

Oligodontia and ectodermal dysplasia

– on signs, symptoms, genetics,
and outcomes of dental treatment

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To individuals affected by oligodontia
and ectodermal dysplasia

Nails, hair and teeth
– those are the magic parts of the body

Quote from Swedish poet Margareta Renberg,

Memoires of a tattooed lady, Norstedt Publishing, Stockholm, 1974

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Abstract

The general aim of this thesis was to broaden our knowledge of the signs and symptoms, genetics, and outcomes of dental implant treatment in individuals with oligodontia or ectodermal dysplasia.

Article I is a population-based study in three Swedish counties of 162 individuals with oligodontia, which was a prevalence of 0.09%. The intent was to explore ways for dentists to assess symptoms from other ectodermal structures than teeth through a clinical interview and chair-side analyses. Thirty per cent had low salivary secretion rates while only 11% with no known syndrome reported symptoms from hair, nails, or sweat glands. These are, together with teeth, the ectodermal structures on which it is proposed that a clinical diagnosis of ectodermal dysplasia (ED) be based.

Article II screened 93 probands with oligodontia for mutations in six genes known to cause oligodontia and hypohidrotic ED. Sequence alterations predicted to be damaging or potentially damaging were revealed in the *AXIN2*, *MSX1*, *PAX9*, and *EDARADD* genes in 14 (15%) of the probands. All mutations but one were novel. For the first time, *EDARADD* mutations were shown to cause isolated oligodontia. No individual who had reported ectodermal symptoms from hair, nails, or sweat glands had a mutation.

Article III assessed orofacial function in individuals with different types of EDs using the Nordic Orofacial Test-Screening (NOT-S) protocol. Individuals with ED scored significantly higher in orofacial dysfunction than a healthy reference sample, especially in the Chewing and swallowing, Dryness of the mouth, and Speech domains.

Article IV surveyed treatment outcome of dental implants in Swedish children up to age 16 years. In a 20-year period, only 26 patients were treated, 5 of whom had hypohidrotic ED and anodontia of the mandible. Individuals with ED had 64% failed implants compared to 6% among subjects with teeth missing due to trauma or agenesis.

The main conclusions of this thesis were that (i) a check of whether one or more permanent incisors are missing will identify 65% of individuals with oligodontia and 84% of individuals missing nine teeth or more, (ii) evaluation of salivary secretion is indicated in children with oligodontia, (iii) a majority of individuals with oligodontia did not report other abnormal ectodermal organ function besides teeth, (iv) no clinical indicator discriminated between individuals with and without mutations in the tested genes, and more unidentified genes are involved in tooth morphogenesis, (v) *EDARADD* mutations are associated with isolated oligodontia, (vi) evaluation of orofacial function is indicated in individuals with ED, and many individuals with ED would benefit from orofacial skills training, (vii) dental implant placement is a rare treatment modality in children, (viii) individuals with hypohidrotic ED seem to present special challenges due to structural as well as direct effects of the mutations on bone, which seem to compromise osseointegration, (ix) central registers on signs and symptoms in individuals with rare disorders would help establish prevalences of various diagnoses and define treatment needs, and (x) quality registers for monitoring treatment outcomes of dental implants would promote early detection of risks and side-effects in individuals with rare disorders.

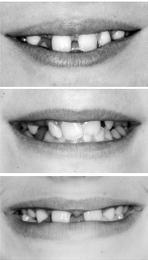
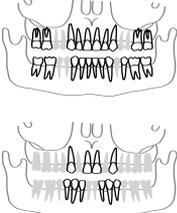
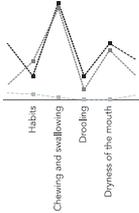
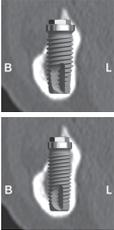
List of publications

This thesis is based on the following original publications, which will be referred to by their Roman numerals:

- I. Bergendal B, Norderyd J, Bågesund M, Holst A. Signs and symptoms from ectodermal organs in young Swedish individuals with oligodontia. *Int J Paediatr Dent* 2006;16:320–326.
- II. Bergendal B, Stecksén-Blicks C, Gabriel H, Norderyd J, Dahl N. Isolated oligodontia associated with mutations in *AXIN2*, *MSX1*, *PAX9*, and *EDARADD*. In manuscript.
- III. Bergendal B, McAllister A, Stecksén-Blicks C. Orofacial dysfunction in ectodermal dysplasias measured using the Nordic Orofacial Test-Screening protocol. *Acta Odontol Scand* 2009;67:377-381.
- IV. Bergendal B, Ekman A, Nilsson P. Implant failure in young children with ectodermal dysplasia: A retrospective evaluation of use and outcome of dental implant treatment in children in Sweden. *Int J Oral Maxillofac Implants* 2008;23:520-524.

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Thesis at a glance

Topic	Materials & methods	
I	Population prevalence of oligodontia and self-reported ectodermal signs and symptoms in individuals with oligodontia in three counties in south-east Sweden.	Through inquiry, dental caregivers identified 162 individuals with oligodontia born 1981–1994. 123 participated in clinical examinations and a structured interview on ectodermal signs and symptoms.
	II	Prevalence of mutations for oligodontia and hypohidrotic ED in clinically characterised individuals with oligodontia.
DGGE and DNA sequence analysis analysed DNA from 93 probands with oligodontia for mutations in the <i>AXIN2</i> , <i>MSX1</i> , <i>PAX9</i> , <i>EDA</i> , <i>EDAR</i> , and <i>EDARADD</i> genes known to cause isolated oligodontia and hypohidrotic ED.		
III	Screening of orofacial function in individuals with different EDs during family conferences in the US and Sweden.	The Nordic Orofacial Test-Screening protocol assessed orofacial function in 46 individuals with EDs, 32 of which (70%) had hypohidrotic ED. The ED group was compared to a reference sample of 52 healthy individuals.
	IV	Retrospective evaluation of treatment outcomes with dental implants in individuals below 16 years of age in Sweden.
A questionnaire to specialist clinics in Sweden found 26 patients had undergone implant treatment at 6 out of 30 centres (20%). Reasons for treatment were agenesis of teeth (14), teeth lost due to trauma (7), and anodontia of the mandible in hypohidrotic ED (5).		

Results

The population prevalence of oligodontia was 0.09%, with great variation between counties. Agenesis of incisors will identify 65% of individuals with oligodontia. Low salivary secretion was found in 30% while only 11% without a known syndrome reported symptoms from hair, nails, or sweat glands.

Sequence alterations predicted to be damaging or potentially damaging were found in 14 probands in the *AXIN2*, *MSX1*, *PAX9*, and *EDARADD* genes. No individual who had reported ectodermal symptoms from hair, nails, or sweat glands had a mutation.

The ED group scored higher (3.5) than healthy controls (0.4) on the NOT-S ($p < 0.001$). The Chewing and swallowing, Dryness of the mouth, and Speech domains had impaired orofacial function most frequently.

Forty-seven implants were placed – 18 in the maxilla and 29 in the mandible. No implants failed in the trauma group, but 2 of 25 (8%) implants in the agenesis group, and 9 of 14 (64%) implants in the hypohidrotic ED group were lost. All implant failures in the ED group occurred shortly after implant placement.

Conclusions

- Subjects with oligodontia can be identified in a majority of cases by checking that all permanent incisors have erupted at the age of 8 years.
- Evaluation of salivary secretion is indicated in children with oligodontia.
- A majority of individuals with oligodontia did not report other abnormal ectodermal organ function besides teeth.

- Hypohidrotic ED was not found in any individual, and mutations were found in only 15% of the probands.
- No dental indicators were found which discriminated between individuals with and without mutations.
- *EDARADD* mutations are associated with isolated oligodontia.

- Most individuals with ED – children and adults – had impaired orofacial function.
- The results point to unmet needs for orofacial skills training and oral habilitation.

- Dental implants is a rare treatment modality in children.
- Individuals with hypohidrotic ED seem to present special challenges due to structural as well as direct effects of the mutations on bone, which seem to compromise osseointegration.

Abbreviations

<i>AXIN2</i>	Axis inhibition protein 2 gene
DGGE	Denaturing gradient gel electrophoresis
DS	Down syndrome
EBM	Evidence-based medicine
ED	Ectodermal dysplasia
<i>EDA</i>	Ectodysplasin-A gene
<i>EDAR</i>	Ectodysplasin-A receptor gene
<i>EDARADD</i>	EDAR-associated death domain gene
FDP	Fixed dental prosthesis
HGMD	Human Gene Mutation Database
IP	Incontinentia pigmenti
<i>MSX1</i>	Msh homeobox 1 gene
NCBI	National Center for Biotechnology Information
<i>NEMO</i>	NF- κ B essential modulator gene
NFED	National Foundation for Ectodermal Dysplasias
NF- κ B	Nuclear factor-kappa B
NIH	National Institutes of Health
NOT-S	Nordic Orofacial Test-Screening
OHRQoL	Oral Health-Related Quality of Life
OMIM	Online Mendelian Inheritance in Man
<i>PAX9</i>	Paired box 9 protein gene
PCR	Polymerase chain reaction
PDS	Public Dental Service
WBDD	Winter-Baraitser Dysmorphology Database

Introduction

Oligodontia

Definitions

The definition of hypodontia is clear and unambiguous: “one or more congenitally absent teeth”¹. Oligodontia, which denotes a stronger clinical expression of hypodontia, has a wide and non-specific definition: the “absence of many teeth, usually associated with small size of the existing teeth and other anomalies”². In the current edition of the Glossary of Prosthodontic Terms, the definition reads “the formation of less than a full complement of teeth; many such teeth are smaller than normal”³. Hobkirk and Brook (1980) considered “severe hypodontia” to be synonymous with oligodontia and defined it as “six or more congenitally missing teeth”⁴. Schalk van der Weide (1992) proposed the criterion “six or more permanent teeth (excluding third molars)”, and since then, this definition has been increasingly used in dentistry⁵. As oligodontia can occur in isolation (I) or as part of a syndrome (S), classifying oligodontia into oligodontia/I and oligodontia/S was suggested.

Syndrome databases for rare disorders use oligodontia as a search term, and a search of two such databases with “Oligodontia” as a search feature yielded matches for 219⁶ and 262⁷ syndromes. However, the search term “oligodontia” in the Winter-Baraitser Dymorphology Database (WBDD) refers to “missing teeth – one tooth or all teeth – without differentiation” (Michael Baraitser, October, 2009, personal communication). In many publications, the definition of oligodontia is indistinct and used as a synonym to hypodontia. In this thesis, oligodontia is used to mean the congenital absence of six or more permanent teeth, excluding the third molars.

Prevalence of tooth agenesis

If third molars are included, tooth agenesis is one of the most common developmental anomalies in man⁸. One or more third molars are missing in more than 20% of humans⁹⁻¹¹. But since acceptable function can be established without third molars, the prevalence of tooth agenesis is normally, and in this thesis, reported within the framework of a 28-tooth dentition.

Polder et al. (2004) in a meta-analysis of 33 studies published from 1936 to 2001 found the prevalence of tooth agenesis to vary between 2.2% and 10.1%¹². Four of the study samples included individuals aged 3, 4, and 5 years. However, a difference in prevalence attributed to insufficient mineralization of mandibular second premolars was reported in 7-year-olds compared to 9-year-olds¹³, so the assessment of permanent tooth agenesis in younger individuals might be inaccurate. The meta-analysis reported variations in tooth agenesis by continent and gender, the female prevalence was 1.37 times higher than for males.

A compilation of tooth agenesis in 10 of the meta-analysis studies, which comprised 48,274 individuals, found that the teeth most often affected were the mandibular second premolar, followed by the maxillary lateral incisor and second premolar. The most stable teeth were the maxillary central incisors, followed by the mandibular first molars and the mandibular canines. Eighty-three per cent of the individuals with tooth agenesis were missing one tooth (48%) or two teeth (35%), and the estimated prevalence of oligodontia was 0.14%.

Fourteen of the studies were from the Nordic countries, and 11 of these reported tooth-agenesis prevalences of 6% or higher. No children under 5 years were included. Table 1 compiles these studies and a more recent Norwegian study that found a tooth-agenesis prevalence of 4.5% in 18-year-olds.

A Swedish study of 1,006 schoolchildren aged 11–14⁹ and a more recent study in 739 7-year-olds in northern Sweden¹⁴ each identified only 1 individual with oligodontia. In Denmark, 9 children (0.16%) in a group of 5,644 Danish schoolchildren with hypodontia were reported to have oligodontia¹⁵. But because children with a known syndrome were not included, the actual prevalence of oligodontia in Denmark must be higher. In Norway, a hypodontia study based on patient records of 9,532 18-year-olds found 8 individuals (0.084%) with oligodontia¹⁶. A British study of 6,000 children referred for orthodontic treatment reported a tooth-agenesis prevalence of 4.3%¹⁷. A calculation made using one of the study figures shows that 3.9% of the children had oligodontia – an oligodontia prevalence of 0.17%.

Table 1. Dental-agenesis prevalence in 15 Nordic studies based on a meta-analysis by Polder et al, 2004¹², and a study by Nordgarden et al, 2002¹⁶.

Year	Author	Country	Age	Sample size	Prevalence (%)
1956	Grahnén	Sweden	11–14	1006	6.1
1956	Grahnén	Sweden	17–43	1064	5.0
1963	Gimnes	Norway	6–15	36 000	4.5
1971	Egermark–Erikson	Sweden	10–16	3327	6.3
1971	Haaviko	Finland	5–13	1041	8.0
1973	Hunstadbraten	Norway	7–14	1295	10.1
1973	Thilander	Sweden	7–13	5459	6.1
1974	Wisth	Norway	9	813	6.8
1977	Bergström	Sweden	8–9	2589	7.4
1977	Magnusson	Iceland	8–16	1116	7.9
1980	Rolling	Denmark	9–10	3325	7.8
1993	Aasheim	Norway	7–10	1953	6.5
1997	Johannsdottir	Iceland	6–7	396	4.3
2001	Bäckman and Wahlin	Sweden	7	739	7.4
2002	Nordgarden et al.	Norway	18	9532	4.5

Associated oral signs and symptoms

Besides congenitally missing permanent teeth, individuals with hypodontia and oligodontia share common tooth characteristics, such as reduced size and aberrant form, anomalies of the enamel, and delayed eruption^{1, 18}. Oligodontia is also associated with reduced salivary secretion rates. Nordgarden et al. (2001) in a study on 68 individuals with oligodontia found unstimulated salivary flow rates below 0.1 ml/min in 15 (22.0%) individuals and chewing-stimulated salivary flow rates below 0.7 ml/min in 25 (36.8%) individuals¹⁹.

Tooth development

To a large extent, tooth development is brought about through conserved signalling pathways that mediate cellular communication in an interaction between ectodermal and mesenchymal tissue^{8, 20, 21}. Mouse models have been used to map sequential signalling involving more than 300 genes. In the early

stages of tooth formation, the epithelium forms ectodermal placodes, which are small thickenings at the sites of each tooth family. Further development is initiated by consecutive cascades of interacting signalling molecules, where the dental lamina transforms into the bud and cap stage of morphogenesis. Cell differentiation in the bell stage precedes matrix secretion in the dental tissues before eruption.

Genetics of tooth agenesis

Mutations in some of the genes involved in mouse tooth development have been identified to cause dental defects in humans. Mutations in the *AXIN2*, *MSX1*, and *PAX9* genes in humans have all been shown to cause familial hypodontia and oligodontia^{8, 22, 23}. Vastardis et al. (1996) found that mutations in the homeobox gene *MSX1* caused autosomal dominant tooth agenesis – primarily of second premolars and third molars – in humans²⁴. Several later studies confirmed this finding and found that *MSX1* mutations also cause oligodontia^{8, 22, 23, 25, 26}. Mutations in the *PAX9* transcription factor also cause human autosomal dominant tooth agenesis and oligodontia^{27, 28}.

A study of 7 kindreds with defined *MSX1* mutations and 10 kindreds with defined *PAX9* mutations and oligodontia found that the probability of missing a particular type of tooth was always bilaterally symmetrical²⁸. Individuals with *MSX1*-associated oligodontia were typically missing maxillary and mandibular second premolars and maxillary first premolars, and the teeth most frequently (75%) absent were the maxillary first premolars. More than 80% of individuals with *PAX9*-associated oligodontia were shown to lack maxillary and mandibular second molars.

Lammi et al. (2004) showed that mutations in the Wnt signalling regulator *AXIN2* were causative for oligodontia and predisposed to colorectal cancer in a large Finnish family and in one unrelated individual – all with severe oligodontia of permanent teeth²⁹. Eleven individuals lacked more than eight teeth, with three individuals having only three permanent teeth. The authors concluded that tooth agenesis, especially severe oligodontia, could be an indicator of cancer susceptibility. Mostowska et al. (2006) showed that mutations in *AXIN2* caused both hypodontia and oligodontia³⁰, and the Callahan et al. study of two families with tooth agenesis found a significant association between agenesis of at least one missing incisor and *AXIN2*³¹.

Ectodermal signs and symptoms

Currently, the most widely used definition of ectodermal dysplasias (EDs) is a group of inherited disorders with developmental abnormalities in two or more of four ectodermal structures – hair, teeth, nails, and sweat glands – and other ectodermal structures^{32, 33}. Of the four criteria structures, teeth is the only criterion with a clear cut-off for distinguishing between affected and unaffected individuals in a clinical examination.

The Schalk-van der Weide et al. study in Holland examined 167 individuals with oligodontia¹⁸. The patients were recruited from specialist clinics in special care dentistry, hospital dentistry, and prosthodontics, and a medical specialist set the diagnoses of syndromes: 89 (53%) had oligodontia/I; 48 (28.7%) had oligodontia/S; and 30 (18%) had oligodontia/I-S, where it was unclear whether or not the patient had a syndrome. Forty-one (85.4%) of the patients with oligodontia/S had ED. And in the patients with oligodontia/I, 28.1% had symptoms of dry skin, 6.7% had sparse hair, 13.4% had abnormal nails, and 9.0% had reduced sweat production.

The Nordgarden et al. study of 68 Norwegian individuals with oligodontia reported that 57% had ectodermal disturbances in the hair, nails, and/or sweat production¹⁹. The research persons were recruited from the Norwegian resource centre for rare disorders (TAKO-centre, Oslo) and in a written request to all orthodontists, prosthodontists, and oral surgeons in Norway. Thus, in neither study was the sample population based, and most probably, many individuals with multiple symptoms were included.

Ectodermal dysplasia

Ectodermal structures

Cutaneous structures or appendages are developed through complex reciprocal signalling interactions between the ectoderm and the underlying mesoderm³⁴⁻³⁶. Individuals with ED manifest variable defects in the morphogenesis of hair follicles, nails, skin, and eccrine glands, such as sweat glands, sebaceous

glands, mammary glands, and mucous producing glands in the upper aerodigestive tract and in the endothelium in the lungs^{33, 37, 38}. A highly conserved signalling pathway is common in vertebrates throughout the animal chain, which regulates the development of ectodermal organs³⁹⁻⁴¹.

Classification of EDs

Feire-Maia (1971) was the first to propose that a definition of EDs be based on four signs: trichodysplasia (hair), dental defects (teeth), onychodysplasia (nails), and dyshidrosis (sweat glands). In the Feire-Maia definition, Group A – a pure ED – comprised disorders with signs affecting at least two of these structures, and Group B – an ED syndrome – comprised disorders involving one of these four structures and another ectodermal malformation⁴².

Today, EDs are described as a diverse group of inherited disorders that share developmental abnormalities of two or more of the following: hair, nails, teeth, sweat glands, and other ectodermal structures^{32, 33}. More than 200 EDs have been described^{43, 44}, but the causative gene is known in only around 30% of defined EDs⁴⁴. The database of the National Foundation for Ectodermal Dysplasias (NFED) – the North American support group for ED – has registered more than 5,200 individuals with ED from 50 states in the US, and from over 70 countries. More than 1,900 (36.5%) of them have hypohidrotic ED, and more than 2,600 (50%) have no specific clinical diagnosis⁴⁵. Only one-third of the registered individuals have a genetically defined diagnosis (Clark Stanford, head of the Scientific Advisory Board of NFED, personal communication, May 2009).

General signs and symptoms

Besides the classical ectodermal signs, other structures derived from the embryonic ectoderm may be affected, among them the mammary gland, thyroid gland, thymus, cornea, conjunctiva, lacrimal gland, lacrimal duct³³, and Meibomian gland⁴⁶. Signs and symptoms of otolaryngologic manifestations in ED are well-known clinically but sparsely reported in the literature. One case report of a patient with hypohidrotic ED who presented dysphagia and pneumonia postulated that laryngeal incompetence was related to recurrent chest infections⁴⁷. In another case, an autopsy, it was found that mucous glands were

absent in the pharynx, larynx, trachea, and oesophagus and were hypoplastic in the colon⁴⁸. Other reports claim that decreased mucous production in the aerodigestive tract cause chronic upper respiratory tract infections, otitis, dysphagia, hoarseness, bronchitis, and sometimes haemoptysis⁴⁹. Atrophic rhinitis with ozaena has been reported to be a symptom of hypohidrotic ED⁵⁰⁻⁵². A study on otolaryngologic symptoms found that, of 75 individuals with ED, 51% reported nasal obstruction and crusting and 72% of these had hypohidrotic ED⁵³.

Individuals with ED tend to be slender with little subcutaneous fat and a study on 138 children with ED found that growth abnormalities measured as weight deficits (weight for height) were present at an early age and persisted through adolescence⁵⁴. In a study of 24 males with hypohidrotic ED, a majority had BMI (body mass index) values which were lower than the normative mean⁵⁵.

Hypohidrotic ED

Genetics and phenotype

Hypohidrotic ED is the most common of the EDs^{41, 45} and can have varying modes of inheritance – x-linked (OMIM 305100), autosomal dominant (OMIM 129490), or autosomal recessive (OMIM 224900) – all with a similar phenotype.

Stevenson and Kerr (1967) estimated that hypohidrotic ED has a birth frequency of 1:100,000⁵⁶. Carter (1977) estimated the syndrome to be within the range of 0.01 and 0.1 per 1000, which equals 1–10 per 100,000⁵⁷. And an encyclopaedia on birth defects reported an occurrence of 1–7:10,000⁵⁸. Thurnam in 1848 was first to describe x-linked hypohidrotic ED, and a few decades later, Darwin in 1875 described a kindred of men from Scinde who had the condition^{33, 59}. Scaling skin and a collodion membrane in the neonatal period have been reported in a few children with hypohidrotic ED, and may be an early indication of the diagnosis⁶⁰⁻⁶². A few publications report on sudden infant death in hypohidrotic ED, related to the relative inability to adjust body temperature due to reduced sweating capacity⁶³⁻⁶⁵.

Kere et al. (1996) showed that a mutation in a gene in the Xq12–Xq13.1 region, which encodes for a transmembrane protein called ectodysplasin, causes x-linked hypohidrotic ED⁶⁶. Monreal et al. later identified the gene in which this mutation occurred and named the gene *EDA*⁶⁷. Males with the full expres-

sion of x-linked hypohidrotic ED have hypotrichosis, hypohidrosis, and severe oligodontia^{41, 68, 69}. Characteristic facial features of frontal bossing, “saddle” nose, maxillary hypoplasia, and hyperkeratotic wrinkles around the eyes give them a similar facial appearance^{41, 68}. Dry skin and eczema, nasal crusting, and thick ear wax are other frequent signs and symptoms, especially in small children.

Since sparse hair, no teeth, and dry skin will not discriminate newborns and infants with x-linked hypohidrotic ED from healthy children, the diagnosis is most often set when the teeth do not erupt within the normal time frame, or when the first tooth erupts in an unusual position and has an aberrant form⁵⁸. A Finnish study, aiming to identify all families in Finland with x-linked hypohidrotic ED, found nine families⁶⁸. They comprised 15 male individuals, of which 4 had died. In 11 living males, the mean number of permanent teeth was 4.7 (range 0–8) in the maxilla and 2.8 (range 0–6) in the mandible. One individual had total anodontia, and three other individuals had anodontia of the mandible.

Autosomal dominant and recessive hypohidrotic ED are caused by mutations in the signal receptor EDAR (EDA Receptor) or the signal molecule EDARADD (EDAR Associated Death Domain) in a signalling cascade leading to the development of teeth and other ectodermal derivatives^{41, 70}. Among mutations for hypohidrotic ED, 25% of non-*EDA* mutations are *EDAR* mutations^{70, 71}.

Oral signs and symptoms in hypohidrotic ED

Oligodontia in the permanent dentition is a frequent feature of the phenotype of hypohidrotic ED. The recent Lexner et al. study of 23 genetically characterised Danish males with x-linked hypohidrotic ED reported a mean of 22 (range 14–28) missing permanent teeth⁷². Impaired salivation is another common clinical sign, and another Lexner et al. study, on 11 males with x-linked hypohidrotic ED, measured a mean whole saliva flow of 0.09 ml/min which was only 25% of the flow rate in male controls⁷³.

Hypohidrotic ED also affects craniofacial growth. Males with this form of ED have typical facial characteristics^{74, 75}, and maxillary hypoplasia is common. At the NFED family conference in 2006, 37 families participated in a 3D facial morphology analysis of individuals with ED. Preliminary results showed that growth rates of boys with ED and unaffected individuals appear to be similar up to the age of about 5 years, after which boys with ED tend to grow slower⁷⁶.

Lind et al. (2006) detected *EDAR* mutations in two large three-generation Swedish families with large phenotypic variability⁷⁷. The affected individuals were missing 3–16 permanent teeth and a common sign observed in all affected persons was the absence of 1–4 permanent mandibular incisors. In addition to the dental anomalies, affected family members showed varying, but generally mild, symptoms of skin and hair. Many reports on hypohidrotic ED are based solely on clinical examination and do not discriminate between x-linked and autosomal forms. Data on autosomal forms of ED – such as number and type of missing teeth in larger materials – are lacking.

Other EDs

Some EDs are well known in dentistry because of specific dental and oral symptoms. Incontinentia pigmenti (IP) (OMIM 308300) is a genodermatosis with x-linked dominant inheritance, caused by mutations in the *NEMO* (NF- κ B essential modulator) gene^{78,79}. The symptoms usually present at or soon after birth. Typical skin symptoms are inflammatory vesicles, which later develop into characteristic hyperpigmented striae. Females are most affected, as the disorder is usually lethal prenatally in males. Symptoms include abnormalities of the skin, eyes, central nervous system, and dentition⁸⁰. The most common dental signs and symptoms found in a study on 16 Koreans with IP were hypodontia (43.8%) and delayed eruption of teeth (37.5%)⁸¹. Besides hypodontia in the primary and permanent dentition, a study on eight females with IP observed delayed primary tooth eruption, cone-shaped teeth, and asymmetrical forms of permanent tooth crowns⁸². A screening of 30 Swedish individuals with IP aged 1–56 years found that 22 of 26 (85%) were missing teeth from agenesis: 11 of these had oligodontia and lacked 6–18 teeth (mean 10.6). Of the 23 who complied with salivary tests, 10 (43.5%) had low salivary secretion rates⁸³.

In ankyloblepharon-ectodermal defects-cleft lip/palate syndrome (AEC, Hay-Wells syndrome, OMIM 106260), ectrodactyly-ectodermal dysplasia-cleft lip/palate syndrome (EEC1, OMIM 129900, EEC3, 604292), and Rapp Hodgkin syndrome (RHS, OMIM 129400), clefts of the lip, the palate, or both are characteristic, but not all individuals with these syndromes have clefting (EEC syndrome without cleft lip/palate, OMIM 129810). Mutations in the *NEMO* gene also cause hypohidrotic ED with immune deficiency (OMIM 300291)⁸⁴, which is a life-threatening condition curable with stem cell therapy⁸⁵.

Sweden has done no population-based studies or systematic registrations of individuals affected by ED. The National Board of Health and Welfare registers birth defects, including congenital malformations detected during the first month of life⁸⁶. ED is not registered, probably because the diagnosis is usually not set at birth but in early childhood⁵⁸.

Orofacial function

The role of the mouth

The mouth is the organ with which the small child first discovers the world. René Spitz, an American psychoanalyst, drafted the saying “The mouth is the cradle of perception” in 1965 to pin-point the central role of the mouth from birth onwards⁸⁷. The mouth is important in many vital functions, such as feeding, swallowing, and synchronization of breathing, and also in functions related to communication and social interaction like speaking, laughing, and crying⁸⁸⁻⁹¹. The homunculus figure, which graphically maps the motor and sensory regions of the body to the motor and primary somatosensory areas of the cerebral cortex, shows the big relative representation of lips, tongue, and oral cavity². Sensory as well as motor oral functions are often disturbed in individuals with congenital diseases or injuries. The rich innervation and the subtle interplay in many different functions increase the vulnerability for damage or disturbed development not only in the foetal period but also after birth. A search in the WBDD, which comprises information on more than 4,000 syndromes, using the search term “Oral region” yields 1,211 syndromes, and the corresponding search for “Mouth” yields 1,074⁹².

Treatment strategies in children with oligodontia

Multidisciplinary treatment planning

Oral habilitation and maintenance care in individuals with many missing permanent teeth is often a long-standing commitment that requires the involvement of different specialists. Hedegård's report from the 1965 European Orthodontic Society Congress recommends that "every effort should be taken to avoid prosthetic treatment as the final stage", and this recommendation has been an overall goal in dental therapy treatment planning for young individuals with missing teeth⁹³.

In an effort to minimise the number of missing teeth that need replacement, different methods can be used, and since the methods are age dependent, an early diagnosis of which teeth are missing is crucial. Growth-adapted measures – such as early extraction of primary teeth to allow eruption of existing permanent teeth into more favourable positions – and orthodontic treatment are the most widely used strategies^{4, 94-97}. Autotransplantation of permanent teeth into positions where teeth are missing can be done with highly predictable results and good long-term outcomes⁹⁸⁻¹⁰⁰.

One of few reports on treatment outcomes in young individuals with many missing teeth is a Danish study on 112 consecutive patients with oligodontia¹⁰¹. Their patients were missing 10 teeth on average, and 10 patients had ED. Ninety-seven per cent had had some kind of orthodontic treatment and 90% of the 51 patients whose treatment was finalised had dental implants.

Bergendal et al. (2005) made a compilation of 61 Swedish patients with oligodontia whose treatment had been planned in a multidisciplinary team and was finalised. The compilation revealed that some type of prosthetic restoration replaced only 42% of teeth absent due to agenesis, the remaining missing teeth had not been replaced. Forty patients (66%) had 174 dental implants – the most frequent form of restoration¹⁰².

Multi-disciplinary treatment planning in teams of dental specialists has many advantages in cases with many missing teeth¹⁰³⁻¹⁰⁷, but little is known about patients' attitudes toward and expectations of treatment. A British study of complaints at a hypodontia clinic revealed that 40% of 451 referred young individuals had "no complaints", while only 14.6% considered "appearance" to be their most important problem¹⁰⁸. A study in Hong Kong on oral health-related quality of life (OHRQoL) targeted individuals with severe hypodon-

tia¹⁰⁹. Twenty-five children aged 11–15 years lacked a mean of 8.9 teeth (range 4–20) and reported considerable OHRQoL impact. A majority (88%) reported functional limitations and impacts on emotional well-being. Locker et al. (2010) studied OHRQoL in 36 Canadian children with hypodontia¹¹⁰. The children were missing a mean of 6.8 teeth (range 1–14), and 75% reported functional and psychosocial impacts “Often” or “Every day/almost every day”.

Dental implant treatment in young individuals

After dental implants were introduced in adults as a highly predictable method for replacing teeth lost to dental disease sequelae, new indications in young individuals were explored. An early attempt to replace an upper central incisor lost due to trauma in a young individual revealed that the implant behaves like an ankylosed tooth and prevents further growth of alveolar bone in the region¹¹¹. Experimental studies in growing pigs^{112, 113} and a long-term follow-up in a child where implants were placed early¹¹⁴ confirm this principle. A Swedish consensus conference on the use of dental implants in young individuals was held in 1995. According to conference statements, although implants should not as a rule be placed in healthy young individuals until growth is completed, implants may be placed in individuals with anodontia or severe oligodontia before the pubertal growth spurt¹¹⁵, a recommendation which is still valid¹¹⁶.

In several cases of dental implant placement in young boys with x-linked hypohidrotic ED – treated from 1.5 to 6 years of age – treatment outcome has been reported to be successful¹¹⁷⁻¹²⁰. A prospective study on six patients who were followed from age 5 years for an average of 7.8 years reported favourable outcomes with the loss of only one implant¹²¹. At present, the Guckes et al. study is the only prospective study done on a large group of individuals with ED¹²². Fifty-one patients aged 8–68 years had 243 implants placed in the anterior mandible and 21 in the anterior maxilla. The survival rates were 91% in the mandible and 76% in the maxilla. Fourteen individuals had lost an implant, and all but two of these were lost before loading.

A retrospective evaluation from Australia reported treatment outcomes in 14 young patients with ED¹²³. Of the 61 implants placed, 88.5% integrated successfully, while 3 of 15 in the anterior maxilla and 4 of 46 in the anterior mandible were lost. Five patients (36%) had lost at least one implant before abutment connection. A review of 12 articles on dental implant treatment in

patients with ED reported implant survival rates to vary between 88.5% and 97.6%¹²⁴. These results are comparable to those of a meta-analysis on implant-supported fixed dental prostheses (FDPs). The meta-analysis reported a 5-year survival of 95.2%, and a 10-year survival of 86.7%¹²⁵. However, both removable and fixed prostheses were used in the ED studies.

Bergendal et al. recently compiled 57 reports of dental implant treatment in individuals with rare disorders. These reports contained 31 (54%) publications that described 110 individuals with ED. Thus, publications on individuals with ED constituted over half of the reports, and the 110 individuals constituted nearly three-quarters of the treated patients⁹². Individuals with ED seem to be predominant among the individuals with rare disorders who undergo oral habilitation with dental implants.

Krieger et al. (2009) assessed failures and complications of prosthetic treatment in patients with birth defects, including hypodontia/oligodontia¹²⁶. After more than 8 years, over 60% of single crowns and 64% of FDPs on implants were free of complications. But when complications with implant- and tooth-supported restorations did occur, they occurred earlier with the implant-supported restorations. Still, the authors recommended implant-supported restorations as the treatment of choice, due to the benefits of avoiding the need for tooth preparation. However, the trend for earlier and more frequent complications must be weighed in, since many years in function can be expected in young individuals. An evaluation of cumulative prosthetic treatment costs for young adult patients with birth defects included 22 individuals with hypodontia/oligodontia. About half of the patients with implant-supported reconstructions experienced no failures or complications over a median observation period of 8 years. Cumulative long-term costs were similar for implant-supported and tooth-supported restorations¹²⁷.

Rare disorders

Definitions

In Sweden, the definition of a rare disorder that is used in the rare disease database of the National Board of Health and Welfare reads “a disorder causing

substantial disability and affecting fewer than 100 individuals per million population”, equivalent to 1 in 10,000 citizens¹²⁸. The concept of a rare disorder has different definitions in different parts of the world. According to the Rare Diseases Act of 2002¹²⁹ and the National Institutes of Health Office of Rare Diseases Research¹³⁰, a rare disease is generally considered to have a prevalence of fewer than 200,000 affected individuals in the US, equivalent to around 1 in 1,500 citizens. The European Organisation for Rare Diseases (EURORDIS), defines a disorder or disease as rare in Europe when it affects less than 1 in 2,000 citizens¹³¹.

In her thesis, Storhaug (1989) emphasised that individuals with rare disorders and their families need special attention concerning information and accessibility to medical and dental care – to prevent unfavourable development and dental disease¹³². Trulsson and Klingberg (2003) used qualitative methods to explore the perspectives of parents of children with rare disorders and found that respect, involvement, continuity, knowledge, and availability were aspects of professional attitude which were considered desirable¹³³.

Level of scientific evidence

The concept of evidence-based medicine (EBM) was originally established to identify the most efficient cures for common diseases and public health problems¹³⁴. Different levels of scientific evidence were defined, the highest level being “strong evidence from at least one published systematic review of multiple well-designed randomised controlled trials”, and the lowest level “opinions of respected authorities based on clinical evidence and descriptive studies or reports of expert consensus committees”. EBM has also had an enormous impact in dentistry on study designs and research funding, besides being indispensable in developing the best methods for preventing and curing major dental diseases and evaluating outcomes of common therapies.

But many clinical problems and types of treatment do not fit into that scheme¹³⁵. Reports on treatment of individuals with rare disorders often reach only the lowest levels of evidence. Editors and clinicians alike often reluctantly refer to case reports as anecdotal and of negligible importance for the advancement of clinical know-how. However, long-term follow-ups of treatment in small groups of individuals can have enormous news value, despite low group size. Clinical knowledge requires an understanding of the patient and the disease, and many important aspects of clinical encounters cannot be

studied using the traditional quantitative research paradigm¹³⁶. Ideally, prospective monitoring of larger cohorts will contribute substantially to clinical knowledge. But in the case of rare disorders and many treatment modalities that cannot be tested in a randomised manner, research designs with lower levels of evidence, as well as the use of qualitative methods, will provide the *best possible* evidence.

Aims

The general aim of this thesis was to advance knowledge on signs and symptoms, genetics, and outcomes of dental implant treatment in individuals with oligodontia or ectodermal dysplasia.

The specific aims were:

- To assess the frequency of ectodermal signs and symptoms in a population-based sample of individuals with oligodontia.
- To determine the frequency of mutations in six genes for isolated hypodontia/oligodontia and hypohidrotic ED in individuals with oligodontia.
- To assess orofacial function in individuals with ED.
- To evaluate the outcomes of dental implant treatment in young individuals with hypohidrotic ED.

Materials

Study designs

The studies in (I) – (IV) were descriptive cohort studies.

Study groups

(I) The study group was recruited from a background population of around 900,000 inhabitants in three counties of south-east Sweden. Public Dental Service (PDS) caregivers in general and specialised dentistry reported patients fulfilling these inclusion criteria: (i) birth year 1981–1994 and (ii) oligodontia. The exclusion criterion was radiation therapy in early childhood. The caregivers worked in areas where over 95% of the children attended the PDS.

Of the 164 reported individuals, two had had radiation therapy in early childhood and were excluded. The remaining 162 individuals were invited to participate in a clinical examination, and 123 (75.9%) consented – 71 females (57.7%) and 52 males (42.3%). Table 2 describes the participants.

Table 2. Study participants in (I): individuals with oligodontia born 1981–1994 and living in one of three south-east Sweden counties.

County	Individuals born	Reported	Excluded	Non-		Examined
	1981–1994*	individuals	individuals	participants		individuals
	(n)	(n)	(n)	(n)	(%)	(n)
Östergötland	73,900	59	0	18	30.5	41
Jönköping	62,686	80	1	11	13.9	68
Kalmar	43,130	25	1	10	41.7	14
Total	179,716	164	2	39	24.1	123

* Statistics Sweden 2000.

(II) The 114 individuals in (I) who had oligodontia but no known syndrome and who participated in the clinical examinations were asked to give a blood sample for genetic analysis; 106 (93.0%) consented. Two were excluded due to syndromology: a boy with hemifacial microsomia and a girl who was clinically diagnosed with Kabuki syndrome after the clinical study. Eight siblings were excluded because only probands (one index person per family) for oligodontia were included. Three samples were destroyed in transport or handling. The final group comprised 93 individuals – 53 females (57.0%) and 40 males (43.0%). Of these, 32 were from Östergötland, 49 from Jönköping, and 12 from Kalmar county (Table 3). The non-participation rate for the whole sample was 7.0%, and varied in the three counties from 1.7% to 15.0%. The flow chart in Figure 1 illustrates participants and non-participants in (I) and (II).

Table 3. Individuals with oligodontia and no known syndrome who participated in the genetic study in (II) and number of analysed samples.

County	Examined	Non-		Excluded	Destroyed	Analysed
	individuals	participants	(%)			
	(n)	(n)			(n)	(n)
Östergötland	40	6	15.0		2	32
Jönköping	60	1	1.7	10	0	49
Kalmar	14	1	7.1		1	12
Total	114	8	7.0	10	3	93

(III) The study group comprised 46 individuals with ED aged 3–55 years who attended family conferences for ED, 31 from the US and 15 from Sweden. The participant or a parent self-reported the ED diagnosis. The ED group comprised 30 males (65.2%) and 16 females (34.8%) with a mean age of 14.5 years (range 3–55). A healthy reference sample of 52 individuals with the same age range – 20 males (38.5%), and 32 females (61.5%), mean age 24.9 years – was selected for comparison.

(IV) The study group comprised 26 patients, 18 girls and 8 boys, reported to be treated with dental implants before 16 years of age. Patients were divided into three subgroups according to treatment reason: tooth agenesis, trauma, or hypohidrotic ED. Table 4 describes the study groups in (I) – (IV).

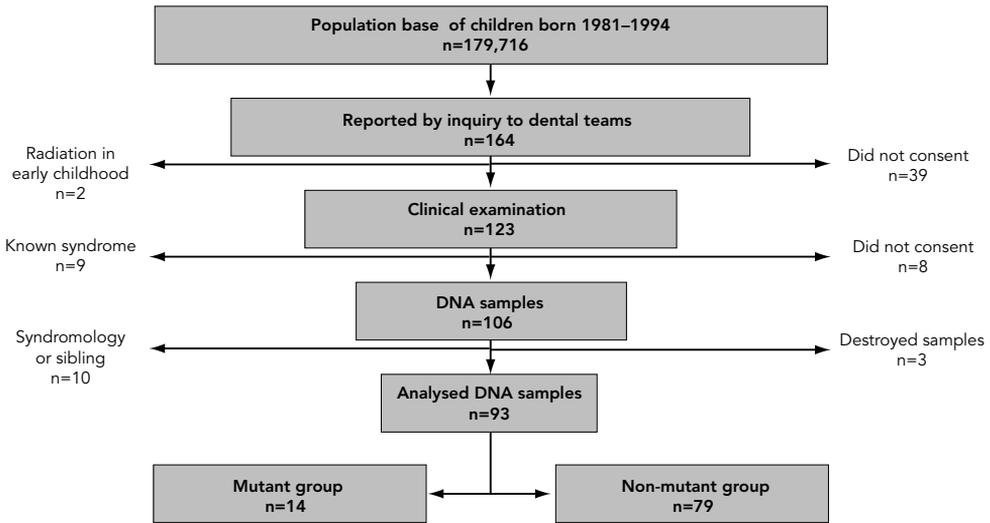


Figure 1. Flow-chart of background population of individuals born 1981–1994 and individuals with oligodontia in (I) and (II).

Table 4. Inclusion criteria and number of included individuals in (I) – (IV).

	Inclusion criteria	Number of included individuals				
		All	Female		Male	
			(n)	(%)	(n)	(%)
(I)	Oligodontia Born 1981–1994	162	91	56.2	71	43.8
(II)	Oligodontia Born 1981–1994	123	71	57.7	52	42.3
(III)	Probanda for oligodontia	93	53	57.0	40	43.0
(IV)	ED 3 years or older	46	16	34.8	30	65.2
(V)	Dental implant treatment Younger than 16 years	26	18	69.2	8	30.8

Methods

Questionnaire to identify individuals with oligodontia (I)

In November 2000, a short questionnaire was sent to PDS clinics in Östergötland, Jönköping, and Kalmar counties. Caregivers in general dentistry, paediatric dentistry, orthodontics, and prosthetic dentistry were asked to report individuals who had been examined during the past 18 months and fulfilled the inclusion criteria of birth year and oligodontia. The questionnaire comprised questions on birth date, initials, gender, number of missing teeth, and known medical diagnoses and syndromes.

Structured interview (I)

The structured interview comprised questions in the following areas: heredity for tooth agenesis; medical history including congenital diseases or syndromes; skin; hair; nails; sweating. The heredity domain contained 2 questions – on tooth agenesis in the family and on hereditary diseases or syndromes. The ectodermal symptom domain contained 10 questions: 4 questions on the skin, 4 on sweating, and 1 question each on the hair and nails (Appendix). The four examiners, all dental specialists, pilot tested the interview questionnaire in a small number of children with hypodontia. The interviews and examinations took place between November 2002 and June 2003. If the answer was unclear, further questions were asked to capture the opinion of the respondent.

Clinical examinations (I)

Assessment of tooth agenesis on panoramic radiographs

The responding caregivers were asked to determine oligodontia by counting teeth missing from agenesis on available radiographs and checking patient records. Of the final group of 162 included individuals, 123 consented to be clinically examined. Tooth agenesis in these individuals was assessed by spe-

cialists from existing panoramic radiographs, or if not optimal or too old, new panoramic radiographs taken in connection with the examinations. One girl with Down syndrome (DS) was unable to comply with a panoramic radiograph, so intra-oral radiographs were used instead. Data on missing teeth for the 39 individuals who did not consent to participate in the clinical study were based on radiographs evaluated by the responding dentists. Panoramic radiographs were used in 37 individuals and in two individuals with DS and autism, respectively, intra-oral radiographs were used. In all, panoramic radiographs were taken from age 9 years in 145 individuals (89.5%), between ages 8 and 9 in 8 individuals (4.9%), and between ages 7 and 8 in 6 individuals (3.7%). In 3 individuals (1.9%) intra-oral radiographs were used.

Saliva testing

Unstimulated whole saliva was collected for 15 minutes, and chewing-stimulated whole saliva for 5 minutes in a graduated measuring glass. The cut-off values recommended by the Swedish Social Insurance Board for low salivary secretion – 0.1 ml/min or less for unstimulated and 0.7 ml/min or less for chewing-stimulated whole saliva – were used¹³⁷. The criterion for low salivary secretion was a value below the cut-off in one or both tests.

Collection of blood samples for genetic screening (II)

After completion of the interview on ectodermal symptoms, the respondents were asked to give a blood sample for later genetic analysis. The samples were sent before noon, and not on a Friday, by mail to the Rudbeck laboratories in Uppsala, Sweden, where DNA was extracted and stored.

Genetic screening: DGGE analysis and DNA sequence analysis (II)

Peripheral blood samples were collected, and genomic DNA was extracted from nucleated cells using standard techniques. Six genes were analysed: *AXIN2*, *MSX1*, *PAX9*, *EDA*, *EDAR*, and *EDARADD*. The analyses were done between November 2007 and March 2009. Hot-start polymerase chain reaction (PCR) generated amplicons for denaturing gradient gel electrophoresis (DGGE) and DNA sequence analysis in 20- μ l reaction mixtures (5 cycles of 1 min at 94°C, 1 min at 58°C, and 2 min at 72°C followed by 5 cycles of 1 min

at 94°C, 1 min at 55°C, and 2 min at 72°C and a final 30 cycles of 1 min at 94°C, 1 min at 52°C, and 2 min at 72°C). DGGE was done according to Myers et al. (1985)¹³⁸, and one of the primers was tailed with a GC-rich clamp to modify melting behaviour¹³⁹. The Melt Ingeny computer program (Ingeny, Goes, The Netherlands), which simulates the melting behaviour of any DNA sequence, was used in primer design and for optimising DGGE conditions for the amplicons. Direct sequencing of PCR products per standard protocols further analysed the DNA sequences that had abnormal migration patterns in the DGGE gel. PolyPhen modelling software^{140, 141} analysed all unclassified sequence variants to predict their functional effects. Variants identified as polymorphisms by these databases were ignored: the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/Genbank/>), the Human Gene Mutation Database (<http://hgmd.cf.ac.uk/ac/index.php>) and the Biobase (<http://www.biobase-international.com/>). Zentrum für Medizinische Genetik in Osnabrück, Germany performed the genetic analyses.

Orofacial function screening with the NOT-S (III)

Orofacial function was assessed with the Nordic Orofacial Test-Screening, NOT-S, protocol and through observation of voice quality and speech clarity. The NOT-S was developed by a Scandinavian network of speech and language pathologists and dental specialists to standardize assessment of orofacial function¹⁴². NOT-S contains 12 domains in two parts: (i) six domains evaluated in a structured interview and (ii) six domains evaluated through clinical observation. In the ED group the examiner also evaluated two symptoms related to voice quality and speech clarity: hoarseness and lisping. Participants were asked to wear their removable dental prostheses during the examination.

Questionnaire search for young implant-treated individuals (IV)

In September 2005 a questionnaire was sent to Swedish specialist clinics in oral and maxillofacial surgery and prosthetic dentistry, asking them to report children and adolescents up to the age of 16 who had been treated with dental implants from 1985 to 2005. The questions comprised initials, birth year, syndrome diagnosis, reason for implant treatment, number of placed implants, and complications; each patient was reported on a one-sheet form. The study

was made in cooperation with the National Board of Health and Welfare and the Swedish ED society.

Review of patient records and clinical outcomes with operators (IV)

The responding specialists who had treated the patients in the ED group were contacted and asked, after informed consent of the parents, to send copies of documentation from the patient records, including radiographs and clinical photographs, for review. Results were discussed with the two oral surgeons who had performed surgery in the two most recently treated patients.

Statistical methods (I–IV)

All data were processed with the Statistical Package for the Social Sciences (SPSS; versions 10.0–15.0; Chicago, IL, USA). Descriptive statistics were reported as means, frequencies, and ranges. The chi-square test was used for statistical testing of proportions of categorical variables, and the one-way ANOVA for testing group differences in missing teeth.

Results

Prevalence of oligodontia (I)

The prevalence of oligodontia was calculated from 162 individuals with oligodontia and data from Statistics, Sweden 2000. For the total sample, prevalence was 0.090% and varied between 0.060% and 0.144% in the three counties. The highest numbers of reported and examined individuals were from Jönköping county, which had the second highest number of inhabitants. Individuals aged 12–18 years at the time of examination were more commonly represented than individuals in other age-groups (Figure 1, I). The calculated prevalence of oligodontia among 12–18-year-olds was 0.115%.

Individuals with syndromes

Ten of 123 examined individuals (8.1%) were coded as having a syndrome diagnosis: six had DS, one ichthyosis, one IP, one Prader-Willi syndrome, and one Kawasaki disease. Another individual had had Kawasaki disease – also called Kawasaki syndrome – in childhood, but both were considered healthy from 3 and 5 years of age, respectively, and should not be regarded as having a syndrome. Thus 9 individuals (7.3%) – 6 females and 3 males – had a syndrome. All but one lived in the same county.

Ectodermal signs

Frequency of missing teeth

The number of teeth missing from agenesis varied between 6 and 20 (median 7). Ninety individuals (55.6%) were missing 6 or 7 teeth; 111 (68.5%) were missing 6, 7, or 8 teeth; and 16 (9.9%) were missing more than 12 teeth (Figure 2, I). The most frequently missing teeth – the second premolars of both jaws followed by the maxillary lateral incisors – constituted more than half (51.3%) of the missing teeth. No individual lacked maxillary central incisors, which were the most stable teeth, followed by the mandibular canines and first molars. Figure 3, (I) shows the distribution of agenesis of individual teeth.

Nine individuals with syndromes were missing between 6 and 16 teeth (mean 8.5, median 8). The highest number of missing teeth was found in a girl with IP.

Salivation

Salivary secretion was tested in 116 individuals. Seven individuals were unable to comply with saliva testing; 4 of them had DS and 1 had Prader-Willi syndrome. Thirty-five (30.2%) had low salivary secretion according to the criteria, and two of them had DS.

Ectodermal symptoms from hair, nails, and sweat glands

Seventeen individuals including 5 with a syndrome, reported ectodermal symptoms. Of 114 individuals with no syndrome, 12 (10.5%) reported abnormal sweating, 5 (4.4%) abnormal hair, and 4 (3.5%) abnormal nails; some reported symptoms in more than one of these ectodermal structures.

Early identification of individuals with oligodontia

Agenesis of one or more permanent incisors was found in 65.0% of the total sample of individuals with oligodontia, in 77.8% of those missing eight teeth or more, and in 84.3% missing nine teeth or more. No other parameter that could serve as a clinical indicator of oligodontia was found.

Mutation analysis (II)

Mutation screening of all coding sequences of the six genes revealed sequence alterations predicted to be damaging or potentially damaging in only 14 of the 93 individuals (15.1%): 7 girls and 7 boys. Mutations were identified in the *AXIN2* gene (4 individuals), the *MSX1* gene (4 individuals), and the *PAX9* gene (4 individuals). Two probands were identified with mutations in the *EDARADD* gene. No mutations were found in the *EDA* or *EDAR* genes. All sequence variants were excluded as polymorphisms in the NCBI database¹⁴³. All variants but one (*AXIN2*, c.1994dupG) were absent in the Human Gene

Mutation Database¹⁴⁴. Thus 13 of the 14 mutations were novel (Table 1, II). No mutation was found in individuals who reported abnormal hair, nails or sweating.

Missing teeth

The mean number of missing teeth was 8.7 (range 6–18) among the 14 probands with mutations, while the corresponding figure in the non-mutant group was 7.7 (range 6–16) ($p>0.05$). In all, 122 teeth were missing in 14 individuals with mutations. Premolars were most commonly missing, 66 (54.1%), followed by molars, 38 (31.1%). Individuals with *AXIN2*, *MSX1*, and *PAX9* mutations had similar mean numbers of missing teeth, 8.0–9.8, and individuals with *EDARADD* mutations had the lowest mean number of missing teeth (6.5). Figure 1 (II) shows the distribution of missing teeth in individuals with mutations. Six individuals with mutations in *AXIN2*, *MSX1*, and *EDARADD* were missing all second premolars. In two individuals with mutations in *EDARADD*, only premolars and molars were missing. All molars were missing in two individuals with a mutation in *MSX1* and *PAX9*. One or two mandibular incisors were missing in 6 individuals with mutations in *AXIN2*, *MSX1*, or *PAX9*.

Five individuals (35.7%) with mutations reported oligodontia in a parent or a sibling compared to 10 (12.7%) in the non-mutant group. Ten probands (71.4%) with mutations reported hypodontia in a parent or sibling, whereas this was reported by 47 (59.5%) in the non-mutant group.

Nine individuals, all without mutations, had self-reported symptoms from hair, nails or sweat glands in a structured interview. They were 5 males and 4 females, and their mean number of missing teeth was 7.9 (range 6–11), which was equal to those without self-reported symptoms ($n=84$), ($p>0.05$).

Screening of orofacial function (III)

The total NOT-S score in the ED group was 3.5 (range 0–8) compared to 0.4 (range 0–2) in the healthy reference sample ($p<0.001$). Dysfunctions were most frequently recorded in the Chewing and swallowing (82.6%), Dryness of the mouth (45.7%), and Speech (43.5%) domains. The added evaluations of hoarseness and lisping were recorded in 32.6% and 47.8%, respectively.

The ED group was divided into one group with hypohidrotic ED, comprising 32 individuals, 26 males and 6 females, and another group with other EDs comprising 14 individuals, 4 males and 10 females. The total NOT-S score for the hypohidrotic ED group was 3.0 (range 0–6), and for the other ED group 4.6 (range 1–8), but the difference was not statistically significant ($p>0.05$). Figure 2 presents the dysfunction profiles of the ED groups and the reference sample.

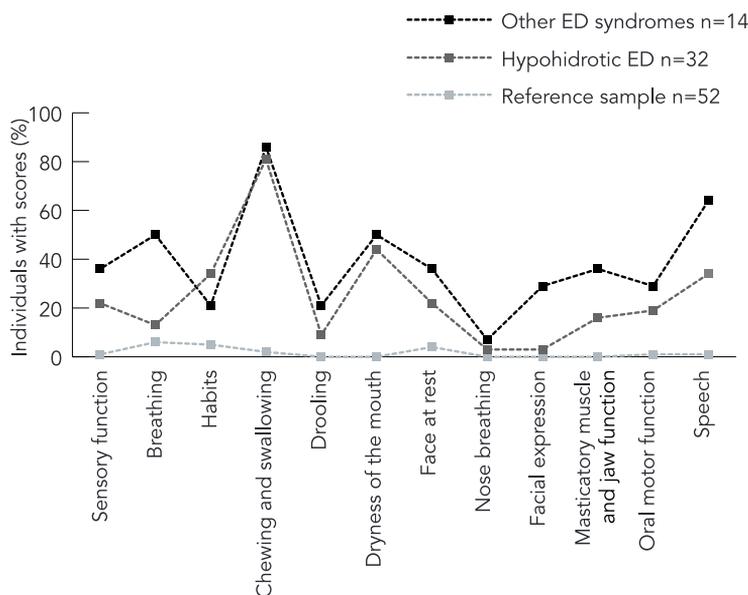


Figure 2. Dysfunction profiles based on NOT-S domain score frequencies in individuals with EDs and a healthy reference sample.

Treatment with dental implants up to age 16 years (IV)

Forty-two clinics representing 30 specialist centres from north to south Sweden responded to the inquiry. Over a period of 20 years, six centres (20%) treated a total of 26 patients under age 16 years: 18 females and 8 males (Table 1, IV). Age at implant placement was 12–15 years in the agenesis and trauma groups, and 5–12 years in the ED group.

Of the 47 implants placed in the three groups, 25 implants (53.2%) were placed in 14 patients for tooth agenesis, and two implants failed. The trauma group comprised 7 patients with 8 implants (17.0%), no implant lost. Thus, in individuals with tooth agenesis or trauma 6.1% of the implants were lost. The

hypohidrotic ED group comprised 5 children with 14 implants (29.8%) in the anodontic mandible. Four of them lost 9 implants (64.3%) shortly after placement and in all cases before abutment connection.

The first patient was treated in 1985 and the last in 2005. The five patients with ED underwent implant surgery under general anaesthesia. The same implant system was used, but with different type, diameter and length of the implants (Table 2, IV). The oral surgeons who had operated on the two most recently treated patients reported that the bone volume was limited and that the bone appeared to be extremely hard, which caused difficulties in implant insertion. The four children with ED whose implants failed were successfully re-operated, two of the children when they were in their teens and two directly after primary healing of the mucosa.

Discussion

Children with few or no permanent teeth present one of the ultimate challenges in dentistry. Despite much attention in the literature concerning the extensive need for dental care compared with other rare syndromes, individuals with ED rarely figure in high-quality studies; most are subjects of case reports with low levels of scientific evidence⁹². Dental treatment of individuals with oligodontia, ED, or both is also a heavy burden on health delivery, in terms of workload (time) and cost.

In recent years, Schalk van der Weide and Nordgarden reported observing other ectodermal symptoms in many individuals with oligodontia^{145, 146}, implying an under-diagnosis of EDs. These studies, however, assessed patient cohorts referred to specialist clinics and a resource centre for rare disorders. Because children with oligodontia often need extensive dental treatment, dentists are ideally placed to help improve diagnostics of ectodermal symptoms in this group of patients.

Oligodontia, according to the dental definition used in recent decades, is a stronger clinical expression of hypodontia. Because of confusion in the use of the term oligodontia, some publications, as well as information in databases on rare disorders, have been misinterpreted. Many publications describe only briefly, if at all, how symptoms from ectodermal structures were diagnosed in individuals with oligodontia/I.

National and international co-operation with medical and dental professionals and with support groups for ED have further disclosed the multitude of problems families face in their efforts to get a diagnosis, master the problems of every-day life, and access the treatment needed for their children. This was the background for choice of topic.

Materials and methodological considerations

Study design, study groups, and methods

(I) and (II) were based on individuals with oligodontia in three Swedish counties. Participants were identified in 2000 through a mailed questionnaire to general and specialised PDS caregivers. More than 95% of children up to age 19 years in these counties receive dental care through the PDS, those treated in private dental practices were not reached. The number of reported individuals born in different years of the 14 years of inclusion varied, and the 12–18-year age-group had the highest number of reported individuals. One reason for this may be that many individuals were reported by orthodontic clinics, where treatment typically begins around 12 years of age. The youngest children, born in 1991–1994, were only 6–9-years-old in 2000, and the diagnosis may not have been established in all affected individuals in this age-group. The three counties reported varying numbers of individuals, and individuals with known syndromes were almost exclusively reported from one of the counties, which affected the prevalence figures. Recall-bias must also be considered in retrospective reporting of patients.

Aside from these shortcomings in participant recruitment, no other systematic reasons for non-report were found. An underreporting of individuals with oligodontia did occur in these three counties, and actual prevalence of oligodontia is certainly higher. A British study of 6,000 children¹⁷ and a Danish study of 5,644 children¹⁵ reported the prevalence of oligodontia to be 0.17%, and 0.16%, respectively. Neither study, however, included individuals with syndromes. A meta-analysis of permanent tooth agenesis found 2.6% of affected individuals to have oligodontia – an overall prevalence of 0.14%¹².

A *prospective* study of a population-based sample of individuals 9–19 years of age, including good monitoring of participating clinics, would have improved inclusion of study persons. With a 0.1%–0.2% estimated prevalence of oligodontia, a population base of one million inhabitants would generate around 125–250 individuals. Ideally, a computerized patient record system where tooth agenesis was strictly defined, discriminating between teeth missing due to trauma, orthodontic extraction, dental disease, or agenesis, would generate more reliable figures on the prevalence of tooth agenesis in general, as well as oligodontia. However, in view of two recent Scandinavian studies on

clinic-referred patients with hypodontia reporting 9 and 8 individuals with oligodontia, respectively^{15, 16}, a study group of 162 individuals with oligodontia represents a comparatively large material.

All but 6 of our 162 study individuals were 8 years or older at the panoramic radiographic examination. Since the Bäckman and Wahlin study re-examined 7-year olds several years later and found only one additional tooth in over 700 examined individuals¹⁴, the low examination age of a few individuals in our study should have had minimal effect on the results.

Because an aim of this thesis was to explore ways of identifying signs and symptoms from ectodermal organs in individuals with oligodontia, clinical methods that the dentist could perform simply and easily – applicable in everyday practice – were chosen. The cut-off values used for hyposalivation – 0.1 ml/min or less for unstimulated and 0.7 ml/min or less for paraffin-stimulated whole saliva – are the values required in Sweden for individuals with Sjögren's syndrome to obtain subsidised dental care¹³⁷. Weighing of saliva samples is a more exact method, but it would not be practicable in routine dental practice.

Self-reported symptoms were assessed in a structured interview that was intended to be discriminative for self-reporting of abnormal skin, hair, nails, and sweating. Diagnostic structured interviews have been used in clinical trials, epidemiologic research, and clinical practice and are reported to improve the reliability of data collection¹⁴⁷. A study that compared interview questionnaires with mail questionnaires found that information recorded in interviews – conducted by district nurses who interpreted the answers – was more consistent than information in self-report questionnaires¹⁴⁸.

Because most examinations occurred in winter, the validity of questions on skin symptoms was probably lower than it should have been. No easily recognised cut-off for hair, nails or sweat glands has been found that will discriminate non-affected from affected individuals. Neither are there any easily applicable clinical tests that can be used in dental practice. None of the other three questions on sweating capacity were interpreted to be discriminative of reduced sweating. Some individuals or their parents commented that the affected individual's hair grew slowly, but this comment was difficult to evaluate. The validity of asking what is normal is questionable, and development of clinically applicable evaluation methods for hair and nail quality and sweating capacity would improve the validity of ectodermal symptom assessment.

In 2003, six genes known to cause isolated oligodontia or hypohidrotic ED were selected for analysis in (II). The intention was to relate clinical findings of ectodermal symptoms with genetic analyses to discriminate individuals with

isolated oligodontia from those with hypohidrotic ED. At that time, no Swedish genetic laboratory was able to analyse mutations in the six genes; this is still true in 2009, and in Denmark, only one gene – *EDA* – can be analysed, in Aarhus. In 2006, a laboratory in Osnabrück, Germany, was contracted to do the mutation analyses. By the time the results were returned in 2009, mutations in *EDA*^{149, 150} and *EDAR*¹⁵¹ had been found also to cause isolated oligodontia.

Ninety-three probands with oligodontia participated in the genetic screening, which was done using well-known genetic techniques. All sequence variants were excluded as polymorphisms from the NCBI database¹⁴³. All variants but one (*AXIN2*, c.1994dupG) were absent in the Human Gene Mutation Database¹⁴⁴. Twelve probands had a mutation in the *AXIN2*, *MSX1*, or *PAX9* genes. Other familial studies on severe oligodontia failed to identify mutations in these genes^{152, 153}. This indicates that mutations in *AXIN2*, *MSX1*, and *PAX9* account for oligodontia in a minor part of affected individuals.

Since Sweden has no registers on individuals with ED and prevalence is low, recruiting individuals at family meetings and inviting individuals from outside Sweden was a feasible way to form a reasonably sized sample in (III). Recruitment of study persons occurred at support group meetings in 2006. At the 2006 annual NFED conference in St Louis, Missouri USA, individuals with ED were asked to undergo screening of orofacial function. The same day data for several other studies were collected, and around 70% of the attendees consented. All individuals with ED at the family meeting of the Swedish ED Society in Enköping, Sweden, participated. The two examiners were well calibrated from participating in the development of the NOT-S protocol.

ED diagnoses were self-reported, and a majority of the study persons had hypohidrotic ED (69.6%), which is an overrepresentation when compared to the NFED database, where around 36% are recorded as having this diagnosis⁴⁵. The study group of individuals with ED had a lower mean age and more males than females compared with the healthy reference sample. A gender- and age-matched reference sample would have been optimal.

Various tests and methods to assess orofacial function have been presented, but no consensus on choice of questions and examinations has been reached¹⁵⁴. Following the 1st Nordic conference on orofacial therapy in 2000, a literature review was done and presented at the 2nd Nordic conference on orofacial therapy in 2002. The review disclosed that nine different tests (7 Swedish, 1 Danish, and 1 Finnish) comprising nearly 250 questions and tasks had not one question or task in common (Anita McAllister, November 2009, personal com-

munication). The conference then elected a Scandinavian network of speech and language pathologists and dental specialists to develop a test for assessing orofacial function. The NOT-S is a screening method with 12 domains of orofacial function and has been shown to discriminate between healthy subjects and individuals with diseases and syndromes¹⁴². On an individual level, changes in orofacial function can be evaluated and monitored; and on group level, dysfunction profiles can be established for certain diagnoses. The outcome of orofacial dysfunction treatment can be assessed, as in a recent evaluation of surgical outcomes in children with adenotonsillar hypertrophy, where differences between affected and healthy children before and after surgery were demonstrated¹⁵⁵.

The proceedings of the Swedish consensus conference on ED¹⁵⁶ presented three cases of boys with hypohidrotic ED and anodontia of the mandible. One patient had had two implants placed in 1985 at age 6 without complications¹¹⁷. The other two cases had lost implants soon after the implant operations in 1988 and 1991. More than 10 years later, members of the Swedish ED Society reported to the National Oral Disability Centre in Jönköping, Sweden about two children with hypohidrotic ED who had lost some implants shortly after placement.

These incidental reports were the incentive to evaluate treatment outcomes of dental implants in young children. The co-operation with the Swedish National Board of Health and Welfare, which was announced in the cover letter mailed with the questionnaire, probably contributed to the high response rate. The questionnaire targeted specialist clinics in oral and maxillofacial surgery and prosthetic dentistry. During the first decade of implant treatment in Sweden, prosthodontists treated most patients. They also supervised other dentists in allocating patients for implant treatment, and until 1993, this was a Swedish Dental Insurance prerequisite for reduced costs. After 1993, decisions on dental implant therapy could be made by all dentists. Due to the system of free dental care for children up to age 20, it is unlikely that younger patients were treated privately. So the patients reported in the study questionnaire are likely to represent most children treated with dental implants. In the case of children with ED, it is unlikely that any children unknown to the Swedish support group or the National Oral Disability Centre were treated.

Evaluation of results

The distribution of tooth agenesis in individuals with oligodontia was similar to what has been reported in many studies of hypodontia. The teeth most frequently missing were the second premolars of both jaws and the upper lateral incisors, findings that are in line with studies of hypodontia^{1,12,17} and oligodontia^{5,16}. Only 9.9% were missing more than 12 teeth. The most stable teeth were the maxillary central incisors, which no individual was missing, followed by the mandibular canines and first molars. These teeth are often reported to be present in individuals with hypohidrotic ED and severe hypodontia^{41,68}.

Nine of the examined individuals (7.3%) had a syndrome, six of the nine had DS. Russell and Kjaer (1995)¹⁵⁷ in a study on 100 Danish individuals with DS found an occurrence of hypodontia that was 10 times higher than in the general population, but they did not report the occurrence of oligodontia – like most other studies of hypodontia in DS. One exception is a study on 70 Brazilian individuals with DS aged 5–40 years. In this study, 60% had hypodontia, mostly with mild expression, and 3 (4.3%) had oligodontia and were missing six or eight teeth¹⁵⁸. In Sweden, the incidence of DS among newborns in 1983–1993 was 20 in 10,000⁸⁶. So for our study group, the expected number of children with this diagnosis and oligodontia would be 15 individuals. Axelsson et al. (2003) in a study on dental characteristics in 41 individuals older than 10 years with Williams syndrome, found 40.5% to have hypodontia and 11.9% oligodontia¹⁵⁹. Hypodontia is a known feature in many syndromes, but in most other rare disorders frequencies of oligodontia have not been reported. So it is difficult to estimate the degree to which individuals with syndromes were missing in the study.

The prevalence of oligodontia found in this thesis – 0.09% – was lower than the estimated prevalence because of under-reporting. Since the Swedish PDS treats most children and now uses computerised patient record systems, the prerequisites for establishing a more accurate prevalence figure would seem ideal. However, registration of missing teeth still does not clearly discriminate between teeth missing from agenesis and teeth lost after eruption.

Before the 1998 consensus conference on ED, a questionnaire on known cases of hypohidrotic ED in the Nordic countries identified 179 individuals, of which 125 were male, 54 were female, and 59 were from Sweden¹⁶⁰. The mean number of missing teeth was 15.3 – ranging from no missing teeth to anodontia – and the maxillary central incisors, the first molars, and the canines of both jaws were the most stable teeth. In the present study sample, no individual had

hypohidrotic ED and only one individual had ED – a girl with IP diagnosed shortly after birth. Today nine children born 1995–2009, the 14 years following the inclusion period for (I) in the same counties of south-east Sweden, have been diagnosed with hypohidrotic ED. To more accurately establish the population prevalence of this diagnosis, a broader population base or a longer period of inclusion would be needed.

Half of the individuals had one to four signs or symptoms from ectodermal structures beside oligodontia. The most common sign was low salivary secretion, while only 11% reported abnormal hair, nails or sweat glands. Cut-off values for hyposalivation are much lower than what is considered normal salivary secretion, and a value less than 0.1 ml/min for unstimulated whole saliva is rare unless there is massive salivary gland impairment¹⁶¹. Girls are known to generally have lower stimulated secretion than boys, and Bardow et al. (2004) recommends a lower cut-off for females at 0.5 ml/min or less for paraffin-stimulated salivary secretion¹⁶². For schoolchildren, stimulated values less than 0.5 ml/min should be considered low with regard to caries risk¹⁶³. The majority in the present material were 10 years or older, and the analysis used the same cut-off values that define hyposalivation in Sjögren's syndrome¹³⁷. Hyposalivation frequency, however, may be slightly overestimated. If a cut-off of 0.5 ml/min or less for stimulated salivary secretion had been used, another 6 females and one male would have been coded as having normal salivary secretion. This equals 24.1% with hyposalivation compared to 30.2% when 0.7 ml/min or less is used as the cut-off. But regardless of cut-off, the recommendation to assess salivary secretion in individuals with oligodontia remains unchanged.

Despite varying methods of assessing ectodermal symptoms, the finding that only 11% reported symptoms from hair, nails, and sweat glands is a much lower figure than has been presented in clinic-referred samples of individuals with oligodontia^{18, 19}. Interestingly, none of the individuals who reported these symptoms had any of the tested mutations for oligodontia or hypohidrotic ED.

The genetic heterogeneity was broad and bore no evidence of genotype-phenotype correlations. *EDARADD* is one of the genes known to cause hypohidrotic ED, and the finding in (II) now adds another member of the EDA signalling pathway, *EDARADD*, to the list of candidate genes behind isolated oligodontia. However, most studies do not describe in detail how disturbances in ectodermal structures were evaluated. The Lind et al. study in Sweden found one or more missing mandibular incisors in individuals with *EDAR*-induced hypohidrotic ED⁷⁷. We found one or two missing mandibular incisors in 6 individuals with mutations in *AXIN2*, *MSX1*, and *PAX9*, which indicates the dif-

difficulties that are encountered in the clinic to distinguish between oligodontia/I and hypohidrotic ED. The EDA-EDAR-EDARADD axis of mutations in ligand, receptor, and adaptor protein is a highly conserved signalling pathway from fish to man, causing the same phenotypic disease in ectodermal appendage development⁴¹. The new findings that all three genes may cause isolated oligodontia show that the clinical expression in individuals with mutations in *EDA*, *EDAR*, and *EDARADD* can vary much more than has been anticipated. Epigenetic factors may cause the variation in dental phenotype^{153, 164, 165} as may differences in the degree of penetration of the mutation¹⁶⁶. A study in eight patients with severe oligodontia (mean 12.5 permanent teeth missing) found no mutation in *AXIN2*, *MSX1*, and *PAX9*, which supports genetic heterogeneity and the involvement in tooth morphogenesis of as yet unknown genes¹⁵². This was confirmed in a study of three sisters with severe isolated oligodontia, which found no mutation in the same three genes¹⁵³.

One individual in (II) had a mutation in *AXIN2* that was identical to the one that Lammi et al. found to be associated with colorectal cancer²⁹. Eleven individuals in the Finnish family lacked at least eight teeth, out of which two had but three teeth, while the individual in (II) with the *AXIN2* mutation lacked eight teeth. To help identify families at risk for cancer, it has been discussed whether dentists should ask patients with oligodontia if the family has a history of cancer^{29, 31}. Lejeune et al. (2006) screened 39 unrelated patients with multiple adenomas or colorectal cancer for mutations in *AXIN2* and in one patient two novel variants in *AXIN2* but no clearly pathogenic mutation was found¹⁶⁷. Thus, mutations in *AXIN2* were rare in this cohort of individuals.

Two US studies on individuals with oral clefts found a trend for an association between *AXIN2* and incomplete cleft palate and also a slightly higher susceptibility to colorectal cancer^{168, 169}. The authors recommended further studies in other geographic and ethnic populations to confirm a correlation between *AXIN2* mutations and colorectal cancer. However, Bille et al.'s population-based Danish study (2005) encompassed over 8,000 cases with oral cleft lip and/or cleft palate and found no evidence for higher overall cancer risk in individuals born with oral clefts¹⁷⁰. As long as evidence for the mutation's prevalence is limited – in our study it was barely 1% in individuals with oligodontia – families have no clear scientific basis for worry. This question has difficult ethical implications, but further evidence is needed before dentists can be advised to change their clinical routine and ask young individuals with oligodontia about cancer in their family.

In (II)'s study group no difference in numbers of missing teeth between individuals with and without mutations was found. Also, the number of individuals with mutations in the same gene was too small to identify patterns of missing teeth. Even though some individuals with identified mutations lacked the teeth that are typically missing in a certain mutation, their pattern of missing teeth could not clearly discriminate them from the other individuals in the mutant or the non-mutant group.

Dentists are ideally placed to identify female carriers for x-linked hypohidrotic ED. As a group they have dental aberrations that differ with statistical significance from unaffected individuals^{68, 72}. But there is a covariance between aberrant tooth form and smaller tooth size in hypodontia in general^{11, 12, 17}, and the chances of identifying female carriers among individuals with hypodontia seem limited.

Orofacial function was found to be compromised in individuals with ED (III), and orofacial skills – especially speech – may be optimised through treatment and training, and chewing capacity through high quality oral habilitation. However, no background information was obtained about type and severity of symptoms in earlier stages of life, performed treatment and training, or dental status before oral rehabilitation, so no generalised conclusions about treatment needs can be drawn from the results. Many individuals with dryness of the mouth would benefit from information on strategies to enhance swallowing and improve oral comfort. The study was an on-the-spot account, which strongly indicates a need for further analyses of orofacial function in individuals with EDs, outcomes of orofacial skills training, and outcomes of oral habilitation.

Very young individuals with hypohidrotic ED and mandibular anodontia have undergone dental implant treatment⁹². Many case reports have described successful outcomes, but a few studies of larger samples have reported higher failure rates than in unaffected individuals^{122, 123}. Stanford et al. (2008) surveyed self-reported implant treatment outcome in individuals with EDs and found higher levels of complications and implant loss than in unaffected populations¹⁷¹. Of the 109 respondents, 50% reported an implant or a prosthetic complication and 24% reported some form of implant therapy failure. All four children in study (IV) with failed implants were successfully re-operated, which supports the idea that implant failures in young Swedish children with hypohidrotic ED may possibly be attributed to surgical difficulties because of the small size of the jaws and perioperative conditions due to “hard bone”, indicating altered bone density.

A picture of higher implant failure risk in individuals with hypohidrotic ED is emerging, and because several reports^{122, 123, 172} discovered early failures before second-stage surgery, the process of osseointegration seems to be compromised. The *EDA* gene is expressed by osteoblast secretion during skeletal development and, thus, may affect bone formation¹⁷³. EDs are not pure “one-layer diseases”³³, so disturbed ectodermal-mesenchymal signalling also affects bone tissue, which may help explain higher rates of complications and failed implants in ED.

Lesot et al. (2009) reported structural and morphological changes in jaw bone of French individuals with x-linked hypohidrotic ED described as trabecular bone hyperdensity and cortical thickening¹⁷⁴. Bone structure alterations were also found in areas without tooth interference, and the researchers suggested a direct effect of the mutation on bone formation and/or remodelling, while interpreting morphological differences in cortical bone thickness as consequences of oligodontia or anodontia.

A genetically induced difference in the bone of individuals with hypohidrotic ED refers to the fact that EDARADD is a downstream effector in EDAR signalling. This strongly supports that impairment in NF- κ B signalling is involved in EDA pathogenesis¹⁷⁵. NF- κ B is a transcription factor that is necessary for intracellular differentiation of osteoclasts and activation of bone resorption¹⁷⁶. Molecular alterations of the NF- κ B pathway are associated with metabolic and structural bone defects in syndromic ED with osteopetrosis, linked to mutations in the *NEMO* gene¹⁷⁷.

Recent studies of arthropathies and osteoporosis have further disclosed the role of the RANK (Receptor Activator of NF- κ B) signalling pathway in the osteoclast^{178, 179}. The *NEMO* gene is further downstream to NF- κ B than *EDA*, so osseointegration in IP, caused by mutations in *NEMO*, may also be compromised. No studies on dental implant treatment in individuals with IP have as yet been published.

Clinical implications

Challenges and strategies

To optimise treatment planning in individuals with oligodontia, early diagnosis is crucial, and the findings of this thesis support a recommendation for dental caregivers to check for missing incisors, which will identify 65% of individuals with oligodontia. The diagnosis could thus be set at around 8 years of age in most cases. Current recommendations to dentists to survey ectodermal symptoms in individuals with oligodontia seem most relevant for salivary secretion, since (I) confirms that many individuals with oligodontia have hyposalivation.

An early diagnosis makes it possible to use growth adapted measures, such as strategic extractions of primary teeth, in treatment planning. Tooth replacement during the child's growing years can be done with less invasive procedures like composite-retained FDPs, preferably in the frontal area, or removable dental prostheses^{94, 97, 100, 105, 180}. Prosthetic intervention with conventional FDPs can then be postponed, which is desirable as the failure rate is higher in young individuals^{181, 182}.

Better clinical diagnostics would more accurately discriminate between oligodontia that occurs in isolation or in conjunction with ED. Because of the small number of individuals with identified mutations in this thesis, and the fact that no individuals with hypohidrotic ED were identified, patterns of typically missing teeth indicative of specific gene mutations were impossible to detect. Instead, an overlap concerning type of missing teeth was observed in individuals with mutations, which indicates that in the individual, conclusions on which gene is mutated do not seem possible.

Since most centres and clinics only see a few, if any, patients with a certain rare diagnosis, multi-centre co-operation among centres of specialists is one way of increasing knowledge. Therapy planning in multi-disciplinary teams of dental specialists has many advantages: continuous information and support to the family, a comprehensive view of oral habilitation, continuity and co-ordination of planning, shared responsibility for therapy decisions, and optimal utilisation of competence, which all add to the group's experience¹⁰³. Typically, a team comprising specialists in paediatric dentistry, orthodontics, oral surgery, and prosthetic dentistry has been advocated.

Six dental resource centres for rare disorders have been established in Scandinavian countries: one in Norway, the TAKO centre, which was established first; three in Sweden; and two in Denmark. Co-operation between the centres and international research projects continually increase knowledge of diagnostics and treatment of oral signs and symptoms in rare disorders. An example of the impact of international networking was a study among orthodontists that aimed to establish the most favourable outcomes in surgical treatment of cleft lip and palate. The study concluded that “standardization, specialization and the participation of high volume operators were associated with good outcomes, and non-standardization and the participation of low volume operators with poor outcomes”¹⁸³. A proposal for a council recommendation on a European action in the field of rare diseases (adopted in February, 2009) addresses the question of designating national and regional centres of expertise within the EU¹⁸⁴.

Education on diagnostics and treatment of rare disorders is important to broaden specific knowledge in the various health disciplines. In 2002, the first international conference on ED was held in Malmö, Sweden. Dental specialists and representatives of support groups from many countries participated. Since then, three more conferences have been held: ECT04 in London, 2004; ED06 in Copenhagen, 2006; and ED09 in Nijmegen, 2009. Conference statements, including core care standards and guidelines for the development of care pathways, were proposed at the 2004 conference¹⁸⁵. In addition, online databases, as Rarelink¹⁸⁶ and Orphanet¹⁸⁷, with up-dated and thoroughly reviewed information on rare disorders currently provide information to health professionals and affected individuals.

The chance that individuals with a rare disorder will meet a health professional with experience of their diagnosis is low. In recent years, patient organizations or support groups have been established in many countries to provide individuals and families with the same diagnosis the opportunity to meet. Family meetings in such organisations provide unique opportunities to share experiences on symptoms, treatment, and strategies for mastering everyday life. The NFED have more than a thousand families in their member records and is the largest ED support group in the world⁴⁵. In 2000, the NFED family conference was held for the first time outside of the US, in Leicester, UK. Many countries in Europe, including Sweden, have founded support groups in the last 10 years, and international co-operations have been initiated.

Future perspectives

A revised classification of EDs was discussed at a 2008 conference in Charleston, SC, USA³². The purpose of the revision would be to integrate clinical and molecular knowledge. Despite no general consensus, the issue of the *American Journal of Medical Genetics*, September 2009, devoted to the state of the art in EDs, serve as a new platform for future research^{188, 189}. The preceding classification focused on structural dimensions in EDs, but a broader focus that includes characteristics of functioning in individuals with ED was also suggested¹⁹⁰. Oral and dental signs and symptoms are important aspects of the clinical diagnosis and require continuing close co-operation between medical and dental specialists.

Assessment of the prevalence of ED in Sweden could serve as a basis for further studies on oral signs and symptoms, as well as genetics. There are no existing registers, and no natural way of reporting, since most individuals with ED do not make regular visits to medical experts and most young individuals are not known to habilitation centres. In an effort to establish the prevalence of x-linked hypohidrotic ED in Sweden, a questionnaire to dental specialists would probably generate good information on affected males from around 2 years of age. Since newborn children are usually not diagnosed until the first tooth erupts⁵⁸, the youngest children would not be included, but this first report could serve as a starting point, with prospective inclusion of newly diagnosed individuals.

A computerised registry would open up possibilities to prospectively monitor outcomes of treatment as well as risks and side-effects. Success stories in other fields of medicine have shown databases to be important ways of improving treatment outcomes. One example is the Scandinavian database for childhood cancer, which has dramatically improved survival¹⁹¹. International co-operation would increase sample size, thus making studies with higher levels of evidence possible. An international multi-centre project on diagnostics and treatment of individuals with ED and severe hypodontia – led by Iven Klineberg in Sydney, Australia and begun in 2007 – used the Delphi method¹⁹² to reach consensus. A prospective clinical trial on dental implant treatment is in the planning stage. The NFED will launch an international ED patient registry in March 2010 in an effort to better characterise EDs, assist the development of care standards, and facilitate the planning of clinical trials¹⁹³.

A goal to improve therapeutic approaches in ED was presented at the 1998 consensus conference on ED¹⁹⁴. Functional knowledge on specific genes and

gene products allows the design of therapeutics, and the first diagnosis where this occurred was x-linked hypohidrotic ED. The *EDA* gene codes for the protein ectodysplasin, and a possible cure for the symptoms of the disorder was first demonstrated in Tabby mice, the transgenic mouse model for x-linked hypohidrotic ED^{195, 196}. Pregnant Tabby mice were injected with recombinant EDA, and the Tabby phenotype was permanently rescued in the offspring. Later, dogs with a similar phenotype were treated postnatally, and their symptoms in teeth as well as sweat glands decreased¹⁹⁷. These advances in research have raised hope in affected families, and in the US, a human clinical trial is planned for start in 2011¹⁹³. Many steps remain, and until we know how these achievements can be implemented in clinical practice, improving diagnostics to identify female carriers of the disorder remains an important task. Only the future can tell how these new possibilities will be addressed in our society from ethical, cultural, and individual standpoints.

A classic paper first published in 1996 stated that “good care is about integrating individual clinical expertise and the best external evidence”¹⁹⁸. Translational research, which aims to promote the transfer of findings between basic research and clinical practice, attempts to understand the discrepancies between what is known and what is done¹⁹⁹. This new field of research also promises quality improvement in the care of individuals with rare disorders. Meanwhile, to improve the way individuals with oligodontia and ED are approached and met in dentistry, development of standards and evaluation of outcomes to establish the *best possible treatment* remain a challenge.

Main findings

- The combined prevalence of oligodontia in three counties in south-east Sweden was 0.090%.
- One in three individuals with oligodontia had low salivary secretion.
- One in ten individuals with oligodontia had self-reported symptoms from the hair, nails, or sweat glands.
- Fifteen per cent of probands with oligodontia had a mutation in the *AXIN2*, *MSX1*, *PAX9*, or *EDARADD* genes.
- *EDARADD* mutations were shown for the first time to cause isolated oligodontia.
- None of the individuals with mutations had self-reported symptoms from the hair, nails, or sweat glands.
- Individuals with ED scored significantly higher in orofacial dysfunction than healthy controls on the NOT-S.
- Orofacial dysfunction was most frequent in the Chewing and swallowing, Dryness of the mouth, and Speech domains.
- Half of the individuals with ED had a lisp and one-third of the individuals with ED had a hoarse voice.
- Dental implants were rarely placed in individuals younger than 16 years.
- Young children with hypohidrotic ED had higher rates of failed implants than other children.
- Young children with ED and failed implants were successfully re-operated.

Conclusions

- A recommendation to check if one or more permanent incisors are missing will identify 65% of individuals with oligodontia and 84% of individuals missing nine teeth or more.
- Measurements of salivary secretion are indicated in children with oligodontia.
- A majority of individuals with oligodontia did not report other abnormal ectodermal organ function besides teeth.
- Registration of tooth agenesis in computerised patient records would generate more reliable cohorts of individuals with oligodontia for future research.
- More, as yet unidentified, genes are involved in tooth morphogenesis.
- *EDARADD* mutations are associated with isolated oligodontia.
- No clinical indicators were found that discriminated between individuals with and without mutations.
- Evaluation of orofacial function is indicated in individuals with ED.
- Many individuals with ED would benefit from orofacial skills training.
- The failure rate of dental implants placed in young individuals with hypohidrotic ED seems to be related to small bone volume and altered bone density.
- Recent publications suggest a direct effect of the mutation in x-linked hypohidrotic ED on bone formation and remodelling, which seems to compromise osseointegration.
- Central databases on signs and symptoms in individuals with rare disorders would help establish prevalences of the various diagnoses and define treatment needs.
- Quality registers for monitoring outcomes of dental implant treatment would promote early detection of risks and side-effects in individuals with rare disorders.

Populärvetenskaplig sammanfattning

Att sakna enstaka tandanlag är relativt vanligt i befolkningen, medan oligodonti, definierat som medfödd avsaknad av sex eller fler tänder förutom visdomständerna, förekommer hos en till två per tusen individer. Avsaknad av tandanlag är också ett av flera kliniska tecken vid en grupp av ovanliga ärftliga tillstånd som kallas ektodermala dysplasier (ED). Vid ED förekommer tecken och symptom från hår, tänder, naglar och svettkörtlar, samt flera andra strukturer, bland annat körtlar i huden och i luftvägarnas och magtarmkanalens slemhinnor.

Den övergripande målsättningen i avhandlingen var att öka kunskapen om tecken, symptom, genetik och utfall av behandling med tandimplantat hos individer med oligodonti eller ED.

I delarbete I undersöktes hur ofta ektodermala symptom förekommer hos personer med oligodonti. Studiepopulationen utgjordes av patienter med oligodonti födda 1981–1994, som rapporterades via folktandvården i tre län i sydöstra Sverige. Sammanlagt 162 barn och ungdomar identifierades, varav 123 deltog i en klinisk undersökning och en strukturerad intervju om symptom från bland annat hår, naglar och svettkörtlar. De saknade mellan 6 och 20 permanenta tänder. Nio barn hade ett redan känt ärftligt syndrom, vanligast var Downs syndrom. Bland individer som inte hade ett syndrom svarade endast 11% att de hade avvikande hår, naglar eller svettförmåga. Låga salivvärden konstaterades hos 30%.

Den genetiska orsaken till oligodonti studerades i delarbete II genom en genetisk screening av sex gener hos 93 av de tidigare undersökta barnen med oligodonti. Mutationer påvisades hos 14 individer (15%) i fyra av de testade generna, *AXIN2*, *MSX1*, *PAX9* och *EDARADD*. Mutationer i de tre första av dessa är kända som orsak till oligodonti, medan mutationer i *EDARADD* tidigare endast var kända för att orsaka hypohidrotisk ED, som är den vanligaste formen av ED. De påvisade mutationerna förklarar således endast en liten del av den genetiska bakgrunden till avsaknad av tandanlag.

För att utvärdera orofacial funktion genomfördes i delarbete III en studie av 46 personer med ED i åldrarna 3–55 år i USA och Sverige. Ett sammanfattande värde på orofacial dysfunktion var nära 10 gånger högre hos dem med ED

jämfört med friska kontrollpersoner i motsvarande ålder. Många personer med ED hade problem med tuggning och sväljning, muntorrhet och tal. Resultaten indikerar ett behov av behandling och träning av orofaciala funktioner, samt av oral habilitering.

Några yngre svenska barn med hypohidrotisk ED har behandlats med tandimplantat i underkäken på grund av total avsaknad av tänder. Via deras familjer och Svenska ED-föreningen rapporterades att några barn förlorat implantat kort tid efter att de opererats in. Därför inventerades i delarbete IV barn som fått implantatbehandling upp till 16 års ålder i Sverige genom en enkät till specialistkliniker. Totalt hade 26 barn behandlats under en period av 20 år. Hos fyra av fem barn med hypohidrotisk ED, som opererats vid 5-12 års ålder, hade 64% av de insatta implantaten förlorats kort tid efter att de installerats jämfört med 6% av implantaten hos övriga barn. Orsaken till implantatförlusterna i ED-gruppen tolkades vara liten benvolym och ovanligt hårt ben. Nyligen har en direkt genetisk effekt på benvävnad beskrivits vid hypohidrotisk ED, vilket också kan bidra till att förklara en ökad risk för tidiga implantatförluster.

Databaser där tecken och symptom hos personer med ovanliga diagnoser registreras skulle öka kunskapen om förekomsten i befolkningen, samt om vårdbehov vid olika diagnoser. Likaså skulle kvalitetsregister för uppföljning av behandling med tandimplantat öka möjligheterna att tidigt upptäcka risker vid behandling av personer med ovanliga diagnoser.

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All web-page references were retrieved January 4, 2010.

Appendix

Excerpt from the structured interview form on heredity and ectodermal symptoms. Questions used as criteria for ectodermal symptoms are marked in bold type.

Heredity

Is there anyone more in your family who is missing some teeth? Yes No

Do you have a congenital disease or syndrome? Yes No

Skin

Do you consider your skin to be dry? Yes No

How often do you rub your skin with lotion? Yes No

Did you have atopic dermatitis as a child? Yes No

Do you have eczema now? Yes No

Sweating

Do you sweat normally? Yes No

Did you have febrile convulsions as a child? Yes No

Can you sit in the sun as long as your mates? Yes No

Can you participate in physical exercise lessons in school? Yes No

Nails

Are your nails normal? Yes No

Hair

Is your hair normal? Yes No