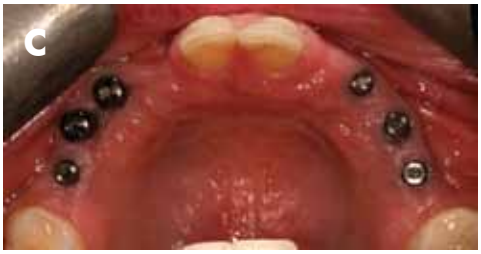
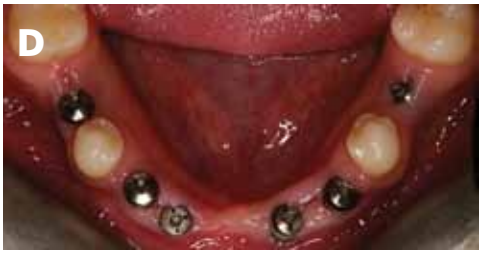


Figure 2. A 17-year old female patient with non-syndromic severe hypodontia, missing nine permanent teeth in the maxilla and ten teeth in the mandible.

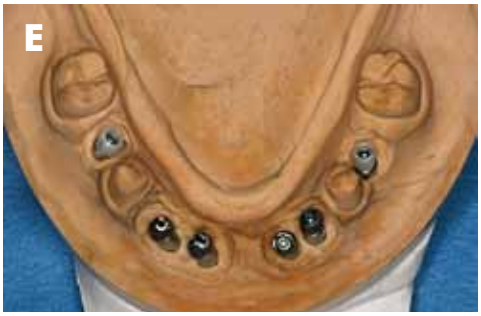
After implant placement.



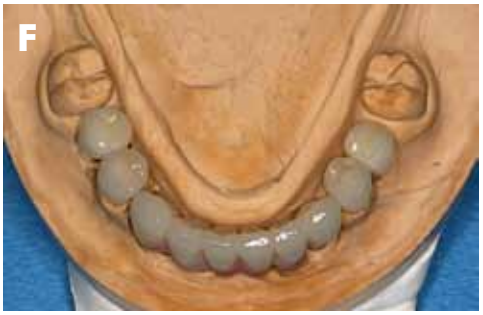
Occlusal view of the maxilla after implant placement.



Occlusal view of the mandible after implant placement.

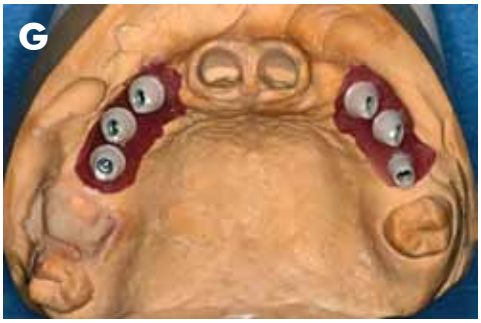


Model of the mandible with abutments.

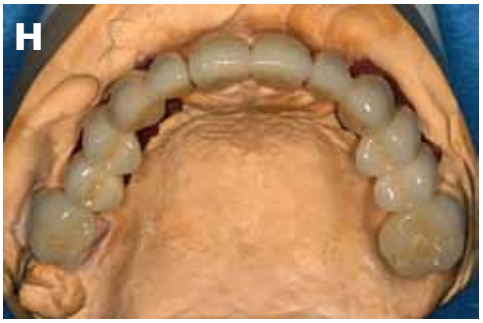


Model of the mandible with crowns and bridge in place.

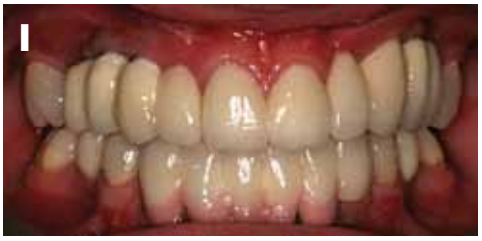
failures occurred thereafter. Most implants failed within the first year, and failure was noted in only a few patients. This clustering of failures is frequently seen in implant dentistry, as well as in non-hypodontia cases.[17, 18] Some previous reports on the use of implant treatment in patients with severe hypodontia have produced conflicting results but give rise to concern nonetheless. Unfortunately, the studied populations are rather small and predominantly involve patients with a verified diagnosis of ED. A 100% implant success rate was reported in a group of 6 ED patients with severe hypodontia[16] and an 88.5% survival rate was reported in 14 ED patients,[14] whereas Bergendal et al noted a 35.7% success rate in 5 young patients.[8] All failures in the latter group occurred before loading. Patient age ranged from 5 to 12 years, which was younger than in the other groups. The disappointing result



Model of the maxilla with all ceramic abutments.



Model of the maxilla with all ceramic crowns.



Intra-oral frontal view in occlusion. The lack of bone and peri-implant mucosa in the anterior region of the mandible, was mimicked by means of pink colored ceramic. Notice the recession of the marginal peri-implant mucosa on the right lateral side of the maxilla.

Pre- and post restorative treatment: extra oral appearance at 18-years and 21 years respectively.

was attributed to the small jaw size, rather than the ED per se. In our study, only a single implant was lost in 1 of the 5 patients with ED. No implants were placed in preadolescent ED patients. This seems to be a more predictable approach. Other authors have documented implant survival rates in patients with severe hypodontia ranging from 86% to 96.6%.[7, 8, 12] These patient groups can only be compared with each other and with the population from our

study up to a certain point because age at implant placement, the severity of the hypodontia, the use of augmentation, the operator, the type of implants used, the evaluation period, and many more factors differ.

It is tempting to compare the survival rate observed in our particular population with that found in 'regular' partially edentulous subjects. A systematic review by Jung et al dealing with the overall implant survival rate of single-implant crowns after 5 years in augmented and non-augmented cases may serve as a reference, although one cannot be sure that this study did not also include hypodontia patients. They estimated implant survival to be 96.8% after 5 years.[19] In a recent systematic review regarding single implants placed in the esthetic zone, an implant survival rate of 95.5% was calculated.[20] It should be realized that the outcome of a single-implant restoration with natural neighboring teeth may be dissimilar to cases in which multiple adjacent teeth are replaced by dental implants, as is frequently the case in patients with severe hypodontia. Single-implant sites benefit from tissue support of the adjacent dentition.[21] The overall implant survival rate in our population of hypodontia patients compares somewhat unfavorably with those described in the literature for what are considered non-hypodontia cases in non-edentulous situations.

Several additional reasons for the impaired implant survival rate can be hypothesized. Because crown formation and root development of permanent teeth are considered to be important for the development of the alveolar process, their absence and the subsequent lack of growth stimuli of the jaw bone will result in reduced alveolar bone volume and possibly also compromised bone structure.[11, 22] The consequences for implant placement may be less severe in cases where deciduous teeth are still present and have erupted properly to the level of the occlusal plane. After extraction, a suitable alveolar ridge may still develop, and conditions for implant treatment are favorable. However, if deciduous teeth have been lost prematurely or have not erupted properly, the alveolar ridge is locally underdeveloped. Implant placement may require extensive and more complex bone augmentation techniques to create an implant recipient site of adequate height and width. There seems to be a tendency for more extensive augmentation techniques to result in impaired implant survival rates.[23] In our study, in more than half of the cases, autologous bone augmentation had to be performed to increase the bone volume. In 14 of 18 failed implants a major augmentation procedure had been performed. The unfavorable anatomic conditions and the subsequent need for bone augmentation in most cases of severe hypodontia present the most likely cause of the compromised implant survival rate.

Genetic factors may also play a role. There is increasing understanding with respect to the molecular mechanisms during cell and tissue interactions. Some homeobox genes, among which is the *MSX1* gene, bear relevance to the process of both tooth and craniofacial morphogenesis.[24, 25] 'Bone quality' is considered relevant with respect to implant prognosis.[26] Recently, a new gene has been identified that, when mutated, causes severe

hypodontia, short stature, and increased skeletal bone density (*LTBP3*).[27] A difference in bone structure could be an influence on the process of osseointegration, as well as other maxillofacial procedures. Little is known about potential differences in trabecular bone structure between hypodontia patients and non-hypodontia subjects, but in a recent study comparing several radiographic parameters of mandibular trabecular bone structure, none could be convincingly demonstrated.[28] This feature is a subject of ongoing research.

Our study must be considered as a non-controlled, retrospective case series with all limitations that are inherent to retrospective studies of small diverse clinical populations, such as selection bias. From retrospective medical record reviews, it is hard to document what the considerations were in choosing a particular treatment. Consequently, considerable heterogeneity of patient material and treatment concepts will have resulted. Within these limitations, the following conclusions are drawn: The hypodontia patients in the studied population who were treated with endosseous implants were treated more recently and were missing fewer teeth than those who were treated with conventional means. Considering the compromised anatomic situation and the complexity of treatment, a 5-survival rate of almost 90% as seen in our study can be considered acceptable.

To improve the standard of care for patients with severe hypodontia, research and in particular prospective studies are needed to evaluate the objective and subjective outcome of various restorative treatment modalities, be it with or without implants in subpopulations of patients with comparable dental and skeletal features. Because of the heterogeneity of hypodontia, this will require vast patient populations, and international collaboration between centers should be encouraged.

Acknowledgment

The authors are grateful to Lennard van Hoften and Alexandra Kesselring for their help during data collection. Dr Jan M. Ruijter of the Department of Anatomy and Embryology, Academic Medical Center, Amsterdam, The Netherlands, is acknowledged for his statistical advice.

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Submitted: Clin Oral Investig 2011

6

Three-dimensional analysis of tooth dimensions in the MSX1-nonsense mutation

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Abstract

A novel, 3D technique to measure differences in tooth crown morphology between *MSX1* cases and non-affected controls was designed, to get a better understanding of dental phenotype-genotype associations.

Eight Dutch subjects from a single family with tooth agenesis, all with an established nonsense mutation c.332 C>A, p.Ser111Stop in exon 1 of *MSX1*, were compared with unaffected controls regarding several aspects of tooth crown morphology of incisor and molar teeth. A novel method of quantitative three-dimensional analysis was used to detect differences.

Statistically significant shape differences were observed for the maxillary incisor in the *MSX1* family compared with the controls on the following parameters: surface area, buccolingual dimension, squareness, and crown volume ($P \leq 0.002$). Molar crown shape was unaffected.

A better understanding of dental phenotype-genotype associations may contribute to earlier diagnosis of some multiple-anomaly congenital syndromes involving dental anomalies. A 'shape database' that includes associated gene mutations resulting from developmental syndromes may facilitate the genetic identification of hypodontia cases.

Introduction

Hypodontia, the congenital absence of one or more permanent teeth, is the most common developmental anomaly in humans, with a prevalence of 5.5% in Europeans and a 1.37:1 preference for women compared with men. Hypodontia appears as part of a syndrome or as a non-syndromic trait [1-5], and has a heterogeneous dental and dento-facial presentation. There is increasing understanding with respect to the genetic background of tooth development and, more specifically, to molecular mechanisms during cell and tissue interactions. The etiology of disturbances of tooth development is considered multifactorial and genetic, epigenetic, and environmental and their interactions factors play a role.[6-9] Over 300 genes have been associated with tooth development to date, at least in mouse embryos. Most of these genes seem to have a function in preserved signaling pathways during repetitive and reciprocal cellular communication between epithelial and mesenchymal tissues.[8-12]

When tooth development is disrupted, a wide spectrum of clinical phenotypes can be expected, including typical patterns of tooth agenesis and variations in tooth morphology and size. For example, in familial non-syndromic oligodontia, it has been suggested that defects in *PAX9* predispose for agenesis of maxillary and mandibular second molars, while in *MSX1*-associated oligodontia, agenesis of bicuspids is typically observed.

More recently, some variations in the *EDA* gene have been demonstrated to cause (X-linked) non-syndromic oligodontia, as well. The patterns of missing teeth associated with *EDA* mutations seem to differ distinctly from those observed with *PAX9* and *MSX1*. There appears to be a tendency towards agenesis of maxillary and mandibular central incisors, lateral incisors, and canines in the presence of an *EDA* mutation. In case of *PAX9* or *MSX1* mutations, maxillary and mandibular first preliminary molars frequently persist.[13, 14] In the case of mutations in the *SHH* gene, fusion of the central incisor tooth buds may result in a single central incisor.[15] In addition, subjects with a single missing central upper incisor can be heterozygous carriers for holoprosencephaly[16], a potentially more serious syndromic condition affecting midline development of the brain and face.

Some examples of aberrations in tooth shape and dimensions that are associated with genetic disturbances are provided below. Mutations in the EDA pathway (*EDA*, *EDAR*, *EDARADD*, *NEMO*) result in hypohydrotic ectodermal dysplasia, commonly with hypodontia and conical, peg-shaped teeth when they do develop.[17, 18] Absence of conical teeth suggests a non-syndromic variation of an EDA mutation.[19] In addition, it has been established that a common variation in *EDAR* (EDAR 1540C) in a Japanese population is strongly associated with the degree to which marginal ridges on the lingual surfaces of upper incisors (tooth shoveling) are developed.[20] Another example of genetically modulated tooth morphogenesis is observed in 47, *XYY* males. The Carabelli's trait and a four-cusp pattern are observed

more frequently in the second maxillary molar than in either male or female controls.[21] A typical combination of morphological tooth features is observed in association with a *DLX3* mutation (amelogenesis imperfecta with taurodontism). [22] In cases of hypodontia, teeth that are formed are generally smaller (microdontia) than those encountered in subjects without tooth agenesis [18, 23-25], while subjects with supernumerary teeth (hyperdontia) generally have larger teeth (macrodontia) than controls.[25, 26] Recently, mutations in the PCNT gene have been shown to be associated with very small teeth, possibly the smallest ever reported.[27] Taurodontia is frequently observed in subjects with hypodontia [28, 29], but not those with hyperdontia.[30] Therefore, a better understanding of dental phenotype-genotype associations may contribute to earlier diagnosis of some multiple congenital anomaly syndromes involving tooth anomalies; additionally, precise measuring tools for shape analysis are desirable.[31]

Morphological tooth traits, parameters of tooth dimension, and agenesis patterns may also serve as biomarkers for a dental phenotype, enabling early diagnosis of syndromes or specific genetic disorders.[18]The National Institutes of Health (NIH) defines a biomarker as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Biomarkers may be anatomic, physiologic, biochemical, or molecular measures that reflect the presence or severity of specific disease states.

Quantitative morphological analysis of teeth poses a fundamental problem because teeth are multi-dimensional, irregular objects that are difficult to measure and quantify.[32] Continuous measures are preferred over descriptive ones, such as the presence or absence of Carabelli's trait or hypocone reduction. In odontometric analyses, linear measurements such as the mesiodistal and buccolingual tooth dimensions are traditionally performed on dental casts by means of analogue or digital calipers. This type of measurement can be obtained with a high degree of inter- and intra-observer reliability.[25] More recently, two-dimensional (2D) image analysis systems became available and non-linear measurements, such as surface areas and perimeters, could be reliably determined.[33] Because both mesiodistal and buccolingual dimensions are generally smaller in subjects affected by hypodontia [25], tooth volume is expected to be an even more discriminative three-dimensional (3D) parameter with which to distinguish small differences in tooth dimension between subgroups of patients.

For this purpose, we have developed a technique to geometrically evaluate the morphological parameters of teeth in three dimensions. This technique was applied to compare a series of patients with a known *MSX1* mutation with healthy controls under the null hypothesis that they are similar. Observed differences in tooth crown morphology between *MSX1* cases and non-affected controls will be discussed in light of the present understanding of the biological regulation regarding some features of tooth crown morphogenesis.

Materials and methods

This study was designed as a case-control study.

Cases

Eight Dutch subjects from one family (4 males and 4 females) with tooth agenesis and cleft palate or cleft lip and palate participated in the study. Subjects' ages varied from 9 to 68 years (mean age, 39 years). All subjects had an established nonsense mutation c.332 C>A, p. Ser 111 Stop in exon 1 of *MSX1*. This particular population has been previously described in detail by van den Boogaard *et al.*, and this mutation was known as p.Ser105Stop. The nomenclature has been changed in accord with the guidelines of the Human Genome Variation Society (HGVS) with reference sequence NM_002448.3[34]. Absent teeth are presented in Table 1.

election of target teeth

Like other families with an *MSX1* mutation, the majority of the family members in the present study lack the premolars, predominantly the second premolars (Table 1)[26]. Therefore, we selected two tooth types that were present in all family members and, in general, are present in even the most severe hypodontia cases: the right maxillary first molar and the central incisor.

Table 1 Congenitally absent teeth in the MSX1 cases

	Maxilla	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
	Mandible	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
Male																	
1	Maxilla	X			X	X							X	X			X
	Mandible	X			X									X			X
2	Maxilla	X	X		X	X							X	X			X
	Mandible	X			X									X		X	X
3	Maxilla	X			X	X							X	X			X
	Mandible	X			X									X		X	X
4	Maxilla	X			X	X							X	X			X
	Mandible	X	X	X	X									X	X	X	X
Female																	
1	Maxilla	X			X	X							X	X			X
	Mandible	X			X									X			X
2	Maxilla				X	X							X	X			
	Mandible	X			X									X			X
3	Maxilla	X			X	X							X	X			X
	Mandible	X	X	X	X									X	X	X	X
4	Maxilla	X	X		X	X							X	X		X	X
	Mandible	X	X	X	X				X	X				X	X	X	X

(X = congenitally missing).

Controls

Healthy Caucasian subjects without hypodontia served as controls (21 males and 21 females). They were selected from the database of the Department of Orthodontics and Craniofacial Biology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands. To be included, all permanent teeth had to be present, including the third molars. The right maxillary central incisor and maxillary first molar should be non-restored and fully erupted. Subjects were excluded when the target teeth were damaged, showed excessive tooth wear, or with severe crowding.

3D measurements of tooth dimensions

Conventional gypsum models were processed into digital dental models and their raw geometric data were obtained for all cases and controls (Digimodel, OrthoProof B.V., Doorn, The Netherlands). Target teeth were virtually cut from the models using commercially available software (Maxilim, Medicim B.V., Mechelen, Belgium). Subsequently, the teeth were loaded into a computer program that enables the mathematical analysis of 3-dimensional shapes (Matlab 2007b, the Mathworks, Natick, USA; Fig. 1).

The teeth must be positioned reproducibly in the geometric model. For this purpose, a reference plane was defined for both the molar and the incisor on the basis of pre-defined reference points, as described below. All geometric measurements were performed from this reference plane.

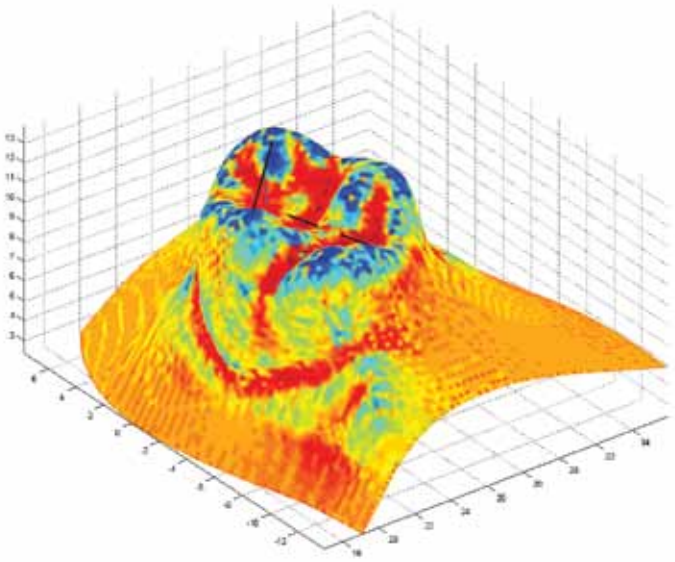

Figure 1 The teeth were loaded into a computer program that enables mathematical analysis of 3-dimensional shapes. For the first molar, a line is drawn between the two mesial cusp-tips. A second line is drawn from the mesial to the distal margin. These two lines are then projected onto each other to form a plane parallel to the occlusal plane.

Maxillary first molar

For the first molar, a line was drawn between the mesial buccal and lingual cusp tips. A second line was drawn from the mesial to the distal margin (Fig. 1). These two lines were projected onto each other to form a plane parallel to the occlusal plane. The molar was then rotated automatically until the constructed plane was parallel to the X-Y plane (Fig. 2). The initial idea was to lower the plane until the largest perimeter of the molar was reached. Unfortunately, this often presents sub-gingivally, where no data regarding tooth dimensions are available. Therefore, the plane was lowered 1.2 mm below the deepest point in the fissure (which was determined automatically). At this depth, all models could be included in the study. The position of the reference plane had now been established.

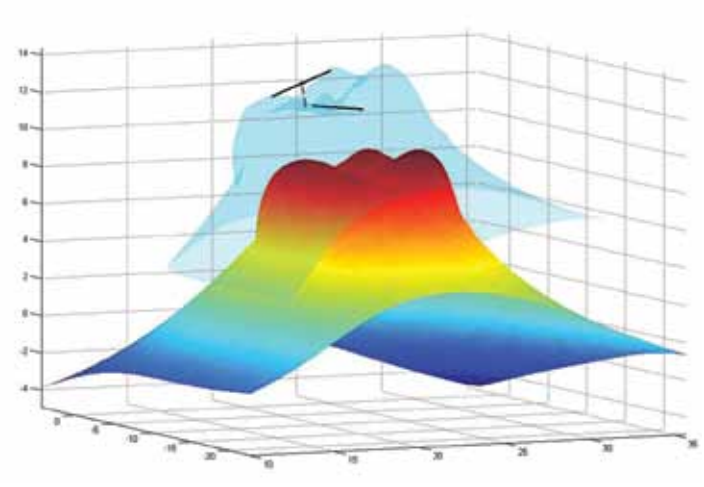

Figure 2 The molar is rotated automatically until the constructed plane is parallel to the X-Y plane.

Maxillary central incisor

For the incisor, a line was drawn through the incisal edge. Subsequently, the most prominent point on the buccal surface and the margin of the cingulum on the palatal surface were determined, and lines were drawn through these two points (perpendicular to the incisal edge line). To create a plane, the model was rotated along the incisal edge line until these two newly constructed lines were equal in length (Fig. 3). Finally, the reference plane was determined 2.3 mm beneath the incisal edge line. This position was chosen because it ensured that the reference plane would be above the gingival margin in all cases.

Morphological quantitative parameters

Tooth crown shape was quantified by means of six parameters that were thought to be representative, all of which were calculated at the level of the reference plane. These parameters were defined as follows:

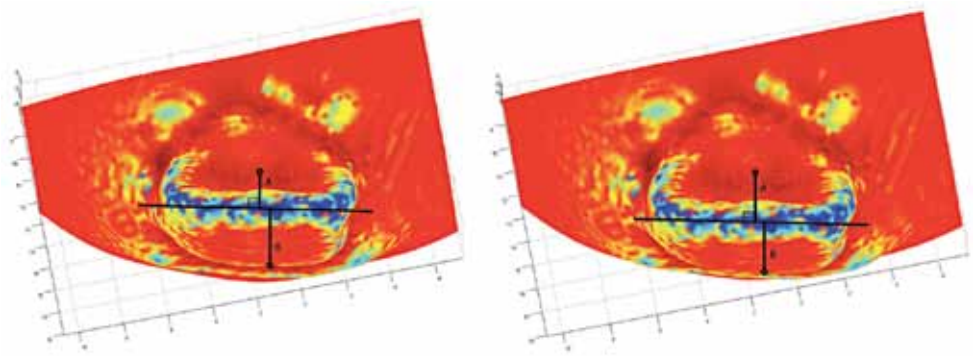

Figure 3 To create a plane, the model is rotated along the incisal edge line until the two newly constructed lines (A and B) are equal in length. Line A is drawn perpendicular to the incisal edge line, through the center of the cingulum. Line B is also drawn perpendicular to the incisal edge line, through the most prominent buccal point.

- Perimeter: the perimeter of the crown at the level of the reference plane (mm);
- Surface area: the surface of the plane at the level of the reference plane (mm^2);
- Buccolingual distance: the maximal distance between the buccal surface and the lingual surface of the crown (mm);
- Mesiodistal distance: the maximum distance between the mesial and distal proximal surfaces (mm);
- Squareness: indicated to what degree the tooth crown shape was square and was the ratio between the mesiodistal and buccolingual distances (mm/mm);
- Volume: the volume of the crown was calculated from the reference plane to the incisal edge and cusps (mm^3).

Repeatability

Twenty randomly selected incisors and 20 randomly selected molars were measured and remeasured by the same observer, and 20 molars and 20 incisors were measured by another observer to assess intra- and inter-observer repeatability. Repeatability of the measurements was expressed as the coefficient of repeatability (CR) in accordance with Bland and Altman[35].

Statistical analysis

Two-way analysis of variance with the geometric parameters as dependent variables and group and gender as fixed factors was applied for the measurements on both the molar and the incisor. Tooth dimensions are likely to be correlated and multiple-testing correction to overcome the increase in type I error was advisable; hence, Bonferroni correction was performed. Consequently, α was set at 0.01. This is considered to be a conservative approach.

Results

The CRs for both intra- and inter-observer repeatability are presented in Table 2. The CR was interpreted in accordance with the guidelines of the British Standards Institution, which states that 95% of the difference between the first and second measurement is expected to be within two standard deviations of the mean difference.[36] This was the case for the inter-observer repeatability of all measurements on both molars and incisors, and was considered satisfactory. Regarding the intra-observer repeatability of the mesio-distal distance, area, and volume, 90% of the differences fell within two standard deviations of the mean difference, while all other parameters were at 95% (Table 2).

The mean values and standard deviations for *MSX1* cases and controls, as well as the statistical comparisons, are presented in Table 3. For the first maxillary molar, no statistically significant differences were noted for any of the six parameters, nor were interaction effects noticeable between gender and group (*MSX1* case or control). Therefore, the null hypothesis

Table 2 Intra- and inter-observer repeatability for incisors (n = 20) and molars (n = 20)

	Coefficient of Repeatability 35, Incisors	
	Intraobserver repeatability	Interobserver repeatability
Perimeter (mm)	0.8	1.1
Mesiodistal distance (mm)	0.3	0.5 *
Buccolingual distance (mm)	0.3	0.3
Area (mm^2)	1.9	1.4 *
Volume (mm^3)	4.5	2.0 *
	Coefficient of Repeatability 35, Molars	
	Intraobserver repeatability	Interobserver repeatability
Perimeter (mm)	1.2	3.2
Mesiodistal distance (mm)	1.0	1.4
Buccolingual distance (mm)	0.3	0.6
Area (mm^2)	3.1	6.6
Volume (mm^3)	12.2	12.3

* 90% of differences fall within two standard deviations of the mean difference. With all other coefficients, 95% of differences fall within two standard deviations of the mean difference.

that there are no differences in dimensions of the maxillary right first molar between *MSX1* cases and non-affected controls cannot be rejected.

Regarding the central incisor, the *MSX1* cases have significantly higher values for the area of the reference plane, as well as its buccolingual distance, squareness, and volume. Again, there were no interaction-effects between gender and group. For the central incisor, the null hypothesis that there are no differences in dimensions between *MSX1* cases and controls can be rejected. Digital models of a typical *MSX1* case and a control are seen in figures 4a and 4b respectively.

Table 3 Descriptive statistics for geometric parameters of crown morphology for molars and incisors in MSX1 cases and controls. Mean values, standard deviations in parentheses. Univariate analysis of variance with geometric parameters as dependent and gender and group fixed factors, P-values given.

	Control (n = 42) Mean (SD)	Case MSX1 (n = 8) Mean (SD)	Gender Group P-value	Gender x Group	
				P-value	P-value
Molar					
Perimeter (mm)	35.9 (2.4)	34.7 (4.6)	0.962	0.264	0.199
Area (mm^2)	93.2 (10.7)	89.8 (23.0)	0.733	0.513	0.191
Buccolingual distance (mm)	9.7 (0.5)	9.3 (1.3)	0.673	0.114	0.699
Mesiodistal distance (mm)	10.7 (0.8)	10.2 (1.2)	0.767	0.193	0.962
Squareness (mm/mm)	1.1 (0.1)	1.1 (0.1)	0.688	0.336	0.848
Volume (mm^3)	151.6 (24.5)	152.1 (57.9)	0.708	0.958	0.159
Incisor					
Perimeter (mm)	21.2 (1.7)	22.1 (1.6)	0.096	0.250	0.598
Area (mm^2)	24.3 (3.7)	31.3 (7.5)	0.079	0.000*	0.484
Buccolingual distance (mm)	3.0 (0.3)	4.2 (0.7)	0.051	0.000*	0.912
Mesiodistal distance (mm)	8.6 (0.6)	8.7 (0.9)	0.127	0.693	0.732
Squareness (mm/mm)	2.8 (0.3)	1.9 (0.3)	0.380	0.000*	0.965
Volume (mm^3)	35.8 (5.6)	45.6 (13.1)	0.301	0.002*	0.986

* Statistically significant values below $\alpha = 0.01$ (Bonferroni correction)

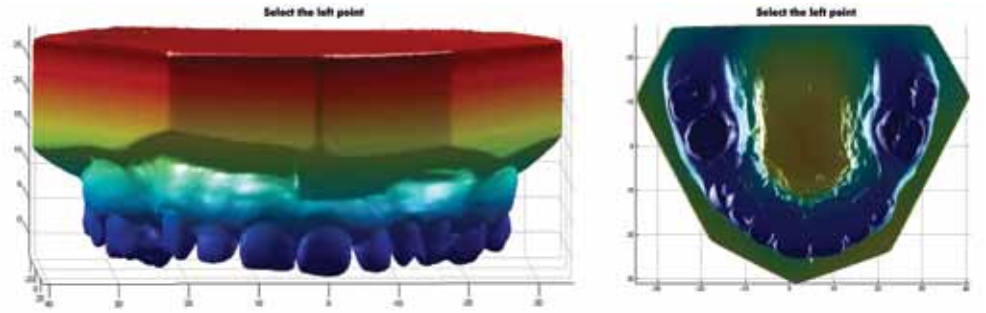

Figure 4a Digital model. Frontal (left) and occlusal (right) view of a typical MSX1 case (female). It is noticeable that the teeth of the MSX1 female are disctinctive in shape and size compared to a control (i.e. figure 4b). In particular the central incisor has a distinctive 'square' appearance.

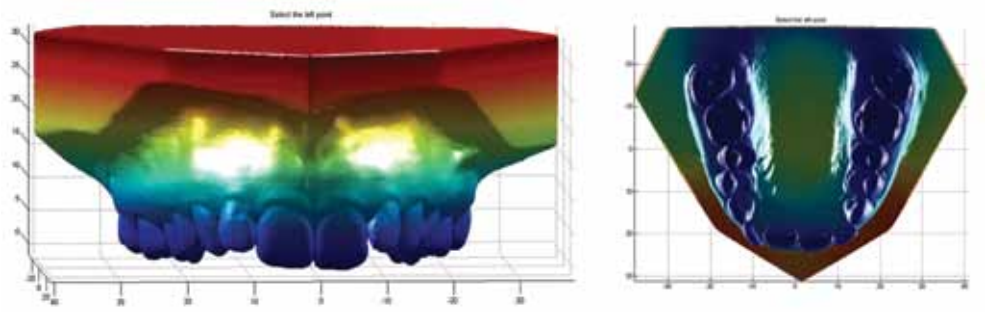

Figure 4b Digital model. Frontal (left) and occlusal (right) view of a healthy control (female).

Discussion

We suggest that quantification of tooth crown shapes may contribute to the early diagnosis of congenital anomaly syndromes involving teeth. A 'shape database' that includes associated gene mutations resulting from developmental syndromes may facilitate the genetic identification of hypodontia cases. So far, the authors have not found an existing technique that enables volumetric measurements in isolated teeth for this purpose; only 2D techniques have been described in literature.

Teeth are multi-dimensional, irregular objects, and are therefore difficult to measure in 2D. To measure 2D parameters such as the perimeter correctly, the 2D picture must be perpendicular to the occlusal plane.[25] However, each tooth has a specific angulation within the jaw. The analysis of multiple teeth would require a separate picture for each individual tooth. Also, additional phenotypes of tooth morphology and dimensions that involve volumetric aberrations can only be obtained from 3D measurements. Because both mesiodistal and buccolingual dimensions in subjects affected with hypodontia are generally smaller, tooth volume is expected to be an even more discriminative parameter.

Because of scattering and related issues, computed tomography (CT) cannot produce 3D data in sufficient detail to allow detailed geometric measurements of teeth. Other disadvantages of CT are that it would require exposure of a subject to radiation and CT equipment is not yet generally available. A laser scanner–based image analysis system that can acquire 3D data of small objects, such as tooth crowns in dental casts, has been described in the literature. The system was considered reliable when comparing the same parameters in 2D and 3D; however, it has not yet been applied to obtain 3D data, such as tooth volume, and would require an experimental setup, which is not very practical.[37] The drawbacks of other techniques have led to the development of the currently described method of 3D analysis to quantify tooth morphology. The method applied is relatively simple, minimally invasive, and inexpensive compared to other methods and conventional gene tests. The only required input is a dental impression of the upper jaw, which is transferred to a 3D digital model. Direct intra-oral scanning of tooth crowns to obtain a 3D digital dataset, without requiring an impression, is also a realistic option. Presently, the described method is rather time consuming, although this concern could be resolved considerably by automating the process, which would probably enhance measurement precision, as well.

In the present *MSX1* family, a specific pattern of tooth agenesis was observed; however, aberrations in tooth morphology were also noted. The central incisor and first molar were chosen because they present in the mouth at an early age (six years of age) and because they are almost never congenitally absent, even in cases of severe hypodontia.[1] Future studies may involve other tooth types as well. This may help diagnose other genotype-fenotype

correlations. Affected teeth may be different among diseases. To ensure accurate and reproducible measurements, the reference points were chosen on the hard tissues and in non-abrasive zones. Reference points in the vicinity of the gingival margin were avoided; they were deemed unreliable because of the soft nature of the gingiva, and variation due to gingivitis. Furthermore, only the supragingival area of a tooth is available for evaluation, and the size of the area is dependent upon the eruption phase.

A striking feature in the *MSX1* cases is that the central incisor has a significantly larger area of the reference plane, a larger buccolingual distance and volume. We did not take body size into account because this information was not available for the controls. This may have helped interpreting these findings. *MSX1* cases also present with incisors that have a more square appearance (outcome variable: 'squareness'). These findings are in contrast with the finding that all teeth that develop in hypodontia are generally smaller than in control groups.[38, 39] A possible explanation for the enlarged incisors in the studied family may arise from proximal-distal patterning during tooth development. Tucker showed that the developing oral epithelium can be divided into two domains, one distal and one proximal.[40] The epithelium of the presumptive incisor domain expresses *BMP4*, which positively regulates the expression of *MSX1* and *MSX2* in the underlying neural-crest–derived mesenchyme. Meanwhile, *FGF8* is expressed in the epithelial presumptive molar region and regulates the expression of *Barx1*. *BMP*4 and *FGF8* negatively regulate each other, thereby restricting *Barx1* expression to the presumptive molar region. The boundary between *MSX1* and *Barx1* demarcates the presumptive incisor forming and the presumptive molar-forming region. Manipulating the expression of these signaling factors could change the crown shape. When beads with Noggin protein, which antagonizes BMP signaling, are placed in the distal mesenchyme, and the expression of *MSX1* is lost, a molar tooth is formed in the presumptive incisor region

Interestingly, in K14-noggin mice, in which over expression of noggin blocks BMP signaling, the incisors were thick, wide, and blunt-ended.[41] They stated that subtle differences in the level, distribution, and timing of signaling molecules could have morpho-regulatory consequences.[41] Modulation of BMP4 signaling can transform a conically-shaped tooth into a tooth with a more complex morphology. Because *MSX1* plays an important role in BMP signaling, one can hypothesize that the incisors in this family have a more posterior, molar-like appearance as a result of decreased *MSX1* expression.

No statistically significant differences were observed for any of the six parameters in the first molar. Clinically, we did observe a deviating morphology in the cusps. The results have a tendency towards a smaller volume and more squared appearance. We expect that these small differences in morphology would also be expressed statistically if the case group were larger. In one case, there was a small extra cusp present. The shape of the tooth crown results from morphogenesis of the epithelium during the cap and bell stage of tooth development,

through differential growth and folding of the epithelium.[42] The enamel knots express growth stimulatory signals. It has been demonstrated that apoptosis in the enamel knot plays an important role in regulating tooth size and shape, and the expression of *BMP4* in the enamel knot is associated with apoptosis.[42, 43]

A model has been presented in which the pattern of tooth cusps is regulated by *FGF4*, which functions as an activator promoting cusp initiation and growth. Inhibitors, such as *BMP4*, control the distance between the enamel knots and negatively regulate cusp growth. It has also been reported that using z-VAD-fmk treatment to block apoptosis results in morphological anomalies. The morphology of mouse molars was similar to that observed in human macrodontia.[44]

MSX1 and *BMP4* are closely associated during tooth development. *BMP4* and *MSX1* regulate one another in a positive feedback loop.[42] Jernvall et al. suggest that *MSX1* is needed for *BMP4* expression, which in turn induces the expression of p21, which is responsible for apoptosis.[45] Thus, apoptosis in the enamel knot is necessary for the proper formation of molar teeth, including appropriate shape and size. It is tempting to speculate that *MSX1*, by inducing *BMP4*, is involved in this stage of tooth morphogenesis, as well. Such speculation could explain the deviating morphology of the molar cusps presented in the studied family. Reduced *MSX1* expression could result in reduced expression of *BMP4* and *p21*, which in turn would inhibit apoptosis.

Conclusions

We introduced a novel, 3D technique to measure aspects of tooth morphology to get a better understanding of dental phenotype-genotype associations. This measurement technique may contribute to earlier diagnosis of some multiple-anomaly congenital syndromes involving teeth anomalies. These findings suggest that *MSX1* may play a role not only in tooth patterning, but also in tooth morphogenesis, as expressed by distinct shape differences, particularly in maxillary incisors, between *MSX1* family members and controls regarding the parameters of area, buccolingual distance, squareness, and volume.

Acknowledgments

We are grateful to the affected members of the *MSX1* family for their continued participation. Dr. J. Ruijter of the Department of Anatomy and Embryology of the Academic Medical Center, Amsterdam, the Netherlands is gratefully acknowledged for his input and statistical advice. We would also like to thank N.A. Nagel, technical medicine student at the University of Twente, The Netherlands, who wrote part of the measuring program.

Conflict of interest

The authors declare that they have no conflict of interest.

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Submitted: J Med Genet 2012.
Accepted 19-03-2012

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Mutations in WNT10A are present in more than half of isolated hypodontia cases

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Abstract

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

In a panel of 34 patients with isolated hypodontia the candidate gene *WNT10A* as well as the genes *MSX1*, *PAX9*, *IRF6*, and *AXIN2* have been sequenced. The probands all had isolated agenesis of between six and 28 teeth (excluding the third molars).

WNT10A mutations were identified in 56% of the cases with non-syndromic hypodontia. *MSX1*, *PAX9* and *AXIN2* mutations were present in respectively 3%, 9% and 3% of the cases. We identified *WNT10A* as a major gene in the etiology of isolated hypodontia. By including *WNT10A* in the DNA diagnostics of isolated tooth agenesis the yield of molecular testing in this condition was significantly increased from 15% to 71%.

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 [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 [1-4]. Congenital dental agenesis can occur as an isolated anomaly or as a one of the features in a large variety of syndromes [2, 4-6]. Hypodontia is also a common feature of ectodermal dysplasia (ED) [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 [7,8]. Genes associated with ED include *EDA*, *EDAR*, *EDARADD* and *WNT10A* [7,8]. Typically, homozygous mutations in *WNT10A* cause various ectodermal dysplasia's, often corresponding to the odontoonychodermal dysplasia (OODD) and Schöpf-Schulz-Passarge-syndrome (SPSS) both combining classic ectodermal developmental anomalies (e.g. hypohidrosis, hypotrichosis, nail dysplasia, lacrimal duct hypo/aplasia, hypo/oligodontia) with additional cutaneous features including facial telangiectases and palmoplantar keratoderma. SSPS is distinguished by the presence of multiple eyelid cysts, histologically corresponding to apocrine hidrocystomas. OODD is apparently characterized by a smooth tongue (i.e. hypoplasia of lingual papillae) [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 *WNT10A* [10,11].

Interestingly, Bohring at al. (2009) suggested that nearly 50% of heterozygotes for *WNT10A* mutations might display isolated ectodermal developmental defects such as missing teeth [11]. According to this original finding, more recently, Kantaputra and Sripathomsawat (2011) demonstrated segregation of a heterozygous *WNT10A* mutation in an American family with autosomal dominant tooth agenesis without recognizable ectodermal features [13].

These observations prompted us to study the contribution of *WNT10A* mutations in comparison with mutations in other genes associated with hypodontia among isolated hypodontia patients consecutively ascertained in a tertiary dental clinic.

Materials and methods

Participants

Individuals with apparently isolated dental agenesis of six or more permanent teeth visiting the Department of Oral and Maxillofacial Surgery, Prosthodontics and Special Dental Care of the University Medical Center Utrecht (UMC Utrecht), were referred to the Department

of Medical Genetics of the UMC Utrecht for syndrome diagnostics and genetic counseling. Tooth agenesis in the patients was assessed by clinical examination by the dentist and on panoramic radiographs.

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. Of 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 ectodermal dysplasia or other syndromes, all patients were physically examined by a single clinical geneticist (MJ van den Boogaard). In addition according to a standardized form, patients were asked about possible symptoms of sweat glands, skin, hair, and nails.

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 gland) suggestive of ectodermal dysplasia. Patients with one major additional ectodermal feature or 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 males (41%) and 20 females (59%)) were classified as non-syndromic and included in this study. The mean age of these patients was 19.7 years (range: 9-53). In 17 patients (10 males (59%) and seven females (41%)) the hypodontia was classified as suspect for ED or syndromic hypodontia due to their additional features (e.g. sparse hair, nail abnormalities, cleft). The mean age of these patients was 20.5 years (range: 7-63).

Blood samples were obtained and DNA analyses of the genes *WNT10A*, *MSX1*, *PAX9*, *IRF6* and *AXIN2* was 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.

In the cohort of 34 non-syndromic probands, a mean of 14.6 (range: 6-28) teeth were missing. In 25 patients (73.5%) there was a positive family history (third degree or more closely related) for tooth agenesis.

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. Amplification was performed by PCR of all exons and their splice site consensus sequences with the Amplitaq Gold 360 Master Mix (Applied Biosystems, Bedford, MA, 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 3130 or 3730 sequencer (Applied Biosystems) was used for analysis. Mutation analysis was performed using the genetic analysis software Sequence Pilot version 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 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). All mutations identified were interpreted as potentially damaging. Heterozygosity for mutations in *PAX9* was identified in three patients (p.Y60*, pY143C respectively p.S49L). In one of the probands a probably pathogenic *MSX1* mutation (p.R223L) was detected. One patient showed a nonsense 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%). (tables 1 and 2). One patient showed a *WNT10A* mutation in addition to an *EDA1* mutation that was previously reported in X-linked hypohidrotic ED (OMIM 305100).

The most frequent mutation p.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 and are part of the normal variation in the population (table 1). Characteristic features of OODD 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,

Table 1: Clinical symptoms and Mutational results in 19 non-syndromic hypodontia Patients with WNT10A Mutations

Patient	1	2	3	4	5	6	7	8
Gender	f	m	f	f	f	f	m	m
Age (years)	22	39	19	11	28	32	18	14
Primary teeth abnormal	–	–	+	–	–	–	–	–
Permanent teeth missing	+	+	+	+	+	+	+	+
Number tooth agenesis	16	15	20	12	10	14	13	28
Abnormal shape teeth	+	+	+	–	+	–	+	+
Smooth tongue	–	–	–	–	–	–	–	
Sparse scalp hair	–	Am	±	–	–	–	–	–
Sparse body hair	–	–	–	–	–	–	–	–
Sparse eyebrows	–	–	–	–	–	–	–	–
Short eyelashes	–	–	–	–	–	–	–	–
Hypohidrosis	–	–	–	–	–	–	–	–
Hyperhidrosis	–	–	–	–	–	–	–	–
Dry skin	–	–	–	–	–	–	E	–
Soft, thin skin	–	–	–	–	–	–	–	–
Palmar hyperkeratosis	–	–	–	–	–	–	–	–
Hyperkeratosis on dorsal hands	–	–	–	–	–	–	–	–
Plantar hyperkeratosis	–	±	–	–	–	–	–	–
Palmoplantar sudation	–	±	–	+	–	–	±	–
Dyshidrotic blistering	–	–	–	–	–	–	–	–
Dystrophic fingernails	–	–	–	–	–	–	–	–
Dystrophic toenails	–	–	–	–	–	–	–	–
Photophobia	–	–	–	–	–	–	–	–
Lid cysts	–	–	–	–	–	–	–	–
Family history *	+	+	+	+	–	+	–	–
Mutational result								
Nucleotide substition	c.321	c.321	c.383	c.487	c.682	c.682	c.918	c.283
(first allele)	C>A	C>A	G>A	T>A	T>A	T>A	C>G	>A
Nucleotide substition	c.=	c.=	c.=	c.=	c.=	c.=	c.=	c.682
(second allele)								T>A
Amino acid substitutions	p.C107*	p.C107*	p.R128Q	p.R163W	p.F228I	p.F228I	p.N306K	p.G95K
	p.=	p.=	p.=	p.=	p.=	p.=	p.=	p.F228I

+: present; - absent; ±: very mild; Am:male alopecia; F: very fair hair; * family members with tooth agenesis

short eyelashes and abnormalities of the toenails. A dry skin was present in several cases (tables 1 and 2).

Dental characteristics in WNT10A cases

The dental numerical characteristics are presented and the tooth agenesis code (TAC) is calculated (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 side

9	10	11	12	13	14	15	16	17	18	19
m	m	f	m	f	m	f	f	m	m	f
10	14	16	18	13	12	15	15	18	19	29
–	–	–	–	–	–	–	–	–	–	–
+	+	+	+	+	+	+	+	+	+	+
14	26	14	26	10	17	13	10	11	15	12
+	+	+	+	+	+	+	+	–	+	+
–	–	–	–	–	–	–	–	–	–	–
– F	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	±	–
–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	–	±	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	–	–	–	–	–	–	–	–	–	–
–	+	+	+	+	+	+	–	+	+	+
c.321	c.321	c.321	c.433	c.682	c.682	c.682	c.682	c.682	c.682	c.682
C>A	C>A	C>A	G>A	T>A	T>A	T>A	T>A	T>A	T>A	T>A
c.682	c.682	c.682	c.433	c.682	c.682	c.682	c.682	c.682	c.682	c.682
T>A	T>A	T>A	G>A	T>A	T>A	T>A	T>A	T>A	T>A	T>A
p.C107*	p.C107*	p.C107*	p.V145M	p.F228I	p.F228I	p.F228I	p.F228I	p.F228I	p.F228I	p.F228I
p.F228I	p.F228I	p.F228I	p.V145M	p.F228I	p.F228I	p.F228I	p.F228I	p.F228I	p.F228I	p.F228I

of the jaw was in line with the population of non-syndromic hypodontia and seen in 58 % respectively 63 % of all non-syndromic *WNT10A* cases for the maxilla and the mandible. In the syndromic *WNT10A* cases this symmetry for the maxilla and mandible was observed in 46% respectively 64% of the cases. Neither patterns of missing teeth that would distinguish the current population from a general population of non-syndromic hypodontia patients nor 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* carriers (tables 1 and 2) was similar 15.6 (range 10-28) respectively 15.4 (range 6-30). The highest

Table 2: Clinical symptoms and Mutational results in 11 syndromic hypodontia Patients with WNT10A mutation

Patient	1	2	3	4	5	6	7	8
Gender	m	m	f	m	f	m	f	m
Age (years)	9	22	34	45	7	8	12	15
Primary teeth abnormal	+	–	–	–	–	–	–	–
Permanent teeth missing	+	+	+	+	+	+	+	+
Tooth agenesis (number)	12	13	11	18	30	18	20	6
Abnormal tooth shape	+	–	–	+	+	+	+	+
Short roots	–	+	+	–	+	?	+	–
Smooth tongue	–	–	–	–	–	–	–	±
Sparse scalp hair	–	–	+	+	+	+	–	–
Sparse body hair	–	–	–	+	–	–	–	–
Sparse eyebrows	–	–	–	+	+	+	+	–
Short eyelashes	–	–	–	+	+	+	+	±
Hypohidrosis	–	–	–	+	–	–	–	–
Hyperhidrosis	–	–	–	–	–	–	–	–
Dry skin	E	+	–	–	+	+	–	±
Soft, thin skin	–	–	–	–	–	–	–	–
Palmar hyperkeratosis	–	–	–	–	–	–	–	–
Hyperkeratosis on dorsal hands	–	–	–	–	–	–	–	–
Plantar hyperkeratosis	–	+	–	–	–	–	–	+
Palmoplantar sudation	–	+	–	–	–	–	–	+
Dyshidrotic blistering	–	–	–	–	–	–	–	–
Dystrophic fingernails	–	–	–	±	+	–	–	–
Dystrophic toenails	+	–	–	±	+	–	–	+
Photophobia	–	–	–	–	–	–	–	–
Lid cysts	–	–	–	–	–	–	–	–
Lacrimal duct stenosis	–	–	–	–	–	–	–	–
Hearing loss	–	–	–	–	–	–	–	–
Cleft	–	+	–	–	–	–	–	–
Family history *	+	+	–	+	–	+	+	+
Mutational result								
nucleotide substition	c.321	c.321	c.682	c.682	c.321	c.321	c.321	c.321
(first allele)	C>A	C>A	T>A	T>A	C>A	C>A	C>A	C>A
nucleotide substition	c.=	c.=	c.=	c.=	c.321	c.682	c.682	c.682
(second allele)					C>A	T>A	T>A	T>A
Amino acid substitutions	p.C107*	p.C107*	p.F228I	p.F228I	p.C107*	p.C107*	p.C107*	p.C107*
	p.=	p.=	p.=	p.=	p.C107*	p.F228I	p.F228I	p.F228I

+: present; -:absent; ±: very mild; E: eczema, Ab: abnormal hair structure * family members with tooth agenesis

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 (patient 5; tables 1 and 2). The mildest hypodontia, with an agenesis of 6 elements, was present in a syndromic patient compound heterozygous for p.C107* and p.F228I (patient 8; tables 1 and 2). The absences of more than 20 teeth were observed in patients that were either homozygous, compound heterozygous or heterozygous for *WNT10A* mutations. The patterns of missing teeth did not differ for the *WNT10A* mutations.

9	10	11
m	f	m
8	45	11
–	–	–
+	+	+
16	14	12
?	+	+
?	–	+
–	–	–
–	+	Ab
–	–	–
–	–	–
–	–	–
–	–	±
–	–	–
+	±	±
–	–	–
–	–	–
±	–	±
–	–	–
–	–	±
–	–	–
–	–	–
+	–	–
–	–	–
–	–	–
–	–	–
–	–	–
–	–	–
+	–	+
c.682	c.682	c.682
T>A	T>A	T>A
c.682	c.682	c.831
T>A	T>A	G>C
p.F228I	p.F228I	p.F228I
p.F228I	p.F228I	p.W277C

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* or heterozygous or homozygous for p.F228I. A patient compound heterozygous for p.C107* and p.F228I showed significant features suggestive for an ectodermal dysplasia (patient 8; sables 1 and 2). A patient with the same genotype did not show additional ectodermal features (patient 10; table 1). A patient carrying the p.C107* mutation had an orofacial cleft (patient 2, 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 (tables 5 and 6). Tooth agenesis was most frequently observed in family members of non-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 apparently isolated hypodontia (56%) a mutation in *WNT10A* could be identified. In five probands a mutations was identified in the candidate genes *MSX1* (one proband), *PAX9* (three probands) respectively *AXIN2* (one proband). No mutations were found in the *IRF6* gene.

A diagnosis of isolated hypodontia is not easily made. Individuals with ectodermal dysplasia show variations in phenotypic expression that may range from prominent to very subtle ectodermal symptoms [3,4,16-18]. The latter can be difficult to classify and might be features of ED or normal variations. Also 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 [19-22].

However, these are not routinely performed or available in daily clinical practice and were not applied in this study.

After careful examination of our patients 67% was finally classified as non-syndromic. This percentage corresponds with previous studies [4,16]. Bergendal et al. (2006) showed that 14.7 % of the oligodontia patients had impaired function of hair, nails and/or sweat glands [3], which is considerably lower than in studies performed in tertiary centers [4,16].

The p.F228I mutation 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 Hardy and Weinberg rules and considering an allele frequency of the c.682T>A *WNT10A* of 1/45 nearly 1 out of 2000 individuals might have a severe oligodontia due to homozygosity for a c.682T>A *WNT10A* mutation. This is approximately half of the prevalence (0,14%) of severe hypodontia reported in the European population.

A mutation screen of *MSX1*, *AXIN2*, *PAX9* and the ectodermal dysplasia genes *EDA*, *EDAR* and *EDARADD* in a population with severe hypodontia identified mutations in 11% [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 required for the 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

Heterozygosity, compound heterozygosity and homozygosity for *WNT10A* mutations can be responsible for severe tooth agenesis. Homozygosity for a nonsense 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 not be confirmed in our study.

Because heterozygosity and compound heterozygosity or homozygosity for *WNT10A* mutations are associated with tooth agenesis pseudo dominant 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 to previously reported patients [9,11,12]. This may reflect a selection bias but may also indicate that other factors, e.g. 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 the *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 6 teeth, excluding the third molar and in probands with a lower number of agenesis but with a positive family history, we recommend to test for mutations in *WNT10A* and if negative, to continue with testing of *MSX1*, *PAX9*, and *AXIN2*. In case of *AXIN2* mutation analysis one should specifically ask for 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. This approach will improve counseling of patients with hypodontia and their family members.

Acknowledgements

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

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

Table 3: Tooth findings in 19 non-syndromic hypodontia Patients with WNT10A Mutations

							upper right		
							94,70%	42,10%	0,00%
Patient	Genotype	Age	Gender	TAOverall	Number of missing teeth	TAC 1st quadrant	18	17	16
1	p.[C107*]+[=]	22	f	210.210.209.209	16	210	x	x	
2	p.[C107*]+[=]	39	m	158.158.144.152	15	158	x		
3	p.[R128Q]+[=]	19	f	158.158.159.153	20	158	x		
4	p.[R163W]+[=]	11	f	142.142.144.144	12	142	x		
5	p.[F228I]+[=]	28	f	16.028.152.152	10	16			
6	p.[F228I]+[=]	32	f	208.152.216.216	14	208	x	x	
7	p.[N306K]+[=]	18	m	216.216.144.112	13	216	x	x	
8	p.[G95K]+[F228I]	14	m	222.252.255.255	28	222	x	x	
9	p.[C107*]+[F228I]	10	m	192.192.219.209	14	192	x	x	
10	p.[C107*]+[F228I]	14	m	222.222.223.223	26	222	x	x	
11	p.[C107*]+[F228I]	16	f	138.138.197.197	14	138	x		
12	p.[V145M]+[V145M]	18	m	222.222.223.223	26	222	x	x	
13	p.[F228I]+[F228I]	13	f	146.146.144.144	10	146	x		
14	p.[F228I]+[F228I]	12	m	150.158.135.135	17	150	x		
15	p.[F228I]+[F228I]	15	f	158.156.144.144	13	158	x		
16	p.[F228I]+[F228I]	15	f	200.204.192.128	10	200	x	x	
17	p.[F228I]+[F228I]	18	m	130.146.152.152	11	130	x		
18	p.[F228I]+[F228I]	19	m	152.152.153.217	15	152	x		
19	p.[F228I]+[F228I]	29	f	152.216.208.144	12	152	x		
19	p.[F228I]+[F228I]	29	f		12	144	x		
18	p.[F228I]+[F228I]	19	m		15	217	x	x	
17	p.[F228I]+[F228I]	18	m		11	152	x		
16	p.[F228I]+[F228I]	15	f		10	128	x		
15	p.[F228I]+[F228I]	15	f		13	144	x		
14	p.[F228I]+[F228I]	12	m		17	135	x		
13	p.[F228I]+[F228I]	13	f		10	144	x		
12	p.[V145M]+[V145M]	18	m		26	223	x	x	
11	p.[C107*]+[F228I]	16	f		14	197	x	x	
10	p.[C107*]+[F228I]	14	m		26	223	x	x	
9	p.[C107*]+[F228I]	10	m		14	209	x	x	
8	p.[G95K]+[F228I]	14	m		26	255	x	x	x
7	p.[N306K]+[=]	18	m		13	112		x	x
6	p.[F228I]+[=]	32	f		14	216	x	x	
5	p.[F228I]+[=]	28	f		10	152	x		
4	p.[R163W]+[=]	11	f		12	144	x		
3	p.[R128Q]+[=]	19	f		20	153	x		
2	p.[C107*]+[=]	39	m		15	152	x		
1	p.[C107*]+[=]	22	f		16	209	x	x	
					Number of missing teeth	TAC 4th quadrant	48	47	46
							94,70%	47,40%	10,50%
							lower right		

f, female; m, male, TAC, ; Tooth Agenesis Code; x, absent teeth

						upper left								
73,70%	63,20%	42,10%	63,20%	0,00%	0,00%	52,60%	52,60%	78,90%	78,90%	5,30%	42,10%	94,70%	TAC 2nd
15	14	13	12	11	21	22	23	24	25	26	27	28	quadrant
x			x			x			x		x	x	210
x	x	x	x			x	x	x	x			x	158
x	x	x	x			x	x	x	x			x	158
	x	x	x			x	x	x				x	142
x							x	x	x				28
x								x	x			x	152
x	x							x	x		x	x	216
x	x	x	x				x	x	x	x	x	x	252
											x	x	192
x	x	x	x			x	x	x	x		x	x	222
	x		x			x		x				x	138
x	x	x	x			x	x	x	x		x	x	222
x			x			x			x			x	146
x		x	x			x	x	x	x			x	158
x	x	x	x				x	x	x			x	156
	x						x	x			x	x	204
			x			x			x			x	146
x	x							x	x			x	152
x	x							x	x		x	x	216
x									x		x	x	208
x	x			x	x			x	x			x	153
x	x							x	x			x	152
											x	x	192
x									x			x	144
		x	x	x	x	x	x					x	135
x									x			x	144
x	x	x	x	x	x	x	x	x	x		x	x	223
		x		x	x		x				x	x	197
x	x	x	x	x	x	x	x	x	x		x	x	223
x				x	x	x		x	x		x	x	219
x	x	x	x	x	x	x	x	x	x	x	x	x	255
x									x			x	144
x	x							x	x		x	x	216
x	x							x	x			x	152
x									x			x	144
x	x			x	x	x	x	x	x			x	159
x	x								x			x	144
x				x	x				x		x	x	209
45	44	43	42	41	31	32	33	34	35	36	37	38	TAC 3rd
84,20%	47,40%	26,30%	21,10%	47,40%	47,40%	31,60%	31,60%	47,40%	84,20%	5,30%	47,40%	100,00%	quadrant
					lower right								

Table 4 : Tooth findings in 11 syndromic hypodontia Patients with WNT10A Mutations

							upper right			
							100,00%	45,50%		9,10%
Patient	Genotype	Age	Gender	TAC overall	Number of missing teeth	TAC 1st quadrant	18	17		16
1	p.[C107*]+[=]	9	m	146.158.144.144	12	146	x			
2	p.[C107*]+[=]	22	m	144.155.145.145	13	144	x			
3	p.[F228I]+[=]	34	f	216.216.192.145	11	216	x	x		
4	p.[F228I]+[=]	45	m	154.154.217.217	18	154	x			
5	p.[C107*]+[C107*]	7	f	254.254.255.255	30	254	x	x		x
6	p.[C107*]+[F228I]	8	m	198.194.211.219	18	198	x	x		
7	p.[C107*]+[F228I]	12	f	214.218.211.199	20	214	x	x		
8	p.[C107*]+[F228I]	15	m	130.130.128.128	6	130	x			
9	p.[F228I]+[F228I]	8	m	152.154.217.153	16	152	x			
10	p.[F228I]+[F228I]	45	f	208.208.209.209	14	208	x	x		
11	p.[F228I]+[W277C]	11	m	146.124.065.065	12	146	x			
11	p.[F228I]+[W277C]	11	m		12	65		x		
10	p.[F228I]+[F228I]	45	f		14	209	x	x		
9	p.[F228I]+[F228I]	8	m		16	153	x			
8	p.[C107*]+[F228I]	15	m		6	128	x			
7	p.[C107*]+[F228I]	12	f		20	199	x	x		
6	p.[C107*]+[F228I]	8	m		18	219	x	x		
5	p.[C107*]+[C107*]	7	f		30	255	x	x		x
4	p.[F228I]+[=]	45	m		18	217	x		x	
3	p.[F228I]+[=]	19	f		11	128	x			
2	p.[C107*]+[=]	22	m		13	145	x			
1	p.[C107*]+[=]	9	m		12	144	x			
						TAC 4th quadrant	48	47		46
							90,90%	54,50%		9,10%
							lower right			

f, female; m, male, TAC, ; Tooth Agenesis Code; x, absent teeth

						upper left															
81,80%	36,40%	27,30%	63,60%	0,00%		9,10%	72,70%	27,30%	72,70%	81,80%	18,20%	54,50%	90,90%	TAC 2nd
15	14	13	12	11		21	22	23	24	25	26	27	28	quadrant
x			x				x	x	x	x			x	158
x						x	x		x	x			x	155
x	x								x	x		x	x	216
x	x		x				x		x	x			x	154
x	x	x	x				x	x	x	x	x	x	x	254
		x	x				x					x	x	194
x		x	x				x		x	x		x	x	218
			x				x						x	130
x	x						x		x	x			x	154
x										x		x	x	208
x			x					x	x	x	x	x		124
				x	x							x		65
x				x	x					x		x	x	209
x	x			x	x				x	x		x	x	217
													x	128
		x	x	x	x		x			x		x	x	211
x	x		x	x	x		x			x		x	x	211
x	x	x	x	x	x		x	x	x	x	x	x	x	255
x	x			x		x			x	x		x	x	217
												x	x	192
x				x	x					x			x	145
x										x			x	144
45	44	43	42	41		31	32	33	34	35	36	37	38	TAC 3rd
														quadrant
63,60%	36,40%	18,20%	27,30%	72,70%		72,70%	27,30%	9,10%	27,30%	72,70%	9,10%	72,70%	90,90%	
						lower left								

Table 5. Clinical Manifestations in Family members of patients with non-syndromic hypodontia and WNT10A mutation

Proband	Age; Gender Genotype Number missing teeth	Family members	Genotype Family Member	Affected Structures				hypohidrosis
				teeth	nails	skin	hair	
1	22 yr; f	father	p.[C107*]+[=]	M	–	–	Am	–
	p.[C107*]+[=]	mother	[=]+[=]	Ex	–	–	Gr	–
	16	brother	[=]+[=]	Ex	–	–	–	–
2	39 yr; m	father		–	–	–	–	–
	p.[C107*]+[=]	mother		–	–	–	Gr	–
	15	son		M	–	–	–	–
		sister		M	–	–	–	–
3	19 yr; f	father	p.[R128Q]+[=]	M	–	–	Am	–
	p.[R128Q]+[=]	mother	[=]+[=]	–	–	–	–	–
	20	SP		T				
		SP		T				
4	11 yr; f	father		M	–	–	–	–
	p.[R163W]+[=]	mother		M	–	–	–	–/Ps
	12	SF		M				
		SM		M				
5	28 yr; f	father		–	–	–	–	–
	p.[F228I]+[=]	mother		–	–	–	–	–
	10							
6	32 yr; f	father		–	–	–	–	–
	p.[F228I]+[=]	mother		M	–	–	–	–
	14	son		M	–	–	–	–
7	18 yr; m	father		M3	–	–	–	–
	p.[N306K]+[=]	mother		–	–	–	–	–
	13							
8	14 yr; m	father		Ex	–	–	Am	–
	p.[G95K]+[F228I]	mother		Ex	–	–	–	–
	28				–	–	–	–
9	10 yr; m	father		–	–	–	–	–
	p.[C107*]+[F228I]	mother	p.[F228I]+[F228I]	–	±	–	–	–
	14							
10	14 yr; m	father	p.[F228I]+[=]	M/T?	–	–	Am	–
	p.[C107X]+[F228I]	mother	p.[C107*]+[=]	M3	–	–	Bo	±
	24?	FM		–	–	–	–	–
		MM		M3/M?	–	–	–	–
11	16 yr; f	father		–	–	–	–	–
	p.[C107*]+[F228I]	mother	p.[F228I]+[=]	–	–	D	–	–
	14	sister	p.[C107*]+[F228I]	M	±	D	–	–
12	18 yr, m	father		M			Am	
	p.[V145M]+[V145M]	mother		T			–	
	26	brother		M			–	
13	13 yr; f	father	p.[F228I]+[=]	M3	–	–	–	–
	p.[F228I]+[F228I]	mother	p.[F228I]+[=]	T	–	–	Af	–
	10	brother		M	–	–	–	–
		SM		M				–

Table 5. *(Cont)*

Proband	Age; Gender Genotype Number missing teeth	Family members	Genotype Family Member	Affected Structures				hypohidrosis
14	12 yr; m	father		–	–	–	–	–
	p.[F228I]+[F228I]	mother		–	–	–	–	–
	17	MP		M	–	–	B	+
15	15 yr; f	father	p.[F228I]+[=]	–	–	_	–	–
	p.[F228I]+[F228I]	mother	p.[F228I]+[=]	–	–	–	–	–
	13	sister		T	±	–	–	–
		brother		M				
16	15 yr; f	father		–	–	–	–	–
	p.[F228I]+[F228I]	mother	p.[F228I]+[=]	–	–	–	–	–
	10							
17	18 yr; m	father	p.[F228I]+[=]	–	–	Ec	–	–
	p.[F228I]+[F228I]	mother	p.[F228I]+[=]	–	–	–	S	–
	11	cousin		M	–	–	–	–
18	19 yr; m	father		C	–	–	Am	–
	p.[F228I]+[F228I]	mother		–	–	–	–	–
	15	brother		M	–	–	–	–
		FP		M	–	–	–	–
19	29 yr; f	father	p.[F228I]+[=]	–	–	–	–	–
	p.[F228I]+[F228I]	mother	p.[F228I]+[=]	–	–	–	–	–
	12	sister	p.[F228I]+[F228I]	M	–	–	±	–

Family: SP, sister of father; SM, sister of mother; FP, brother father; PP, paternal grandfather
+, present; −, absent; ±, mild affected
Teeth: C, cleft; Ex, no data on agenesis due to extractions M, agenesis of 2 to 6 permanent teeth except third molars without further information available; M3, absent third molars; T, single tooth absent, excluding M3; ?, one or two teeth might be extracted;
Skin: D, dry skin; Ec eczema;
Hair: Af, female pattern hair loss, Am, male alopecia; Bo, bold until 2 years of age; B, sparse body hair, Gr, early grey hair;
S, sparse scalp hair;
Hypohidrosis: Ps, Palmoplantair sudation
A blank entry indicates no information is available

Table 6. Clinical Manifestation in Family members of patients with syndromic hypodontia and WNT10A mutation

Proband	Age; Gender Genotype Number missing teeth	Family members	Genotype family members	Affected Structures				hypohidrosis
				teeth	nails	skin	hair	
1	9 yr, m	father		–	–	–	–	–
	p.[C107*]+[=]	mother	p.[C107*]+[=]	M	±	–	Gr	–
	12	brother		B				
2	22 yr, m	father		–	–	–	–	–
	p.[C107*]+[=]	mother		M	–	D	–	–
	13	SM		M	–	–	–	+
3	34 yr, f	father	[=]+[=]	–	–	–	–	–
	p.[F228I]+[=]	mother	p.[F228I]+[=]	Dt*	–	–	S	–
	11							
4	45 yr, m	father		–	–	–	–	–
	p.[F228I]+[=]	mother		G	–	–	S	±
	18	sister		A	–	–	–	–
		son	p.[F228I]+[=]	M	–	–	–	–
		niece	p.[F228I]+[F228I]	M	I	–	S	–
		nephew		G				
5	7 yr; f	father	p.[C107*]+[=]	–	–	–	–	–
	p.[C107*]+[C107*]	mother	p.[C107*]+[=]	–	–	–	–	–
	30	PM		T	–	Ec #	–	–
		PP		–	–	–	Am*	–
6	8 yr; m	father		–	–	–	Bo	–
	p.[C107*]+[F228I]	mother		–	–	–	–	–
	18	sister	p.[C107*]+[F228I]	M	–	D	E	–
		sister	p.[C107*]+[F228I]	M	–	D	S, E	–
		FM		M			S	
		4 cousins mother		M				
		4 cousins once removed		M				
7	12 yr; f	father		–	–	–	–	–
	p.[C107*]+[F228I]	mother		M3	–	–	–	–
	20	brother	p.[C107*]+[F228I]	M	–	–	E, L, S (±)	–
8	15 yr; m	father	p.[F228I]+[=]	–	–	–	–	–
	p.[C107*]+[F228I]	mother	p.[C107*]+[=]	A	–	D,Ec	–	±
	6	MM					S	
9	8 yr; m	father	p.[F228I]+[=]	–	–	–	Am*	–
	p.[F228I]+[F228I]	mother	p.[F228I]+[=]	M	–	–	–	–
	16	sister	p.[F228I]+[F228I]	M	–	–	–	–
		MM		M				
10	45 yr; f	father		–	–	–	–	–
	p.[F228I]+[F228I]	mother		–	–	–	S/F	–
	14	sister		–	–	–	S/F	–
11	11 yr; f	father		M3	–	–	–	–
	p.[F228I]+[W277C]	mother		–	–	–	–	–
	12	cousin		A/Sp	–	Ec	Ab	–
		FP		T	–		–	

+, present; - , absent; (±), mild; Teeth: A, markedly small, or abnormal shape or missing upper lateral permanent incisors; Sp, wide spaced teeth; B, agenesis permanent incisors; C, agenesis of upper permanent canines; Dt, dental prostheses; G, permanent teeth are markedly small; M, agenesis of 2 to 6 permanent teeth except third molars without further information available; M3, absent third molars;
T, single tooth absent, excluding M3; Nails: I, indentations nails, very mild; Skin: D, dry skin; Ec, eczema;
Hair: Ab: abnormal structure; Am, male alopecia; Bo, bold until 3 years; E, sparse eyebrows F, fragile hair; Gr, early grey hair; L, sparse eyelashes; S, sparse scalp hair *: At young age #: Hands, transient
A blank entry indicates that there was no information available.

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8 General discussion and future perspectives

General discussion

In general, the use of clinical protocols allows health care providers to offer appropriate diagnostic treatment and clinical care to patients, based on the best available evidence. They provide a locally, nationally or internationally agreed standard to which individual clinicians or centers can work and against which they can be audited. They can also be used as treatment guidelines. Results obtained by following these guidelines should be monitored and meticulously evaluated. Disappointing or unexpected results as well as deviations from the guidelines should be reported and lead to re-evaluation of the guidelines over time.

Reports in the literature regarding treatment planning of and outcome in patients with severe hypodontia are predominantly case reports, describe rather small and heterogeneous populations and are of a retrospective nature.[1, 1-3] Generally accepted guidelines for the treatment of severe hypodontia are missing to date, yet as stated above are paramount to high quality (dental) health care.

The research that is presented in this PhD thesis focuses on patients with (severe) non-syndromal hypodontia. The general aim has been to characterize affected patients on several aspects: dental, dentofacial/skeletal, osseous, genetic and morphological. Treatment outcome in combination with endosseous implants was evaluated. The type of information that was collected should help in obtaining more insight into the backgrounds of severe non-syndromal hypodontia and eventually allow formation of patient categories with more or less similar features. This could form the basis for restorative guidelines in the treatment of severe hypodontia.

During the course of this PhD project new evaluation and classification tools were developed. An overall Tooth Agenesis Code (TACoverall). It was designed to provide a *per person*, numerical and positional description of tooth agenesis. The TACoverall code is consistent with a unique pattern of tooth agenesis from which the number of missing teeth can also be calculated.

A measuring tool to describe teeth geometrically / morphologically in three dimensions. This technique can be utilized to pinpoint specific genotype-phenotype associations. It was developed in collaboration with the Department of Oral and Maxillofacial Surgery (Head: prof. dr. S Bergé), and the department of Oral Biology and Orthodontics (Head: prof. dr. AM Kuijpers-Jagtman) of the Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.

Already existing, validated tools in other areas were applied on populations of patients with (severe) hypodontia:

A measurement technique to study parameters of mandibular trabecular bone structure on panoramic radiographs, initially used for research on osteoporosis, was now applied to a

population of patients with severe non-syndromal hypodontia. The technique was originally developed at the department of Oral and Maxillofacial Radiology (Head: prof. dr. PF van der Stelt), Academic Centre for Dentistry Amsterdam (ACTA), Amsterdam, the Netherlands. Dentofacial characteristics of hypodontia patients were analyzed on lateral cephalometric radiographs using commercially available software. Data were compared with a normative population originating from the Nijmegen growth study. A suggestion for a skeletal classification in patients with severe hypodontia was made on the basis of cluster analysis.

Furthermore, treatment outcome of implant placement in patients with severe hypodontia treated in the department of Oral and Maxillofacial Surgery, Prosthodontics and Special Dental Care of the University Medical Center Utrecht (Head: prof. dr. R Koole) was evaluated retrospectively. The use of implants in cases of severe hypodontia is relatively new, and outcome should be monitored in order to get an impression regarding its effectiveness, merits and drawbacks and how these results relate to application in non-hypodontia patients. The genetic research was performed in close collaboration with drs. MJ van den Boogaard, clinical geneticist (Department of Medical Genetics, University Medical Center Utrecht, Head: prof. dr. WA Knoers). This particular study was initiated to build on the limited knowledge regarding genetic involvement in the etiology of hypodontia. Patients classified as having non-syndromal severe hypodontia from the departments of Oral and Maxillofacial Surgery and Special Dental Care of the University Medical Center Utrecht (Head: prof. dr. R Koole) and the St. Antonius Hospital Nieuwegein were referred for extensive genetic counseling. Prior to the initiation of our study, a limited number of genes were known to cause non-syndromal hypodontia, but penetration was low.
In this discussion, the relevance of these new and existing methods and the obtained results are discussed. Throughout this discussion, suggestions will be made for future research.

The original aim of the study was to characterize patients with (severe) hypodontia on several aspects; dental, dentofacial, skeletal and genetic in order to clinically evaluate former, current and future restorative treatment strategies in severe hypodontia. All different aspects were addressed. Not all fields could be characterized distinctively. Below, these different aspects are discussed separately and suggestions for future research are made.

Dental aspects: TACoverall

In the literature, patients who suffer from (severe) hypodontia are usually characterized in terms of the number of absent teeth and not in patterns and position of absent teeth. As described in chapter 2, we developed a method to uniquely characterize tooth agenesis

throughout the mouth in a single number: the overall Tooth Agenesis Code or TACoverall. This can be practical for several reasons.

First and foremost for epidemiological reasons i.e. in multicenter studies. Identical patterns within populations can be easily identified by means of the TACoverall. Symmetry of agenesis patterns between the left and right side as well as the upper and lower jaw can be calculated. A web-based tool was created for (inter-) national epidemiological data collecting. Members of Cobijt, a national society for dentists involved in centers for special dental care, can register their patients in this database via http://www.cobijt.nl/Hypodontie.aspx (see appendix I). Internationally we encourage other groups to publish their data on oligodontia or (severe) hypodontia patients in a similar manner, so that data exchange is facilitated.

Potential disadvantage of the TACoverall code is that patterns that are very similar / nearly identical cannot be easily identified at first glance. A single additional missing tooth leads to a completely different code and higher TACoverall numbers do not necessarily imply more absent teeth. This is a mathematical challenge that needs to be solved. Manually clustering closely related patterns is a feasible, but a time consuming alternative.

Such clusters of TACoverall codes should reflect the restorative challenge and optimal treatment given a specific cluster. Depending on the position and number of missing teeth both aesthetic and/or functional problems may arise and it seems logical that different clusters of TACoverall's reflect this. These may be implemented in general guidelines for restorative treatment protocols. The former can also be a solution when a general guideline for the reimbursement of treatment costs in severe hypodontia is to be defined by health care legislators (see below as well). We found that the researched population in chapter 2 was so diverse, that making similar groups was not as easy as we initially assumed or rather hoped.

Another reason why the TACoverall may be practical has to do with the fact that tooth agenesis patterns may pinpoint to specific genetic disorders. Severe hypodontia presents itself in numerous clinical variations (patterns) as a result of different missing tooth numbers and locations. Certain tooth patterns are proven to be an indicator for specific gene mutations. For example, patients with an *MSX1* gene mutation, have a high agenesis prevalence of the second premolars in the upper and lower jaw. In patients that have a *PAX9* gene mutation, a high agenesis prevalence of the second and third molar is seen, in both the upper and lower jaw.[4] Consequently, certain agenesis patterns, or TACoverall scores, can be linked to specific gene mutations. Specific agenesis patterns or dental phenotype could be an early indication tool that points to a specific genetic cause (genotype). Further research is needed to explore this assumption.

Osseous aspects: The use of dental implants

Over the years, we see an obvious shift from conventional prosthetic solutions without implants towards implant treatment . Because we gained more experience and success in bone augmentation procedures and implant survival has risen over the last decades, the indication area of dental implants expands. This phenomenon is also seen in cases with (severe) hypodontia. Implant treatment reduces the need to sacrifice sound dental tissues for indirect restorations. Also a recent published article, concerning the quality of life, supports the restorative choice for the use of implants in favor of conventional restorative options. Quality of life as measured by the Oral Health Impact Profile (OHIP) score was lower in rehabilitated patients with tooth agenesis, compared to a control group of patients without tooth agenesis. They reported a better aesthetic outcome in patients rehabilitated with implant supported prostheses as compared to patients treated with tooth supported fixed dental prosthesis.[5]

However, is it justified to use implant treatment instead of conventional prosthetic treatment today, given the lack of long-term data of their use in patients with severe hypodontia? As explained in chapter 5 the success rate of implant survival in cases of severe hypodontia varies. Hypodontia from or syndromic or non-syndromic origin seems to affect implant survival. This could be caused by the fact that generally, syndromic patients have more agenetic teeth, i.e. the bone volume is more compromised than the average non-syndromic hypodontia patient as shown in chapter 7. Lack of bone in these severe cases is often (more) severe, causing a less favorable anatomic starting point. Consequently, pre-implant bone augmentation is indicated in most cases when implant treatment is chosen. Implant survival in augmented cases is less favorable than in non-augmented cases. Also, implants placed in children, who were still in their pre-puberty or pre-adolescent phase have a worse prognosis than implants placed in older patients. It is felt that failures are 'underscored' in literature; especially in younger patients with ectodermal dysplasia.[6-9] The genetic profile may play a role. There is an increasing understanding of the molecular mechanisms during cell and tissue interactions. Some homeobox genes, among which is the *MSX1* gene, seem to play a role in the process of both tooth and craniofacial morphogenesis. The unexplained clustered implant loss we see in our researched population could support this theory.[10, 11]

A difference in bone structure could influence the process of osseointegration, orthodontic procedures as well as other maxillofacial surgical procedures. In chapter 4, we found some evidence that the trabecular bone structure is different in the (severe) hypodontia group, compared to a healthy control population. Jawbone density appears to be increased in patients with severe hypodontia in X-linked hypohydrotic ectodermal dysplasia (XLHED) patients compared to controls, when measured using computed tomography, densitometric

profiles and 3D reconstructions. The alterations in bone structure found were also observed in locations where the presence or absence of teeth could not have interfered. In other words, the changes in bone structure seem to be tooth-independent and suggest a direct effect of the gene mutation on bone formation and/or remodeling.[12]
In addition, bone from young female individuals (age ranged from 14 to 20 years) with ectodermal dysplasia was found to be denser and more compact than in male ectodermal dysplasia patients, using microcomputed tomography (micro-CT) analysis on bone biopsy samples. The authors hypothesize that external force transfer of mastication may be different between male and female ectodermal dysplasia patients, which may influence outcome.[13, 14] We did not differentiate between gender in our study on implant survival in non-syndromic hypodontia patients as presented in chapter 5.

At the University Medical Center Utrecht, many hypodontia patients have been treated with implants over the years and a total of 294 could be included in our retrospective study. The cumulative proportion of implant survival is estimated at 90% after 5 years and beyond. Jung et al dealing with the overall implant survival rate of single-implant crowns after 5 years in augmented and non-augmented cases may serve as a reference in a 'normal' population, although one cannot be sure that this study did not also include hypodontia patients. They estimated implant survival to be 96.8% after 5 years. Considering the compromised anatomic situation and the complexity of treatment, a 5-survival rate of almost 90% as seen in our study, can be considered acceptable. Choosing implants as a preferred treatment option, especially if neighboring teeth are sound, is in our view defendable. It is essential though, that these figures and the complexity of the anatomic situation are discussed at an early stage with the patient, before deciding on the final plan of treatment. It is evident that there is room for improvement on implant survival.
We did not investigate the number of suprastructures that were lost or could not be made because of implant failure, which may also be considered a clinically relevant outcome parameter.

Dentofacial/skeletal aspects

Orthodontic challenges

In chapter 3 we found that the population of hypodontia patients was best divided into 4 subgroups, based on the cephalometric outcome. By not making a classification in advance, we found that the groups formed by our statistical program resemble that of the Angle classification, commonly used in orthodontics. Implant treatment in most of the *severe* hypodontia patients can only be started in post-adolescence, when major maxillofacial facial

growth has ceased. Hence, if one commences with the orthodontic treatment at the age of 13 years old, the retention period will be several years. Retention of teeth for such a long period is virtually impossible, regardless of the type of retention device used. Because of the absence of neighboring teeth that can be used for retention, stabilizing the situation after the orthodontic treatment relies depends on removable appliances. Young people find it hard to wear these devices for such a long period of time. Also, even if they do wear them, the apices tend to migrate anyway because of continued growth or lack of fit of the removable appliance. Frequently, prior to definitive restorative treatment in post-adolescence and after years orthodontic treatment, new orthodontic treatment is indicated. This can be very disappointing for the patient with the prospect that he still has a long treatment ahead of him. This is why orthodontic treatment just prior to restorative treatment is generally preferred. This reduces the orthodontic retention period to a minimum. An exception can be made when there is a large skeletal deviation, and orthodontic simulation of growth is indicated. Orthodontic treatment is best split into 2 phases: orthodontic stimulation of growth at a younger age and the second phase, the positioning of the teeth during post-adolescence, just prior to restorative treatment.

It is generally also a good idea to perform a minor orthodontic intervention at younger age to align the frontal teeth for esthetical reasons (see figure 3 in the introduction).

Furthermore, the use of orthodontic bone anchorage in the form of mini-implants can be a useful adjunct. In the severe hypodontia cases sufficient anchorage is often difficult to find because of missing molars.

Genetic and morphological aspects

The WNT10A gene

The results from the study in chapter 7, regarding genetic make up of patients that presented severe, non-syndromic hypodontia are very exciting. Two dentists referred a cohort of severe hypodontia patients which they considered to be 'non-syndromic' for genetic evaluation. After genetic screening and counseling, some of these patients were categorized as 'syndromic' after all. Sometimes the syndromic features are very mild and easily overlooked during a regular visit to the dentist. Nevertheless, additional training of dentists that work in centers for special dental care may be advisable.

Patients were screened for genetic abnormalities, that were known to be causing non-syndromic hypodontia, presented as isolated. The frequency of mutations is known to be low, so we did not expect to find many patients with genetic mutations. Interestingly, when we included the WNT10A gene, over 50 % (!) of the population had a mutation in this gene. By including *WNT10A* in the DNA diagnostics of isolated tooth agenesis the yield of molecular

testing in this condition was significantly increased, from 15% to 71%. Consequently, it is strongly advised to include this gene in the standard series of genetic tests when screening non-syndromic hypodontia patients genetically. In discussions with experts in the field of genetics it has become quite clear that some have doubts if severe hypodontia is truly isolated, without other physical features being present. That poses the question whether 'non-syndromic' hypodontia just has a mild expression of ectodermal dysplasia features?

'Shape database'

For the study in chapter 6 we developed and tested a technique to evaluate tooth dimensions in a three dimensional manner, enabling us to estimate tooth volume as well. In the past, most methods described used two dimensional techniques. Within the *MSX1* patients that were compared with age and gender matched controls, especially the incisor differed, and not so much the molar teeth. It remains to be seen if this would also be the case for other gene mutations. The technique is useful and reliable but in its present state of development rather time consuming. Further software development should help automate steps and make the program more user friendly.

As already suggested in chapter 6, a 'shape database' could contribute to an early diagnosis of congenital disorders involving tooth abnormalities. Because differences are often small, we consider that the technique we developed as useful in recognizing small shape differences. In the near future, it would be of interest to analyze the teeth of more families with a known genetic disorder besides *MSX1*, like for example *IRF6, PAX9*, *WNT10A* or *AXIN2*. If we analyze large numbers of people with the same gene mutations, we expect to find 'norm measurements' with respect to tooth dimensions or similar TACs that are typical for these different gene mutations. The TAC codes or norm measurements can then be used as biomarkers that would raise suspicion for or point at distinct gene mutations.

Ethical considerations in genetic counseling regarding hypodontia

People with severe hypodontia who are referred to the Department of Special Dental Care of the University Medical Center Utrecht, can in broad terms be divided into two groups. One group of patients present themselves with anamnestic, clinical or radiographic features that can be linked to a syndrome. We advise them to seek genetic counseling and will refer. Counseling can give patients insight into the risk for their children to have the same disorder. Also, other medical complaints, such as problems with sweating, might be explained.

The second group of severe hypodontia patients has no apparent additional anamnestic, clinical or radiographic syndromic features. This PhD thesis predominantly deals with this group of patients.

Some ethical dilemmas are illustrated on the basis of two findings, one from our study, one from the literature. In chapter 7 we found 1 patient of the 34 referrals who had an *AXIN2*

gene mutation. This mutation can cause severe hypodontia, but can also be linked to a higher risk of colon cancer. Of 14 family members that Lammi investigated, 11 were carriers of an *AXIN2* gene mutation. Eight out of these 11 were known with colon cancer.[15] Others later substantiated their findings[16], but so far little research has been performed on the correlation between severe hypodontia and colon cancer.

The ethical question would be whether or not a physician should inform the isolated severe hypodontia patient about the possible risk of developing (or having) colon cancer and whether or not referral for genetic counseling should be the 'standard of care'? This does not seem practical, nor very efficient given the low frequency of *AXIN2* mutations in the general population. But consequences for a non-identified but *AXIN2* affected patient may be severe. It should be seen in light of the question whether or not there is a 'fair chance' on colon cancer in patients with severe hypodontia. 'Fair chance' is a term used in the WGBO (the law on the medical treatment agreement). This law obligates physicians to inform their patients about their findings and possible risks. Although quickly improving, costs of genetic counseling and testing are still substantial and testing can be time consuming. These costs are better justifiable, if we know more about the risks those patients of this specific patient group have. To date these risks are unknown. Presently, a pragmatic advice may be to refer any person with severe hypodontia and a family history of colon cancer on a routine basis.

On a similar subject, fifty patients with epithelial ovarian cancer (EOC) were compared with 100 matched controls regarding their dental status. Twenty percent of the patients with ovarian cancer had hypodontia, compared to 3% of patients in the control group, suggesting a statistical association between hypodontia of the permanent dentition and EOC. Women wit EOC are 8.1 times more likely to have hypodontia than women without EOC.[17] From a different perspective: should all female hypodontia patients be screened vigorously for EOC? Questions like these will arise ever more frequently these days because new techniques in the genetic field such as next generation sequencing with microarray technology are applied and make genetic profiling cheaper and more accessible. Also genetic counseling itself is readily available to the patient. We also found an increased interest among our patients in referral to the department of clinical genetics. Patients are interested if their dental disorder is part of a syndrome and are keen to find out what the consequences are for their kin. As clinicians we gladly oblige and a referral is easily organized.

Genetic research will surely shed more light on the etiology of dental abnormalities. So far, genetic profiling is not part of any regular healthcare program in the Netherlands. However, in chapter 7 the *WNT10A* gene was identified as a frequent cause of hereditary tooth agenesis. In former days, referral to a geneticist and DNA profiling would have a poor chance on identifying the genetic cause of the hypodontia. Now this chance has increased by more than 50 percent. In the clinic, this means that far more patients can be offered advice

because the genetic cause can be frequently identified. This is a vast step forward. Patients should be made aware however that a 'cure' for hypodontia is not available to date.

Future research

3d imaging

Nowadays, it is possible to digitally fuse skeletal, soft tissue and dental data into one 3-dimensional model. The extensive and elaborate orthodontic, orthognatic, surgical (bone augmentation and implants) and restorative treatment that most hypodontia patients undergo affect all these three parameters. If we want to measure more precisely what the effect of our treatments on these patients is, image fusion models are ideal for analysis of these issues. By analyzing the effects of treatment one can expect to improve the functional and esthetical outcome of treatment.

Restorative classifications and guidelines

In this thesis, studying different aspects that influence the outcome of treatment helped the clinical decision-making process in patients with severe hypodontia. In our first study described in chapter 2, we found that the severe hypodontia population is a very diverse population. Only three pairs of a total of 116 patients had exactly the same agenesis pattern. Developing of specific clinical guidelines for such a diverse group is challenging and, in hind sight even unrealistic. Nevertheless, it is challenging to try and apply the findings of the different studies to patient care. For this purpose, appendix III is added. The feasibility of distinguishing groups of patients that possess similar traits will also depend for what specific purpose the classification is made. For example: groups made for restorative guidelines will differ form groups made for genetic profiling. The TAC code can already be linked to specific gene mutations, but groups can only be made afterwards. The combination of the content of this thesis and three dimensional profiling might give us more insight what restorative groups we are looking for.

Quality of life

The impact of hypodontia using the Child Perception Questionnaire (CPQ) can be studied.[34, 35] The results for both studies showed the oral health impact on quality of life was immense. It can be questioned whether or not the CPQ is suitable for this specific patient group.
Others also used the CPQ to assess the psychosocial impact of hypodontia and compared this with the results from a routine orthodontic treatment group who completed the CPQ.[36] Their results conflict with other studies, as they did not report a significantly larger psychosocial impact of hypodontia compared with non-hypodontic patients undergoing orthodontic

treatment. However, patients with hypodontia in this study did report greater difficulty in chewing.

Another way of testing our treatment outcome is to describe the quality of life before and after treatment of the severe hypodontia patient group. Akram made a new tool for assessing the quality of life of patients with hypodontia. The measure was designed to acquire a research method to identify issues of importance for patients with hypodontia. A questionnaire based on these issues was created to assess the impact of non-syndromic hypodontia on quality of life, for patients aged between 11 and 18 years old.[37] We are planning to conduct further research on this field.

Genetics

Our research collaboration with the medical genetic department should be continued. We think we are able to find out even more about the genetic causes of hypodontia, based on the outcome of our research so far. Genetically, well-defined pathways play a role in this disorder. The size of the population of this rare disorder makes it a unique collaboration because in international literature most reports are based on single families or small groups. Also, we would like to match TACoverall codes and shapes (as described in chapters 2 and 6), to specific gene mutations. More research on larger populations groups is necessary to find these expected correlations.

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Summary

Summary

The general aim has been to characterize patients severe non-syndromic hypodontia on several aspects: dental, dentofacial, skeletal/bone quality, treatment outcome and genetic. Purpose of these characterizations has been to evaluate former, current and future restorative treatment strategies in severe hypodontia and to support restorative choices.

The purpose of **chapter 2** was to characterize a population of oligodontia patients and identify patterns of tooth agenesis. A total of 116 patients with non-syndromic oligodontia were studied, and the Tooth Agenesis Code (TAC) per quadrant was calculated. Oligodontia was defined as the congenital absence of 6 or more permanent teeth, excluding the third molars. The TAC is a unique number, consistent with a specific pattern of tooth agenesis. The authors suggest the use of an overall Tooth Agenesis Code by means of which the dentition throughout the mouth can be presented in a single number. The pattern of agenesis of each quadrant remains recognizable. Frequency analysis was used to study the prevalence of various patterns.

There was a great diversity of TACs. In the maxilla, agenesis of both premolars and the lateral incisor or mere presence of the central incisor and first molar were the most common patterns. In the mandible, agenesis of the second premolar or both premolars occurred most frequently.

No single pattern of agenesis occurred more than twice when the full mouth is viewed. Hence, the presentation of the dentition in oligodontia is very heterogeneous. Evaluation of treatment strategies in oligodontia patients is a methodological challenge because homogenous, comparable subgroups of patients are not available.

Chapter 3 aimed to identify distinctive dentofacial characteristics of hypodontia patients. For this purpose, 189 young hypodontia patients (cases) were divided into subgroups, based on criteria from literature. Normalized differences between cases and controls were calculated for various parameters of dentofacial form. Subsequently, cluster analysis was applied to disclose subsets of hypodontia patients with distinctive dentofacial features.

The ANB angle, interincisal angle and lower anterior face height were consistently significantly different amongst the subsets. Four clusters of patients with an increasing number of missing teeth and distinctive dentofacial characteristics could be identified. Patients in cluster 1 display a high-angle facial pattern. Patients in clusters 2 and 3 exhibit markable dentoalveolar characteristics (a relatively small and a large interincisal angle, respectively). Patients in cluster 4 exhibited notable sagittal–skeletal discriminative features predominantly because

of a retrognathic maxilla. The smallest nasolabial angle and lower anterior face height were seen in this cluster.

It is concluded that the anterior– posterior relationship between the jaws, the interincisal angle and the lower anterior face height are discriminative parameters of dentofacial form in hypodontia patients. Patients with hypodontia can be clustered in four groups, each with distinctive vertical–skeletal, dentoalveolar and sagittal–skeletal characteristics. This categorization of patients with hypodontia into meaningful groups may be useful for treatment planning, interdisciplinary communication and as a means of identifying groups of patients that qualify for reimbursement of costs. Other dental factors should be appreciated as well during restorative clinical decision making in patients with hypodontia.

In **chapter 4**, radiographical parameters of mandibular trabecular bone structure between 67 subjects having hypodontia and those without were studied on digital panoramic radiographs.

Three Regions of Interest (ROI) were defined: the ascending ramus, apical of the mandibular molar and mesial of the first mandibular molar. The effects of the presence of hypodontia and the ROI on the mandibular trabecular bone structure were tested for statistical significance by means of multivariate analysis.

Radiographical parameters of trabecular bone architecture were found to differ between various regions of the mandible *($p=0.000$)*, but not between the group of hypodontia subjects and their controls *($p=0.23$)*. There was no interaction effect between the ROIs and the two groups *($p=0.79$)*. For people having hypodontia, some directional parameters of trabecular bone have a reverse correlation with the number of missing teeth. The fractal dimension and the number and perimeter of white segments in the binarized image correlate positively with the number of congenitally missing teeth.

A limited number of parameters of radiographic mandibular trabecular bone structure correlate with the number of missing teeth. However, a markable difference in radiographic parameters of mandibular trabecular bone structure between hypodontia and non-hypodontia subjects could not be demonstrated.

The aim of **chapter 5** was to retrospectively study and evaluate the result of implant treatment in patients with severe hypodontia and compare some basic characteristics of patients with severe hypodontia who received conventional dental treatment or no treatment at all with those who were treated in combination with endosseous implants.

All patients who had been referred to an academic center of special dental care between 1990 and 2008 and who had been classified at their first visit as having 'oligodontia' or 'severe hypodontia' were selected from the hospital's database. Their charts were reviewed,

and surgical treatment details and outcomes of the implants were registered from those patients who received endosseous implants.
Of the 294 patients who met the inclusion criteria, 44 patients were treated in combination with endosseous implants. The cumulative chance of implant survival of the 214 placed implants after 5 years was 89.8% (SE, 2.6%), with a mean observation period of 2.9 years (minimum, 0.1 years; maximum, 18.3 years). No implants failed thereafter. Patients who received implants were missing fewer teeth and were treated more recently compared with those who received conventional restorative treatment or no treatment at all.
Considering the compromised anatomic situation and the complexity of treatment, a 5-year survival rate of 89.8% in patients with severe hypodontia, as seen in this study, is regarded as acceptable.

In **chapter 6**, a novel 3D technique to measure differences in tooth crown morphology between *MSX1* carriers and non-affected controls was designed, to get a better understanding of dental phenotype-genotype associations. Eight Dutch subjects from a single family with tooth agenesis, all with an established nonsense mutation c.332 C>A, p.Ser111Stop in exon 1 of *MSX1*, were compared with unaffected controls regarding several aspects of tooth crown morphology. A novel method of quantitative three-dimensional analysis was used to detect differences.
Statistically significant shape differences were observed for the maxillary incisor in the *MSX1* family compared with the controls on the following parameters: surface area, buccolingual dimension, squareness, and crown volume ($P \leq 0.002$). Molar crown shape was unaffected. A better understanding of dental phenotype-genotype associations may contribute to earlier diagnosis of some multiple-anomaly congenital syndromes involving dental anomalies. A 'shape database' that includes associated gene mutations resulting from developmental syndromes may facilitate the genetic identification of hypodontia cases.

Chapter 7 describes that mutations in *WNT10A* are present in more than half of isolated hypodontia cases. Mutations in *MSX1*, *PAX9*, *AXIN2*, *IRF6* and the ectodermal dysplasia genes *EDA*, *EDAR* and *EDARADD* have been detected in familial severe tooth agenesis. However, until recently, in the majority of cases (~90%) the genetic factor could not be identified, implying that other genes must be involved. Recent insights into the role of *WNT10A* in tooth development and the finding of hypodontia in carriers of the autosomal recessive disorder odontooncychodermal dysplasia due to mutations in *WNT10A* (OMIM 257980; OODD) make *WNT10A* an interesting candidate gene for dental agenesis.
In a panel of 34 patients with isolated hypodontia the candidate gene *WNT10A* as well as the genes *MSX1*, *PAX9*, *IRF6*, and *AXIN2* have been sequenced. The probands all had isolated agenesis of between six and 28 teeth (excluding the third molars).

WNT10A mutations were identified in 56% of the cases with non-syndromic hypodontia. *MSX1*, *PAX9* and *AXIN2* mutations were present in respectively 3%, 9% and 3% of the cases. We identified *WNT10A* as a major gene in the etiology of isolated hypodontia. By including *WNT10A* in the DNA diagnostics of isolated tooth agenesis the yield of molecular testing in this condition was significantly increased from 15% to 71%.

In **Chapter 8** the findings are discussed and put in perspective with respect to some clinical implications. Recommendations for future research were formulated.

Nederlandse samenvatting
(Dutch summary)

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(Dutch summary)

Het gemeenschappelijke doel van de studies die in dit proefschrift worden beschreven (**hoofdstuk 1**), was om patiënten met niet-syndromale ernstige hypodontie op verschillende aspecten van deze aandoening in beeld te brengen. Het betreft dentale, dentofaciale, skeletale, botkwalitatieve en genetische aspecten. Ook resultaten van in het verleden uitgevoerde behandelingen worden geëvalueerd. Dit alles met de bedoeling om huidige en toekomstige behandelstrategieën voor de behandeling van patiënten met ernstige hypodontie beter te kunnen evalueren en onderbouwen.

In **hoofdstuk 2** wordt een groep patiënten met oligodontie gekarakteriseerd en worden patronen van agenesie van gebitselementen geïdentificeerd. Er worden116 patiënten met niet-syndromale oligodontie bestudeerd en van hen werd de Tand Agenesie Code (TAC) per kwadrant berekend. Oligodontie wordt hier gedefinieerd als het niet aangelegd zijn van 6 of meer blijvende gebitselementen, de verstandskiezen niet meegerekend. De TAC is een kenmerkende cijfercode, behorend bij een bepaald agenesiepatroon. Het gebruik van een TAC voor de hele dentitie wordt aanbevolen, waarbijhet agenesiepatroon in alle vier de kwadranten in een cijfermatige code wordt gekarakteriseerd, terwijl de agenesie per kwadrant herkenbaar blijft in de code. Er werd een frequentieanalyse uitgevoerd om patronen van agenesie te onderkennen. Er bleek een grote diversiteit in de TAC's te bestaan. In de bovenkaak bleken de volgende agenesiepatronen het meest frequent voor te komen: agenesie van de laterale incisieven en eerste of tweede premolaren en het alleen aangelegd zijn van de centrale incisieven en de eerste molaren. In de onderkaak werd agenesie van de tweede premolaren en agenesie van beide premolaren het meest gevonden. Geen enkele TAC voor de hele dentitie werd meer dan twee keer aangetroffen. De verschijningsvorm van oligodontie blijkt dus zeer variabel. De evaluatie van uitgevoerde behandelstrategieën bij patiënten met oligodontie is dus een methodologische uitdaging, want vergelijkbare patiëntengroepen van voldoende omvang voor evaluatie zijn moeilijk samen te stellen.

De dentofaciale karakterisering van patiënten met hypodontie is beschreven in **hoofdstuk 3**. Voor dit doel werden 189 jeugdige patiënten met hypodontie onderverdeeld in groepen, volgens criteria zoals in de literatuur beschreven. Genormaliseerde verschillen tussen patiëntengroepen met hypodontie en controlegroepen zonder hypodontie werden berekend met parameters voor de dentofaciale verschijningsvorm. Vervolgens werd een clusteranalyse verricht om subgroepen te onderscheiden van patiënten met hypodontie met bepaalde dentofaciale kenmerken.

De hoek ANB, de interincisale hoek en de hoogte van het ondergezicht (van voren bezien) waren steeds significant verschillend tussen de subgroepen. Vier clusters van patiënten, met een oplopend aantal niet aangelegde gebitselementen en de daarbij behorende onderscheidende dentofaciale kenmerken konden worden onderscheiden:

Cluster 1: divergente aangezicht opbouw
Cluster 2: kleine interincisale hoek
Cluster 3: grote interincisale hoek
Cluster 4: retrognathie van de bovenkaak, met kleine nasolabiale hoek en klein onderste gezichtshoogte(van voren bezien).

De conclusie is dat de voor-achterwaartse kaakrelatie, de interincisale hoek en de hoogte van het ondergezicht (van voren bezien) onderscheidende dentofaciale parameters zijn bij patiënten met hypodontie. De categorisering van patiënten met hypodontie kan nuttig zijn voor behandelplanning, interdisciplinaire communicatie en om groepen te onderscheiden die in aanmerking komen voor vergoeding van de behandelkosten door de zorgverzekeraar.

In **hoofdstuk 4** wordt een röntgenologische studie beschreven naar de botstructuur van 67 personen met hypodontie in vergelijking met die van een controlegroep van gelijke omvang zonder hypodontie.

Drie specifieke gebieden van onderzoek (zogenaamde 'Regions Of Interest', ROI's) werden gedefinieerd:

een gebied in de opstijgende tak van de onderkaak;
een gebied apicaal van de eerste molaar in de onderkaak;
een gebied mesiaal van de eerste molaar in de onderkaak.

De verschillen in botstructuur tussen personen met hypodontie en de controlepersonen werden getest met een multivariate statistische analyse. De radiologische parameters voor de botstructuur bleken te verschillen tussen de onderscheiden ROI's in de onderkaak ($p=0.000$), maar niet tussen de groep personen met en zonder hypodontie ($p=0.23$). Er werd geen interactie-effect gevonden tussen de ROI's en de twee groepen personen ($p=0.79$). Bij personen met hypodontie bleken enkele indicatieve parameters in het trabeculaire bot een omgekeerde relatie te hebben met het aantal niet aangelegde gebitselementen. De 'fractile dimentions' en het aantal en de omtrek van de witte segmenten in het gebinariseerde beeld correleerden positief met het aantal ontbrekende gebitselementen.

Een beperkt aantal parameters in de botstructuur blijkt te correleren met het aantal ontbrekende gebitselementen. Een duidelijk verschil tussen de radiologische parameters voor de botstructuur tussen personen met en zonder hypodontie kon echter niet worden aangetoond.

In **hoofdstuk 5** worden in een retrospectieve studie behandelingen met implantaten bij patiënten met ernstige hypodontie geëvalueerd en vergeleken met enkele karakteristieken van behandelingen bij patiënten met ernstige hypodontie die conventionele tandheelkundige behandeling ontvingen of in het geheel niet werden behandeld.
De patiënten die in de periode 1990-2008 naar de afdeling Bijzondere Tandheelkunde van het UMCU werden verwezen en bij de intake waren benoemd als hebbende "oligodontie" of "ernstige hypodontie" werden geselecteerd uit de database van het UMCU. Hun behandelverslag en de resultaten bij patiënten die met implantaten waren behandeld werden bestudeerd. Het bleek dat van de 294 personen die aan de inclusiecriteria voldeden, er 44 waren behandeld met implantaten. De cumulatieve kans op implantaatoverleving na 5 jaar, van de in totaal 214 geplaatste implantaten was 89.9% (SE 2.6%), bij een gemiddelde observatieperiode van 2.9 jaar (min. 0.1 jaar; max. 18.3 jaar),. Patiënten die behandeld werden met implantaten bleken minder gebitselementen te missen en werden recenter behandeld dan degenen die conventioneel of in het geheel niet werden behandeld.
De complexe anatomische verhoudingen en complexiteit van de behandeling in aanmerking genomen wordt geconcludeerd dat een 5-jaars overleving van 89.9% in patiënten, zoals in deze studie gevonden, als acceptabel te beschouwen is.

Om meer inzicht te krijgen in dentale fenotype/genotype associaties bij personen met *MSX1*-mutaties, wordt in **hoofdstuk 6** een nieuwe 3-D tandmorfologische meettechniek beschreven. Acht personen, behorend tot één familie waarin agenesie bij alle familieleden voorkomt, allen bekend met een non sense mutatie c.332>A, p.Ser111Stop in exon 1 van *MSX1*, werden vergeleken met 42 niet aangedane controle personen voor wat betreft meerdere aspecten van de tandmorfologie.
Statistisch significante vormverschillen werden gemeten voor de centrale incisief in de bovenkaak voor de *MSX1* familie in vergelijking met de controlepersonen, voor de volgende parameters: oppervlakte grootte, buccolinguale afmeting, haaksheid en kroonvolume ($p \leq 0.002$). De vorm van de molaren bleek niet aangedaan.
Een beter inzicht in dentale fenotype/genotype associaties zou kunnen bijdragen aan een vroegere onderkenning van enkele congenitale syndromen met multipele anomalieën, waaronder ook dentale. Een vormendatabase met daaraan gekoppeld geassocieerde genetische mutaties zou de genetische identificatie van personen met hypodontie beter mogelijk kunnen maken.

In **hoofdstuk 7** wordt onderzoek gedaan naar het voorkomen van verschillende genmutatie sin een populatie niet-syndromale ernstige hypodontie patiënten. Mutaties in *MSX1, PAX9, AXIN2* en de ectodermale dysplasie genen *EDA, EDAR* en *EDARADD* werden aangetoond in familiair voorkomende ernstige hypodontie van gebitselementen in de literatuur. Echter, tot

op heden kon in de meerderheid van de gevallen (± 90%) de bepalende genetische factor niet worden geïdentificeerd. Dus moesten er wel nog andere genen betrokken zijn. Recente inzichten in de rol van *WNT10A* bij de tandontwikkeling en het aantreffen van hypodontie in dragers van autosomaal recessieve odonto-onychodermale dysplasie (OODD) als gevolg van mutaties in *WNT10A* (OMIM 257980; OODD), maakte *WNT10A* een interessant kandidaat gen voor agenesie van gebitselementen.

In een groep van 34 patiënten met niet-syndromale hypodontie werden zowel mutaties in het het kandidaat gen *WNT10A* als in de genen *MSX1*, *PAX9* en *AXIN2* aangetoond. De probanden hadden allemaal niet-syndromale hypodontie, variërend tussen de 6 en 28 ontbrekende gebitselementen, de verstandskiezen niet meegerekend.

WNT10A mutaties werden aangetoond in 56% van de personen met niet-syndromale hypodontie. *MSX1*, *PAX9* en *AXIN2* mutaties bleken aanwezig in respectievelijk 3%, 9% en 3% van de gevallen.

In ons onderzoek werd *WNT10A* dus als het meest bepalende gen in de etiologie van niet-syndromale hypodontie aangetoond. Door *WNT10A* in de DNA-diagnostiek van niet-syndromale ernstige hypodontie te betrekken, wordt het rendement van de moleculaire testen bij deze aandoening verhoogd van 15% naar 71%.

In **hoofdstuk 8** worden de resultaten uit de onderzoeken, die beschreven staan in hoofdstukken 2 t/m 7 bediscussieerd in het perspectief van enkele klinische implicaties en worden aanbevelingen voor verder onderzoek gedaan.

Dankwoord

Dankwoord

Gezien mijn angst voor de tandarts, had ik nooit gedacht dat ik voor het beroep als tandarts zou kiezen. Na mijn eerste jaar tandheelkunde was het me duidelijk: het is een briljante keus geweest, die ik zonder hulp van velen niet had kunnen maken.

Mijn vader, **Hans Créton**: door zijn kritische vragen (tot vervelens toe) en zijn interesse in mijn leven, heeft hij mijn gedachten en keuzes altijd scherp weten te krijgen. Dankzij zijn tomeloze energie, geduld en blind vertrouwen, heb ik meer kunnen bereiken dan ik ooit had gedacht.
Mijn moeder, **Annemarie Créton-van Roon**, bij wie ik altijd thuis kom, om vervolgens in de watten gelegd te worden met een overvloed aan liefde, hapjes en gezellige kopjes koffie. **Michiel Créton**, omdat hij de beste, sterkste, stoerste grootste broer is die je als zusje kunt wensen. **Robbert-Jan Créton**, die me altijd aan het giechelen kan krijgen met zijn unieke humor en die ik enorm mis omdat hij zo ver weg woont. Ik ben zeer vereerd dat ze achter me willen staan tijdens de promotie en kan me geen veiliger gezelschap bedenken. **Rolien Créton**, (ja, ik ben het trotse zusje van!) die me heeft leren stappen in Utrecht en warm heeft gemaakt voor het studentenleven.

Na mijn afstuderen ben ik terecht gekomen in het UMCU. Dit bleek een zeer vruchtbare omgeving om mij verder te kunnen ontwikkelen en ik wil graag de volgende mensen hiervoor bedanken:

Allereerst **Prof.dr. MS Cune**, Marco, die me enthousiast heeft kunnen maken voor 'de wetenschap' en heeft me meegesleurd, geduwd. gemangeld en getrokken om me door dit proefschrifttraject heen te krijgen. Ik ben hem daar zeer dankbaar voor en ben trots dat hij bovendien een geweldige vriend is geworden.
Prof.dr. C de Putter, Kees, heeft als vader van onze afdeling me een nieuw thuis gegeven in het UMCU.
Prof.dr. R Koole, Ron, en **Harmien Ruules**: dankzij de vrijheid en het vertrouwen die jullie mij van begin af aan hebben gegeven, heb ik kunnen bloeien. Ik heb veel respect voor jullie en jullie leiderschap.

De medewerkers van de afdeling Mondziekten, Kaak- en Aangezichtchirurgie en Bijzondere Tandheelkunde wil ik allen bedanken voor de bijzonder goede sfeer en een prettige werkomgeving:

Ralph van Brakel waar ik altijd bij terecht kan als ik een afdruk niet uit een mond kan krijgen en zijn familie omdat zij hem 2x per jaar afstaan voor ons jaarlijkse 'wetenschaps weekje' en werkoverleg. Ik waardeer onze vriendschap enorm.
Verder **Dirk Bittermann** (voor zijn interesse en hartelijkheid), **Wouter Bouman** (voor het redden als ik in paniek raak in de keuken), **Willem van den Braber** (omdat je je haren niet verliest), **Erik Dik**, **Robbert van Es**, **Erica Etty**, **Willem Fennis**, **Frits van Kampen**, **Marvick Muradin** (die op de meest ingewikkelde verzoeken durft in te gaan), **Christi Oortgijsen**, **Rick van der Pas** (ook omdat je me op de hoogte houd van alle rugby perikelen), **Stephanie Philippen**, **Jac Quirijnen**, **Ad de Ruiter**, **Toine Rozenberg**, **Jacqueline Schrijen**, **Willem Verhoeven** (voor de 'buur-lockerpraatjes') en **Kees Zachariasse** .

De mondhygiënistes, die ervoor zorgen dat onze patiënten aan alle kanten worden opgevangen: **Nicolette de Boer, Gerrita van Dijk, Sanne Hermsen, Emily Mannaerts, Veronique Roosendaal, Simone Sluys** en **Petra Statema**
Het dreamteam aan assistentes: **Mariette , Nienke, Claudia, Saskia** (ook voor het mij in conditie houden)**, Laura, Jose , Barbara en Wanda**. Ook wil ik **Dalila** (voor al haar vrolijkheid), **Astrid** (voor haar tomeloze inzet) en **Riet** (waar ik nog steeds vlaggerig van wordt) bedanken voor de afgelopen jaren.
De assistentes kaakchirurgie: **Carla**, **Evelien**, **Lydia**, **Marjan**, **Monique** en **Wafaa,** ook omdat ze me meehelpen aftellen en glucoseniveau op pijl houden.
De dames en heer van de administratie: **Judith, Jeanette, Janny, Els, Betina, Rudy, Wanda, Yvonne** en **Thilly**.
De verpleegafdeling D5 en **Monique Kippersluis** en **Iris Stokhof**.

De medewerkers van het tandtechnisch laboratorium in het UMCU en tandtechnisch laboratorium Oosterwijk, voor alle geweldige tandtechnische werkstukken en de zeer prettige samenwerking. Jullie zijn de echte kunstenaars. **Mathieu Steijvers** en **Patrick Oosterwijk** wil ik in het bijzonder bedanken voor al het geduld dat ze de afgelopen jaren met mij gehad hebben en voor de vriendschap.

De afdeling medische fotografie die altijd voor ons klaar staat en **Petra Boddé** voor het realiseren van de 3-D fotografie.

Marie-José van den Boogaard, die mij enthousiast gemaakt heeft voor de genetica.

Thomas Maal en **Luc Verhamme**: de 'wizz kids' uit Nijmegen. Veel dank voor de zeer plezierige samenwerking.
De **patiënten** die ik in het UMCU heb behandeld, voor al het vertrouwen en enthousiasme.

Mijn fantastische collega's van Tandartsen en Verwijspraktijk Wilhelminapark: **Maaike, Merle, Evelijn, Esther** en **Ingrid**. Doordat de praktijk zo goed loopt ben ik ook wel eens op tijd thuis.

Dr. Giner Tarrida, Lluís, die me de kans heeft gegeven een jaar op de faculteit tandheelkunde van de Universitat International de Cataluña te werken. Gracias por su confianza y por pronunciar mi nombre perfectamente.

Bestuur NVGPT: **Willem Fennis, Paul Goedegebuure, Liesbeth Starmans, Kees Zachariasse, James Huddleston Slater en Paul Schmitz**. Dankzij jullie ben ik vergaderen zelfs leuk gaan vinden.

Arien, Barbara, Jeroen, Maaike, Merle, Opla, Rick en **Roland**: jullie hebben mij er de afgelopen jaren voor behoed dat ik alleen met mijn werk bezig was en daar ben ik jullie erg dankbaar voor.

'Kees': Behalve de geboorte van dit proefschrift is er een andere geboorte op komst (en je bent op het moment aan het trappelen in mijn buik). Ik kan niet wachten tot je er bent.

Willem: je hebt een zeer waardevolle dimensie aan mijn leven toegevoegd en ik kijk enorm uit naar onze toekomst samen.

Curriculum Vitae

Curriculum Vitae

Marijn Créton was born on September 28th, 1973 in Utrecht as the youngest of four children.

After attending a long high school traject of MAVO, HAVO and then VWO, she took one year off to learn French in France (Besançon) and work as a carpenter in Val d'Isère. In this year she was accepted in the dental school at the University of Nijmegen, where she started her dental studies in 1993.

In 1999 she got her dental degree at the Radboud University in Nijmegen, and in the same year started working for the Air Force at 'GGW de Peel' where she worked part-time for 7 years as a general dentist. From 2000 until currently she works at department of Oral and Maxillofacial Surgery, Prosthodontics and Special Dental Care of the University Medical Center Utrecht (Head: prof. dr. R Koole), where she specialized in Maxillofacial Prosthodontics. In 2003-2004 she was lecturer at the International University of Barcelona in Spain. Presently, she also runs a private (referral) practice.

She is board member of the Dutch Association of Orofacial Pain and Prosthetic Dentistry (NVGPT) since 2008.

Her clinical interests at the University Medical Centre cover the whole work field of maxillofacial prosthetics, with a focus on cleft palate, oncology and severe hypodontia.

Published on website:
www.cobijt.nl/Hypodontie

Appendix I
The Dutch hypodontia database

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Marco S Cune

Treatment of patients with severe hypodontia: A Dutch perspective

In the Netherlands (population 16.600.000 inhabitants), dental treatment for patients with severe physical, mental or medical limitations as well as for a wide range of acquired or congenital dental disabilities is covered within the National Health Insurance Scheme. For patients with severe hypodontia full reimbursement of treatment costs is possible, including orthodontic, surgical and dental treatment throughout life.

The 'average' Dutch general practitioner has to cope with approximately 3-4 patients with oligodontia in his practice, based on a patient population of 2500 and an estimated incidence of 0.15 %.[3] Although treatment itself can be delivered by every dentist, there is consensus that dental care in specialized centers is to be preferred because of the concentration of knowledge and experience as well as the desired interdisciplinary approach (Algemene richtlijn ernstige hypodontie, workshop on severe hypodontia, CoBijt, Enschede, september 2007). In the Netherlands approximately 25 of such centers for Special Dental Care exist. They range in size and are associated with both academic and non-academic hospitals whereas some of them are independent centers. Through structural and incidental initiatives from the Dutch Society for Gnathology and Prosthetic Dentistry (NVGPT) and from the Society that represents people working in Centers for Special Dental Care (CoBijt), there are regular meetings and workshops.

Because of the considerable dental[2], skeletal[1] and genetic variation of patients with severe hypodontia, even concentration in centers does not allow the formation of large-size subpopulations of cases with more or less similar clinical presentation. The latter is a pre-requisite for therapy evaluation. An initiative was developed to document cases of (severe) hypodontia on a national level.

The Dutch hypodontia database

The Dutch hypodontia database is a web-based application to document hypodontia cases. In designing it, there were some legal limitations to consider that predominantly had to do with safe guarding the privacy of the patient. It can be approached by members of CoBijt after entering a password. (http://www.cobijt.nl/Hypodontie.aspx). After registration, centers can add patients with hypodontia to the database. There is a limited number of boxes to check and questions to answer, so it takes little time per patient. Variables include:

- the number and type of missing teeth. It is documented by checking the appropriate box when a tooth is missing ('ontbrekende elementen'). The overall tooth agenesis code is automatically calculated (TAC-score). By means of the overall Tooth Agenesis

Code the pattern of agenesis is represented by means of a single number, whereas the agenesis pattern of each quadrant remains recognizable. It allows frequency analysis to study the prevalence of various patterns.[2-4];

- gender ('geslacht') and date of birth ('geboortedatum');
- whether it is a syndromic or a non-syndromic case ('is er sprake van een syndroom'): if answered affirmatively, a list of syndromes commonly associated with hypodontia is displayed;
- whether or not the hypodontia is combined with alveolar clefting ('is er sprake van een schisis').

When de data are saved ('gegevens opslaan'), the applicant receives an e-mail with a unique case number to be saved with this particular case. It is the only link between the database and the patient data.

Final remarks

To date the database has 137 entries from 7 different centers. The data in its raw format (excel) are available upon request (secretariaat@cobijt.nl). By this initiative we hope to have stimulated nationwide collaboration among physicians and centers that are interested in the etiology, characteristics and treatment of patients with severe hypodontia.

Acknowledgments

The authors are grateful to CoBijt for hosting the hypodontia web page and to Wouter The in particular for designing it.

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Appendix II
Full-Mouth Tooth Agenesis Code in 106 oligodontia patients

This appendix was added because the original articled lacked space for it (Chapter 2). We thought the list to be important to show the possibility to share information among the research community. These are the TAC codes of the severe hypodontia population that was included in our research. It is part of the severe hypodontia population of the University Medical Center in Utrecht.

TAC1.TAC2.TAC3.TAC4 count prevalence

4.087.087	1	0.9%
2.002.003.003	1	0.9%
2.002.007.007	1	0.9%
2.010.081.065	1	0.9%
6.006.016.016	1	0.9%
6.006.017.017	1	0.9%
6.022.003.011	1	0.9%
8.016.080.080	1	0.9%
10.002.016.024	1	0.9%
10.008.019.019	1	0.9%
12.012.007.003	1	0.9%
12.076.072.112	1	0.9%
14.014.003.007	1	0.9%
14.014.023.087	1	0.9%
14.030.023.087	1	0.9%
18.010.016.016	1	0.9%
18.018.024.024	1	0.9%
24.016.024.024	1	0.9%
24.024.016.016	1	0.9%
24.024.024.024	1	0.9%
24.024.025.024	1	0.9%
24.024.088.024	2	1.7%
24.088.080.016	1	0.9%
24.088.088.080	1	0.9%
26.018.113.113	1	0.9%
26.024.016.016	1	0.9%
26.026.002.002	1	0.9%
26.026.008.024	1	0.9%
26.026.016.016	1	0.9%
26.026.024.016	1	0.9%
26.026.024.024	2	1.7%
26.026.026.026	1	0.9%
26.026.027.027	1	0.9%
26.026.027.091	1	0.9%
26.026.031.031	1	0.9%
26.026.121.120	1	0.9%
26.030.016.016	1	0.9%
26.098.016.000	1	0.9%
28.020.003.007	1	0.9%
28.028.016.016	1	0.9%
28.028.024.016	1	0.9%

TAC1.TAC2.TAC3.TAC4	count	prevalence
30.020.016.016	1	0.9%
30.030.020.020	1	0.9%
30.030.023.023	1	0.9%
30.030.031.027	1	0.9%
30.094.008.016	1	0.9%
30.094.025.021	1	0.9%
30.094.074.018	1	0.9%
30.094.088.088	1	0.9%
48.024.025.024	1	0.9%
48.080.032.048	1	0.9%
62.004.029.017	1	0.9%
62.014.073.081	1	0.9%
64.064.085.071	1	0.9%
66.066.067.003	1	0.9%
66.066.103.103	1	0.9%
68.028.016.016	1	0.9%
70.014.091.091	1	0.9%
70.068.072.073	1	0.9%
70.086.030.071	1	0.9%
72.008.073.009	1	0.9%
76.012.080.080	1	0.9%
76.084.049.049	1	0.9%
78.078.017.089	1	0.9%
78.082.079.070	1	0.9%
78.094.127.095	1	0.9%
80.016.065.001	1	0.9%
82.018.112.113	1	0.9%
82.026.056.032	1	0.9%
82.082.099.099	1	0.9%
82.086.002.082	1	0.9%
84.086.068.084	1	0.9%
86.002.087.087	1	0.9%
86.070.049.049	1	0.9%
86.086.091.091	1	0.9%
88.088.088.088	2	1.7%
88.090.016.016	1	0.9%
88.090.089.089	1	0.9%
90.026.002.010	1	0.9%
90.074.026.026	1	0.9%
90.090.016.024	1	0.9%
90.090.017.017	1	0.9%
90.090.091.091	1	0.9%
90.090.112.112	1	0.9%
90.094.027.027	1	0.9%
90.094.095.095	1	0.9%
92.092.065.065	1	0.9%
92.094.001.003	1	0.9%
94.006.091.090	1	0.9%

TAC1.TAC2.TAC3.TAC4	count	prevalence
94.028.113.113	1	0.9%
94.030.024.024	1	0.9%
94.030.027.091	1	0.9%
94.070.019.019	1	0.9%
94.090.024.080	1	0.9%
94.094.029.087	1	0.9%
94.094.067.079	1	0.9%
94.094.083.083	1	0.9%
94.094.087.095	1	0.9%
94.094.089.089	1	0.9%
94.094.090.090	1	0.9%
94.094.095.091	1	0.9%
98.098.123.095	1	0.9%
104.104.097.097	1	0.9%
106.106.105.115	1	0.9%
110.106.080.080	1	0.9%
112.112.097.097	1	0.9%
114.114.081.065	1	0.9%
114.114.097.097	1	0.9%
118.126.023.087	1	0.9%
122.122.121.123	1	0.9%
123.127.119.127	1	0.9%
124.118.113.112	1	0.9%
126.126.095.031	1	0.9%

Appendix III
Restorative aspects and reflections on clinical decision-making in severe hypodontia

Clinical decision-making in severe hypodontia patients can be complex. This is illustrated on the basis of a typical clinical case. The knowledge acquired in the different parts of this thesis is integrated when discussing the observations and the decision-making process in this particular case. Clearly, the discussions that are raised can be argued and alternative treatment modalities would also have been possible.

Case history, requests and expectations

The patient is first referred to the department of Oral-Maxillofacial Surgery, Prosthodontics and Special Dental Care of the University Medical Center Utrecht after orthodontic treatment, at the age of 18 (figures 1 and 2). The referring practitioner requests prosthodontic planning and treatment. He argues that (partial) orthodontic closure of the diastemas would have been comprehensive and technically complex. That is why it was decided to open up the diastemas. Thus far, orthodontic treatment took 2.5 years (figure 3).
Patient desires an esthetic, functional and permanent, fixed solution for her missing teeth. She finds the present protrusive position of her central incisors, as well as the relatively small teeth esthetically disturbing. Practical problem is that she plans to attend a musical academy and wishes to finish treatment before the start of her first year, which is eminent.

Medical and dental history

Her medical and dental history are unremarkable. The deciduous teeth appeared normal and were all present.
Both her parents and her sister were dentally examined too:
Her father has 3 agenetic teeth (15, 25 and 45). His third molars are agenetic and all his teeth seem smaller than average. The upper incisors have a 'shovel' shape.
Her mother misses the upper right second premolar, but it may have been extracted. Tooth shape seems normal, except for the upper lateral incisors, which appear rather small.
Her sister has no missing teeth and normal tooth shape.
Subsequent genetic research of the patient showed a mutation on the *MSX1* (as has her mother) and the *PAX9* gene (as has her father), which can explain the hypodontia and the derogatory tooth shape.

Clinical data collection

Intra oral findings

The patient has a sound dentition with good oral hygiene. Tooth dimensions seem relatively small and the upper central incisors have a straight, shovel-like shape, like those of her father.

This kind of shape deviation was also seen in the MSX1 family described in chapter 6. Geometric analysis of teeth might in future give an indication as to the genetic cause of severe hypodontia in individual cases from clinical observations (Chapter 6).

Besides the third molars, 7 teeth are agenetic. The numbers in red illustrate the missing teeth:

18 17 16 15 14 13 12 1121 22 23 24 25 26 27 28
48 47 46 45 44 43 42 4131 32 33 34 35 36 37 38

This agenesis pattern corresponds with Tooth Agenesis Code-overall (TAC): 018.018.024.016 as explained in chapter 2. The mandibular TAC-scores 16 and 24 show the highest prevalence in our research group (Chapter 2). In the maxilla 26 and 94 were the most common TAC-scores; this case shows a less severe pattern.

Agenesis of lateral incisors and premolars is rather common in the general population, but even more so in the more severe hypodontia patient (Chapter 2). Oligodontia patients hardly ever present with those tooth types. On the other hand, central incisors and first molar teeth are far more stable.

We saw 49% symmetry between the left and right side of the jaw. In this case, only the upper jaw is symmetrical. The patterns of the left and right maxilla and mandible are not symmetrical; this was the case in only 7% of our research group.
The single deciduous tooth present during clinical inspection (74) has no successor. The vertical eruption did not seem inhibited and there is no sound difference while 'tapping' with a metal instrument on the occlusal plane of the deciduous tooth and the neighboring permanent teeth. Also, the 74 occludes with its antagonist. Because of these clinical and radiographic findings it is considered non- ankylotic.

Ankylosis of deciduous teeth with no successors can, in general, be a reason to extract them prematurely. They obstruct vertical development of the jaw. The horizontal bone loss caused by tooth extraction, is more easily solved surgically than inadequate vertical bone height.

The horizontal bone volume in the areas of the agenetic teeth is small, mainly in the lower jaw. There is adequate vertical bone height. It is unclear whether the deciduous teeth were lost due to physiological root resorption or whether they were extracted to facilitate orthodontic treatment.
The diastemas where the lateral upper incisors are missing are 10 mm wide on each side. This poses a restorative problem, since 7 or 8 mm is optimal. There is a horizontal overbite

of 2 mm. The occlusion at the molar region is not ideal (figure 2). Upper and lower incisors are proclined. With the orthodontic equipment still in place, adaptations can still be made.

In general, the initial treatment plan is based on the desires of the patient and results from the views of the restorative dentist, who coordinates the course of treatment, the orthodontist and the surgeon who will eventually place the implants (if these are planned). Therefore an interdisciplinary instead of a multidisciplinary approach is advisable. Treatment span is generally long and may require adjustments, that are discussed with the patient and among the members of the treatment team. Treatment of hypodontia goes through various stages of planning, performing initial treatment, re-evaluation of treatment goals, fine-tuning, finishing of treatment and maintenance. Treating pre-adolescent patients is particularly challenging because of their young age and understandable esthetic desires during puberty. There is generally little tooth material to work with. Adolescent patients commonly become fed up with treatment and are in between schools, which occupy their lives.

Extraoral findings

The patient has a harmonic profile on the soft tissue level (figures 1 and 4). No obvious ectodermal features are observed: her hair, nails and gland function show no abnormalities.

Radiographic findings

There is one retained deciduous molar (74). Resorption of the apices of the permanent teeth is suspected when comparing the original panoramic radiograph with that after 2.5 years of orthodontic treatment (figure 3).

The main radiographical feature of this case is that the upper and, to a lesser degree, the lower incisors are retroclined (Table 1). This places her in Cluster 3: the respective normalised values for the interincisal angle differs more than 2 standard deviations from the norm. Subjects in cluster 3 exhibit the largest interincisal angle of all four clusters (Chapter 3). From table 1 it can be seen that orthodontic treatment mainly induced dental and no skeletal changes. There are however changes in the soft tissue profile as a result of the proclination of the incisors in the maxilla and the mandible. The interincisal angle has decreased and the lip profile changed accordingly, as seen in figure 5.

Diagnosis and proposed treatment

We diagnosed the patient as healthy, non-syndromal, having severe hypodontia and missing 7 permanent teeth excluding the third molars as a result of gene mutations on the *MSX1* and *PAX9* homeobox genes. The corresponding TAC-overall score is: 018.018.024.016. The profile is pre-orthodontically diagnosed in Cluster 3: 'retroclined upper and lower incisors'.

Table 1. Cephalometric analysis of the pre- and post orthodontic lateral radiographs.

Angles	Description	Pre orthodontic	Norm	SD	Post orthodontic
SNA	SNA angle: measures the anterioposterior position of the maxilla in relation to the anterior cranial base	79.2	81.8	3.7	77.6
SNB	SNB angle: measures the anteroposterior position of the mandible in relation to the anterior cranial base	75.7	79.2	2.3	75.7
ANB	ANB angle: measures the relative position of the jaws to each other (difference SNA and SNB)	3.5	2.6	2.4	1.9
NSLML	Mandibular plane angle: measures the inclination of the mandible relative to the cranial base	28.7	31.3	3.1	28
NLML	Palatal to mandibular plane angle: measures the inclination of the maxilla relative to the mandibular plane	23	23.2	3.7	22.9
NSLNL	Palatal plane angle: measures the inclination of the maxilla relative to the cranial base	5.7	8	2.2	5.2
ILSNL	Maxillary incisors angle to palatal plane: measures the relative forward to backward inclination of the upper incisors relative to the palatal plane	82.6	111.1	6.2	112
INTERINC	Interincisal angle: measures the inclination of the maxillary incisors and the mandibular incisors relative to each other	165.5	133.6	13	128.6
ILIML	Mandibular incisor plane angle: measures the forward or backward inclination of the lower incisors relative to the mandibular plane	88.9	92	9.4	96.5
NSLBOP	Cant of occlusal plane: measures the inclination of the bisected occlusal plane relative to the cranial plane	15	14.4	2.5	13.4
NASOLAB	Nasolabial angle: measures the upper lip protrusion relative to the inferior border of the nose. It is formed by two lines, namely a columella tangent and an upper lip tangent	120.9	110.3	10.7	104.8
AMSMEN	Anterior face height ratio: depicts the ratio between the distance from the Anterior Nasal Spine to Menton and Nasion to Menton.	0.53			0.53

The referring practitioner normalized this using orthodonic treament . She has a harmonious profile.

The following problem areas were defined:

- diastemas in areas where the patient desires an esthetic, functional and fixed solution as soon as possible because of the eminent start of her professional training;
- local bone deficiencies at areas where teeth were missing;
- the width of de diastemas 12 and 22 is not ideal for restorative treatment (10 mm instead of 7 or 8 mm);
- labial eversion of the central incisors;

- small (mesio-distally) and 'shovel-shaped' central incisors, which the patient finds esthetically disturbing;
- a horizontal overbite of 2 mm;
- unstable occlusion in the posterior region;
- resorption of the apices of some of the permanent teeth;
- a limited amount of available treatment time.

Treatment proposal was as follows. The buccal eversion of the central incisors, the horizontal overbite of 2 mm and the excessive width of the diastemas 12 and 22 might be resolved orthodontically by rotating the 11 and 21 in- and slightly upward. Also the occlusion could be improved by additional and longer orthodontic treatment. However, this would further burden the already resorbeded roots and lengthen treatment time, so it was decided against. A temporary, diagnostic phase was suggested as an alternative, so the braces could be removed on short notice and an esthetic try-out could be made. Occlusion can be stabilized by restorative means and implants were proposed, after augmentation of those areas with insufficient volume with bone from the retromolar area. After implant placement and osseointegration crowns can be made.

Actual treatment

After removal of the orthodontic appliances, composite veneers were made on the 13, 11, 21 and 23. Essix retainers with teeth integrated were made to temporary fill up the diastemas. In this phase the size and future position of the teeth and implants could be analyzed. By enlarging the central incisors, the diastemas became smaller and more suitable for replacing lateral incisors. Also, small positional changes could be simulated. The composite resin was adjusted according to the wishes of the patient. After local bone augmentation, 7 implants were placed according to a surgical guide at the positions: 15, 12, 22, 25, 34, 35 and 45. Unfortunately, during the restorative phase 3 out of the 7 implants placed proved not osseointegrated (figures 6 and 7).

Implants in severe hypodontia patients have a reasonable, though compromised prognosis compared to implants in non-hypodontia patients (Chapter 5). This might be due to more challenging local, anatomic conditions, or as a result of genetic factors. We were unable to demonstrate major differences in bone architecture radiographically between hypodontia and non-hypodontia subjects (Chapter 4). Some homeobox genes, among which are the MSX1 and PAX9 genes, seem to play a role in the process of both tooth and craniofacial morphogenesis (Discussion and Chapter 5) .This may also explain why failing implants cluster.

Temporary acrylic crowns were made on the remaining implants and an adhesive bridge was made to fill up the diastema 22 (figure 8). Now osseointegration of the remaining implants could be evaluated.

After 6 month, the remaining implants were re-evaluated and further planning was discussed with the patient. The remaining implants seemed to be well integrated. There were no clinical or radiographical indications otherwise. Additional implant placement was considered. However, because there was no obvious explanation for the implant losses and because of the emotional setback for the patient we decided to proceed with treatment on the remaining implants.

The final result of treatment is shown in figures 9 and 10. Because of the high esthetic demands a conventional zirconia bridge 21-23 was made. Figure 9 shows that only little sound tooth structure had to be sacrificed to prepare the central incisor because of the original 'shovel' shape of the tooth. On the 11 and 13 zirconia crowns were made in the same fashion. Crowns were made on the implants 12, 15 and 45. The fixed partial denture made in the third quadrant is an unconventional one and is checked regularly. Partial porcelain restorations were made on the upper molars to stabilize the occlusion and to fill up the diastema in the second quadrant. Until the last checkup (February 2012) the treatment result proved stable, and has been for 5 years now. Figure 12 shows pictures of the patient at the time of intake and after treatment. She is pleased with the final result.

Acknowledgements

Surgical procedures: Prof. dr. G.J. Meijer, presently UMC St. Radboud University, Nijmegen
Dental technician: Jaron Roubos of Oosterwijk Dental Laboratory Utrecht.
I would personally like to thank the patient extensively for all her patience with us and for allowing me to present the course of her treatment in this PhD thesis.

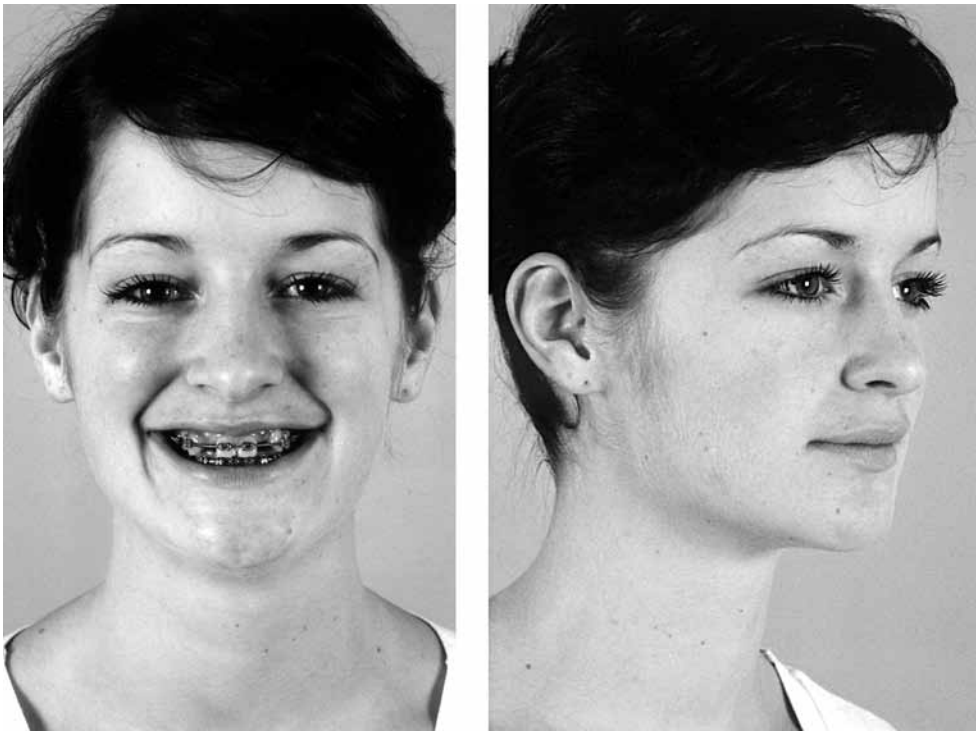


Figure 1 Frontal and lateral picture of patient at time of referral (18 years) .

Figure 2 Intraoral situation; status after 2.5 years of orthodontic treatment. Note the horizontal overbite and rather wide diastemas in the anterior region.

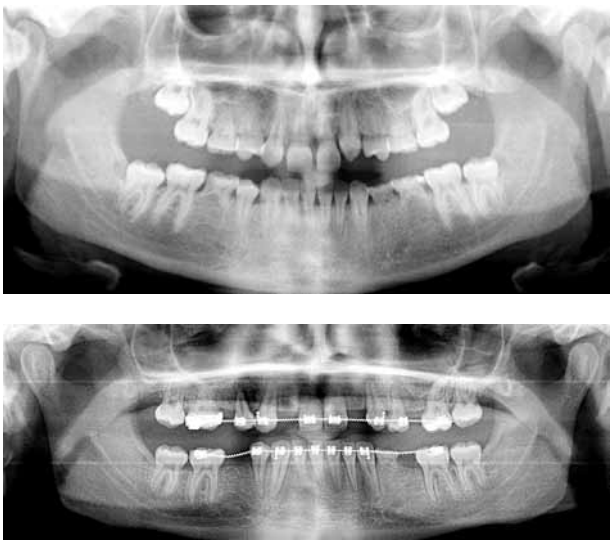


Figure 3 Panoramic radiographs before and after 2.5 years of orthodontic treatment.
Seven teeth are agenetic. Local resorption of the apices is suspected.

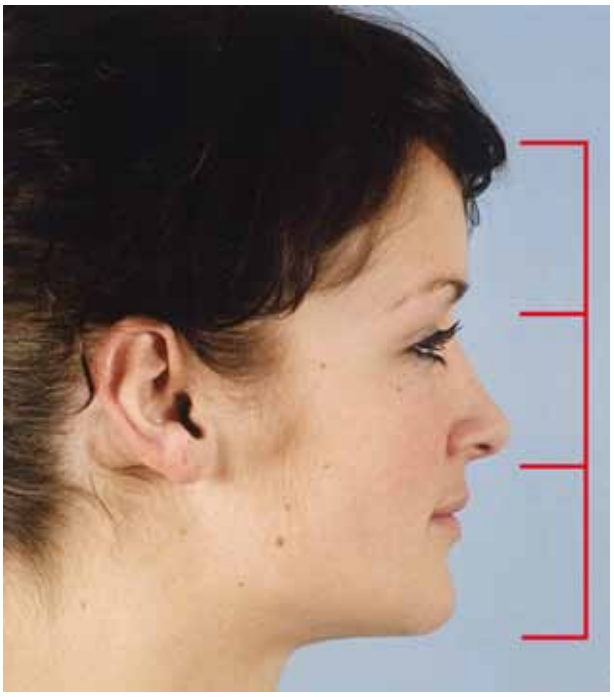


Figure 4 Lateral perspective depicting a normal, harmonious profile.

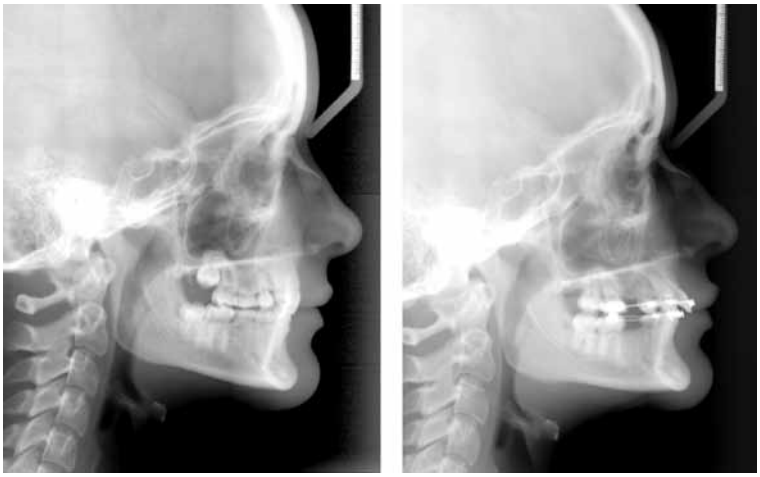


Figure 5 Pre- and post orthodontic lateral radiographs. Skeletally, not much has changed after 2.5 years of orthodontics. The interincisal angle has decreased with an effect on the nasolabial angle.

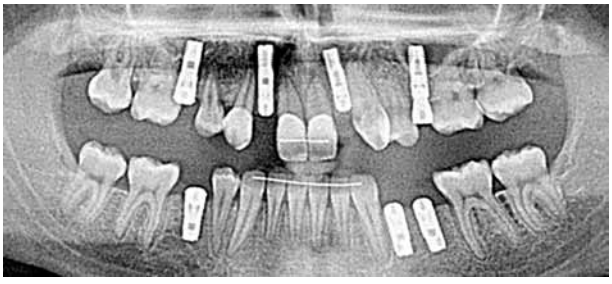
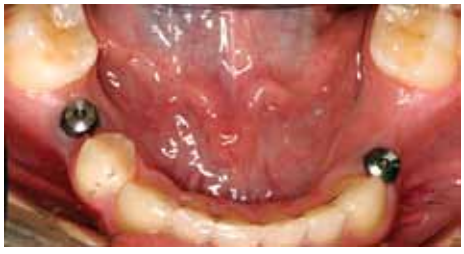


Figure 6 Panoramic radiograph after implant placement: 3 of the 7 implants placed were eventually lost (22, 25 and 35).



Figures 7 Clinical situation during the restorative phase: 3 of the 7 implants placed were lost.

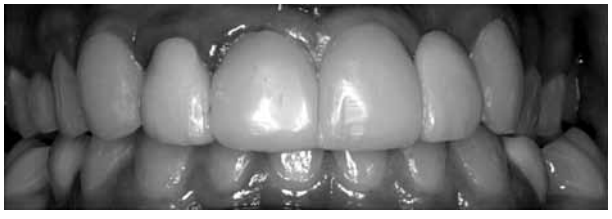


Figure 8 Temporary, acrylic crowns were made on the remaining implants and an adhesive fixed partial denture was provided to fill up the diastema at the 22 area.

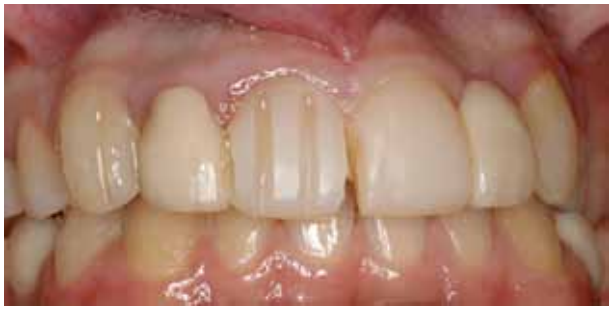
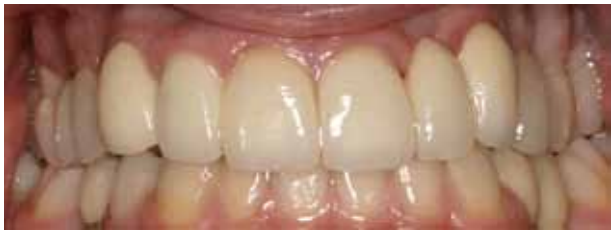
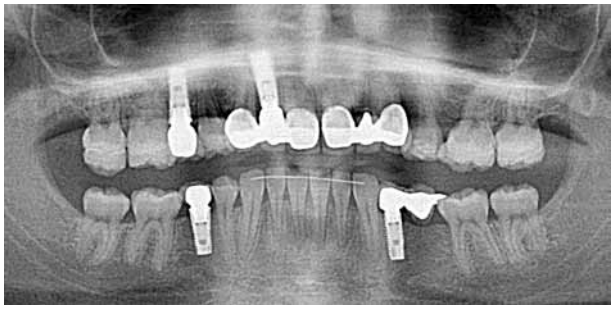


Figure 9 Preparation during the final phase of treatment: minimal invasive preparation was possible by only removing the resin. The original 'shovel' shape of the tooth allowed for this.





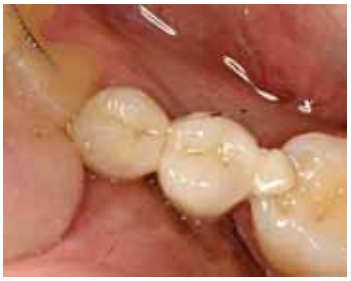


Figure 10 Intra-oral view at the end of treatment.

Figure 11 Before and after restorative treatment.