THE UNIVERSITY OF SYDNEY

COPYRIGHT AND USE OF THIS THESIS

This thesis must be used in accordance with the provisions of the Copyright Act 1968.

Reproduction of material protected by copyright may be an infringement of copyright and copyright owners may be entitled to take legal action against persons who infringe their copyright.

Section 51 (2) of the Copyright Act permits an authorized officer of a university library or archives to provide a copy (by communication or otherwise) of an unpublished thesis kept in the library or archives, to a person who satisfies the authorized officer that he or she requires the reproduction for the purposes of research or study.

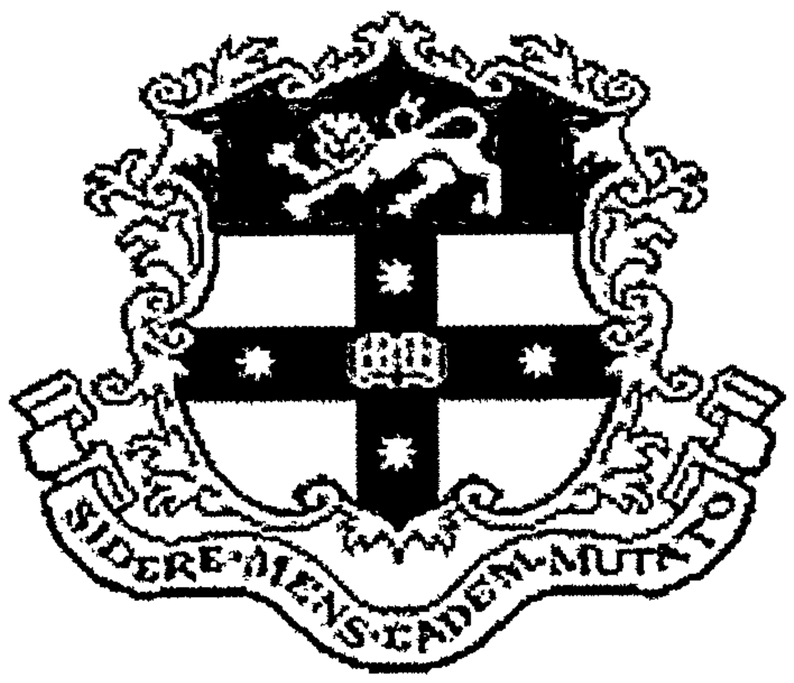
The Copyright Act grants the creator of a work a number of moral rights, specifically the right of attribution, the right against false attribution and the right of integrity.

You may infringe the author's moral rights if you:

- fail to acknowledge the author of this thesis if you quote sections from the work
- attribute this thesis to another author
- subject this thesis to derogatory treatment which may prejudice the author's reputation

For further information contact the University's Copyright Service.

sydney.edu.au/copyright



A Clinical and Molecular Genetic Study of Oligodontia in Three Australian Families

Michael Malandris

BDS (Adelaide)

Thesis submitted in partial fulfilment
of the requirements for the degree of
Master of Dental Science (Paediatric Dentistry)

Discipline of Paediatric Dentistry
Faculty of Dentistry
University of Sydney
Australia

ACKNOWLEDGEMENTS

Writing this thesis has been an amazing journey for me that would not have been possible without the contributions provided by many people. In particular I would like to extend my greatest thanks to my chief supervisors. Associate Professor Richard Widmer, a mentor and great role model for all Paediatric Dentists you have taught, I am indebted to you for bringing together this eminent group of supervisors and for helping to fuel my fascination in this new and exciting area of research.

Thank you also Associate Professor John Christodoulou for your tremendous positive energy, your clarity of thought, your guidance and your incredible efficiency in helping me bring this work to fruition. To Dr Meredith Wilson, thank you for instilling me with passion and providing me with much needed guidance in the exciting field of Clinical Genetics. Thank you also Dr Bruce Bennetts for tirelessly providing me with so much knowledge in the field of Molecular Genetics. You too have been an inspiration.

To my assistant supervisor Associate Professor Angus Cameron I wish to thank you for assisting me in ascertaining subjects for this study and for sharing with me some of your wealth of knowledge and clinical experience in the field of Paediatric Dentistry. You have been an incredible teacher and you have introduced me to many of the greatest leaders in this area of research.

Many thanks also to Dr Maureen Rogers for your wonderful insights into hair anomalies and for kindly providing me with exquisite photographic examples and conducting the hair sample analyses. Thank you also to Associate Professor Jenny Donald and Dr Zhixin Liu for putting everything on hold to help me crunch those numbers! I appreciate the enormous effort that was required. To Professor Roger Hall, I thank you for passing on some of your incredible knowledge in Paediatric Dentistry and for kindly providing me with illustrations on dental anomalies.

Thank you to Dr Sam Gue for initially encouraging me to take the first tentative steps into pursuing what I always knew I wanted to do and for sticking by me for the years that have passed since and the years to come!

To Dr Soni Stephen, Dr Erin Mahoney, Dr Sally Hibbert and Dr Philippa Sawyer, I thank you for your support and encouragement throughout these last three years. You have all contributed to what I know and appreciate about Paediatric Dentistry.

Thank you Heidi Marr, Vicki Tadic, Craig Kennedy, Gemma Jenkins and Julianne Jackson for your much needed assistance in conducting the clinical and laboratory work for this research. I am also grateful to Mr Stephen Haynes, professional director of GC Australia, for kindly providing me with the saliva test kits for use on all participating subjects.

I also wish to thank my fellow registrars Dr's Juliette Scott, Fiona Bell and John Camacho who have had to put up with me over the last few years and for sharing with me the challenges of postgraduate student life! Thank you also to the consultants, senior registrars and visiting specialists I have had the pleasure of working alongside, including Dr's Simrit Malhi, Sherene Alexander, Neeta Prabhu, David Finucane, Eduardo Alcaino and Peter Wong.

Finally, I wish to dedicate this thesis to those closest to my heart who have been by my side throughout my life and my career. To Mum, Dad and Vassil, I know it's been difficult to see so little of me over the last few years and I am grateful that you have been so understanding. Most of all, to my wife Manjiri and my two wonderful children Sophie and Eleni, you are the reason I have come so far and the reason I keep striving for the best. Manjiri, thank you for all the love and support you have provided me with and for always having faith in me.

ABSTRACT

Tooth agenesis is one of the most common human polymorphisms, with an estimated prevalence of approximately 20%. Oligodontia is generally considered to be a rare, severe form of tooth agenesis that may occur in isolation but can also be an important clinical feature of a large group of syndromes and in particular the ectodermal dysplasias. The implications for patients of inheriting an isolated dental condition as opposed to a syndrome or dysplasia are immense. It is therefore of great clinical interest to be able to differentiate isolated (non-syndromic) oligodontia from that which is associated with a syndrome or dysplasia. In addition, enormous advances have occurred in recent years in determining the mechanisms involved in odontogenesis at a molecular genetic level by studying which genes are affected when tooth agenesis manifests.

The objectives of this research were to characterise the clinical features (both dental and non-dental) of families presenting with autosomal dominant oligodontia and then explore the molecular genetic basis of the oligodontia conditions in these families.

The clinical component of the study was achieved by ascertaining subjects from a clinical database held at the Department of Paediatric Dentistry, Westmead Centre for Oral Health and then conducting a comprehensive clinical examination on all affected and unaffected family members who agreed to participate. Clinical characterisation of all subjects was based on data obtained from a full medical and dental history, a physical examination by a clinical geneticist, a dental examination as well as any necessary special investigations by a paediatric dentist and an analysis of a sample of hair under light microscopy by a paediatric dermatologist.

The molecular genetic component of the study was achieved by extracting DNA from peripheral blood lymphocytes provided by each patient via venipuncture, amplifying PCR fragments of this DNA at specific loci using microsatellite markers and then undertaking a linkage analysis to these loci pre-selected because of their proximity to potential candidate genes.

The results of the clinical study were that two pedigrees were diagnosed with non-syndromic oligodontia (NSO) and one was diagnosed with autosomal dominant hypohidrotic ectodermal dysplasia (HED). Differentiating all affected from unaffected individuals was problematic for all three pedigrees, as some individuals presented with agenesis of one or two teeth (excluding third molars) and had other associated mild clinical features while others had severe oligodontia and more definitive associated clinical features. Diagnostic criteria were required in order to differentiate affected from equivocal and unaffected individuals prior to molecular genetic assessment.

In those individuals regarded as definitely affected, an average of 10 teeth were congenitally missing in the NSO pedigrees (range 5-20) and an average of 11 teeth (range 10-12) were congenitally missing in the autosomal dominant HED pedigree. The only associated clinical features for the NSO pedigrees were microdontia and variable absence of distopalatal cusps on upper first permanent molars (U6's) and distobuccal cusps on lower first permanent molars. For the affected members of the autosomal dominant HED pedigrees, the associated clinical features were moderate to severe hypohidrosis, moderate to severe dysphagia and choking, variable but mild hair, skin and fingernail abnormalities as well as absence of distopalatal cusp on U6's, variable microdontia, cervical constriction, tapered incisors and prominent/conical buccal premolar cusp form.

The results of the molecular genetic study were that linkage to the known NSO candidate genes *MSX1* and *PAX9* was excluded for one pedigree and linkage to the only known autosomal dominant HED candidate gene, *EDAR*, was excluded for the HED pedigree. The second NSO pedigree was too small and uninformative to confirm or exclude linkage to known NSO candidate genes using microsatellite markers. Sufficient power was predicted in the autosomal dominant HED pedigree to conduct a genome-wide linkage study, with microsatellite markers spread on average 10cM apart, in an attempt to identify other loci that segregated with disease. Linkage analysis provided evidence of linkage to the D17S784 marker at locus 17q25.3, with a maximum logarithm of odds for linkage (LOD) score of 3.32 at a recombination fraction (θ) of 0. Further investigations are underway to determine whether a major susceptibility locus for autosomal dominant HED exists on chromosome 17.

In summary, clinical characterisation of oligodontia conditions depends on careful consideration of the pattern of oligodontia and other associated dental anomalies as well as a multitude of other possible non-dental features observed. A multidisciplinary approach to such an investigation is considered ideal. Recently, the ability to reach an accurate diagnosis is becoming more reliant on molecular genetic techniques as many of the causative gene mutations are being identified and an efficient way of mapping disease to these genes has developed. The research conducted here showed that not all cases of NSO and autosomal dominantly inherited HED are a result of mutation in the most commonly reported genes. A susceptibility locus for autosomal dominant HED may have been identified on chromosome 17.

TABLE OF CONTENTS

Acknowledgements ii

Abstract iv

Table of Contents vii

List of Tables xi

List of Figures xii

List of Abbreviations xiii

Statement of Authorship xvi

Presentations/ Abstracts xvii

1. LITERATURE REVIEW 1

1.1 Introduction 1

1.2 Clinical Background 4

1.2.1 Definition and Description of Oligodontia 4

1.2.2 Nosology, Malformations and Dysplasias 6

1.2.3 Epidemiology of Oligodontia 11

1.2.4 Diagnosis of Oligodontia 12

1.2.4.1 The Dentition 14

1.2.4.2 Craniofacial and Other Features 18

1.2.5 Comorbidities 19

1.2.5.1 Psychosocial Impact 20

1.2.5.2 Speech and Mastication 21

1.2.5.3 Hypohidrotic Ectodermal Dysplasia 23

1.2.6 Management of Oligodontia 35

1.3 Embryology and Genetic Background of Odontogenesis 37

1.3.1 General and Craniofacial Embryology 37

1.3.2 Odontogenesis Revisited 42

1.3.3 Dental Patterning 43

1.3.4 The Genetic Basis of Odontogenesis and Dental Patterning 46

1.4 Molecular Genetic Research 54

1.4.1 Introduction to Molecular Genetics 54

1.4.2 Molecular Genetic Laboratory Techniques 57

1.4.2.1 DNA Markers: Microsatellites and Single Nucleotide Polymorphisms 57

1.4.2.2 Polymerase Chain Reaction (PCR) Technique 59

1.4.2.3 Gel Electrophoresis 59

1.4.3 Data Analysis 60

1.4.3.1 Linkage by Standard LOD Score Analysis 62

1.4.3.2 Candidate Gene Approach 64

1.5. Genetic Background of Oligodontia 65

1.5.1 Inheritance Patterns 65

1.5.2 Candidate Genes 67

1.5.2.1 Non-Syndromic Oligodontia Candidate Gene Studies 67

1.5.2.2 Autosomal Dominant Ectodermal Dysplasia Genes 68

1.5.2.3 Other Potential Candidate Genes 70

1.6. Research Outline and Objectives 71

1.6.1 Objectives of the Research 71

1.6.2 Research Outline 72

1.6.3 Significance of the Research 73

2.	**CLINICAL STUDY OF OLIGODONTIA**		74
2.1	**Materials and Methods**		74
	2.1.1	Study Design	74
	2.1.2	Subject Ascertainment	74
	2.1.3	Consent	76
	2.1.4	Clinical Genetics Assessment	77
	2.1.5	Dental Assessment	79
	2.1.6	Special Investigations	82
	2.1.7	Blood Sample Collection	85
2.2	**Results**		86
	2.2.1	Three Autosomal Dominant Oligodontia Pedigrees	86
	2.2.2	General Clinical Features of the Oligodontia Pedigrees	87
	2.2.3	Oral Features of the Oligodontia Pedigrees	94
2.3	**Discussion**		99
2.4	**Conclusion**		102
3.	**MOLECULAR GENETIC STUDY OF OLIGODONTIA**		103
3.1	**Materials and Methods**		103
	3.1.1	Study Design	103
	3.1.2	Candidate Gene Selection	104
	3.1.3	Microsatellite Marker Selection	104
	3.1.4	DNA Extraction and Quantitation	105
	3.1.5	PCR Amplification	106
	3.1.6	Agarose Gel Electrophoresis	107
	3.1.7	Gene Scan Analysis of Microsatellite Markers	108

3.1.8 Linkage Analysis of Candidate Gene Markers 110

3.2 Results 111

3.2.1 Uninformative markers 111

3.2.2 Linkage to Non-Syndromic Oligodontia Candidate Genes 111

3.2.3 Linkage to Hypohidrotic Ectodermal Dysplasia Candidate Gene 113

3.2.4 Linkage to Genome-Wide Microsatellite Markers 113

3.3 Discussion 115

3.4 Conclusion 118

4. DISCUSSION AND FUTURE DIRECTION 119

4.1 Summary of Results 119

4.2 Future Directions 124

4.2.1 Further Investigations into Oligodontia 124

4.2.2 Genotype-Phenotype Correlations for a Better Nosology of Oligodontia Conditions 126

4.2.3 Stem Cell Research, Pharmacogenomics and Oligodontia 128

Glossary of Terms 130

References 131

Appendix 142

LIST OF TABLES

Table 1.1 Oligodontia syndromes (excluding ectodermal dysplasia)

Table 1.2 Ectodermal dysplasia syndromes associated with oligodontia

Table 1.3 Tests for Hypohidrosis

Table 1.4 Transcription factor gene mutations linked to diseases associated with numeric anomalies of the dentition

Table 1.5 Transcription factor gene mutations in knockout mice

Table 1.6 Extracellular signalling ligand and receptor gene mutations in humans

Table 1.7 Signalling ligand and receptor gene mutations in knockout mice

Table 1.8 Miscellaneous gene mutations linked to diseases associated with oligodontia

Table 1.9 Intracellular molecule gene mutations in knockout mice

Table 1.10 Reported mutations in non-syndromic oligodontia candidate genes

Table 1.11 Mutations in *EDAR* responsible for autosomal dominant hypohidrotic ectodermal dysplasia

Table 2.1 Special investigations performed

Table 2.2 Clinical features of members of pedigree 1

Table 2.3 Clinical features of members of pedigree 2

Table 2.4 Clinical features of members of pedigree 3

Table 2.5 Pedigree1 dentition, selected members

Table 2.6 Pedigree 2 and 3 dentition, selected members

Table 3.1 Microsatellite markers used for linkage analysis

LIST OF FIGURES

Figure 1.1 Dysplasias, disruptions, deformations and malformations

Figure 1.2 The human dentition

Figure 1.3 Comparative prevalence of tooth agenesis

Figure 1.4 Dental characteristics of ectodermal dysplasia

Figure 1.5 Typical facial features of X-Linked hypohidrotic ectodermal dysplasia in males

Figure 1.6 Photomicrograph of hair shaft displaying pseudomonilethrix.

Figure 1.7 Caudo-cephalic folding of a 28 day human embryo

Figure 1.8 Neural crest cell migration to the developing jaws

Figure 1.9 Fusion of medial nasal and maxillary processes in 7 week old embryo

Figure 1.10 Meiosis and genetic variability

Figure 1.11 Capillary gel electrophoresis

Figure 1.12 Determining linkage

Figure 1.13 Pedigree displaying autosomal dominant inheritance

Figure 2.1 Prefabricated stainless steel crown form

Figure 2.2 Saliva test kit

Figure 2.3 Pedigree 1

Figure 2.4 Pedigree 2

Figure 2.5 Pedigree 3

Figure 2.6 Dentition in autosomal dominant ectodermal dysplasia pedigree member

Figure 3.1 Slab gel electrophoresis

Figure 3.2 ABI 310 genetic analyser

Figure 3.3 Electrophoretogram of pedigree 1

Figure 3.4 *MSX1* and *PAX9* linkage map of pedigree 2

Figure 3.5 *MSX1* and *PAX9* linkage map of pedigree 3

Figure 3.6 *EDAR* linkage map of pedigree 1

Figure 3.7 Chromosome 17 linkage map of pedigree 1

LIST OF ABBREVIATIONS

AD	Autosomal dominant
AGRF	Australian Genome Research Facility
AR	Autosomal recessive
Barx1	BarH-like homeobox gene
BMP	Bone morphogenetic protein, an extracellular signalling molecule
bp	Base pairs
CARD14	Caspase recruitment domain protein molecule
CLP	Cleft lip and/or palate
cM	Centimorgan, a unit of measurement of genetic distance
DDX48	DEAD box protein
Dkk-1	Dickkopf-1
Dlx	Distal-less homeobox gene, a transcription factor gene
dNTP's	deoxyribonucleotides
DNA	Deoxyribonucleic acid
DTDST	Diastrophic dysplasia sulphate transporter
ED	Ectodermal dysplasia
EDA	Ectodysplasin gene
EDAR	Ectodysplasin receptor
EDARADD	EDAR associated death domain
EDTA	Ethylenediaminetetraacetic acid
EEC	Ectrodactyly, ectodermal dysplasia and cleft lip/palate
ELB	Erythrocyte lysis buffer

FGF	Fibroblast growth factor, an extracellular signalling molecule
HED	Hypohidrotic ectodermal dysplasia
Hh	Hedgehog, a group of genes coding for extracellular signalling molecules involved in growth and patterning
HMT	Hot master Taq
HOX	Homeobox
Lef1	Lymphoid enhancer-binding factor 1 gene
Lhx	Lim-homeobox domain gene
LOD	Logarithm-of-odds
M	Molar (a unit of measurement of concentration)
MLE	Maximum likelihood estimate
MSX	Muscle segment homeobox gene
NaCl	Sodium chloride
NEMO	Nuclear factor-kappa-β essential modulator gene
NSO	Non-syndromic oligodontia
OD	Optical density
OMIM	Online Mendelian Inheritance in Man
PAX	Paired box gene
PCR	Polymerase chain reaction
PITX	Pituitary homeobox gene
PRKGIB	Type 1 cGMP-dependent protein kinase
PVRL1	Cell adhesion molecule
RNA	Ribonucleic acid
RUNX2	Runt-related transcription factor 2 gene

rpm	Revolutions per minute
SDS	Sodium dodecyl sulphate
SHH	Sonic hedgehog, a gene that codes for an extracellular signalling molecule
SNP	Single nucleotide polymorphisms
STR	Single tandem repeats
TE buffer	Tris-EDTA
TGFβ	Transforming growth factor beta, an extracellular signalling molecule
TNF	Tumour necrosis factor, an extracellular signalling molecule
TNFR	TNF receptor
Traf	TNFR associated intracellular signalling molecule
UV	Ultra-violet
WBC	White blood cell
WFI	Water for injection
Wnt	Wingless-type MMTV integration site family of genes
XD	X-linked dominant
XEDAR	X-linked EDAR
XR	X-linked recessive

STATEMENT OF AUTHORSHIP

Except where reference is made in the text, this thesis contains no material which is published in any other thesis, or extracted in whole or in part from any other thesis. The material presented has not previously been submitted for a degree or diploma in any university, and to the best of my knowledge contains no material previously published or written by another person except where due acknowledgement is made in the main text of the thesis itself.

Michael Malandris

PRESENTATIONS/ ABSTRACTS

2004

Poster: "A Clinical and Genetic Study of Oligodontia in Three Families", presented at Ect04, the International Conference on the Management of Ectodermal Dysplasia and Severe Hypodontia, The Eastman Dental Institute for Oral Health Care Sciences, University College, London, UK

Abstract: "A Clinical and Genetic Study of Oligodontia in Three Australian Families" submitted for publishing in Synopses, the Australian and New Zealand Society of Paediatric Dentistry (ANZSPD) newsletter

Presentation: "A Clinical and Genetic Study of Oligodontia in Three Australian Families", the ANZSPD Colgate Postgraduate Research Award, ANZSPD Federal Convention, Melbourne

2003

Presentation: "A Clinical and Genetic Study of Oligodontia in Three Australian Families", the University of Sydney, Faculty of Dentistry Research Day, Westmead Centre for Oral Health, Sydney

Presentation: "A Clinical and Genetic Study of Oligodontia in Three Australian Families", annual Australasian Academy of Paediatric Dentistry meeting, Sydney

1 LITERATURE REVIEW

1.1 Introduction

> *"I've had dentures since I was five years of age. My dentist had to send for specially made plates for me because most dentists have never made dentures on such a small scale. They were perfect once the ulcers went down; I bet all the kids out there can identify with that. Even at such a young age I filed them down myself to make them more comfortable. As the years went by the dentures got better...I always had the top dentist, they loved the challenge, and I am a regular at the Charles Clifford Training Hospital. Only the best will do!*
>
> *"Due to the pressures of work I had to visit a local dentist...he made me a set of dentures, but they weren't right. I went back to him and complained 3-4 times (until) he sent me to the local hospital dentist. They offered me implants but wanted to charge me £6,000 – I couldn't afford that – they kept saying it was cosmetic. At this time I'd still no idea of what my condition was called. I left the hospital very angry.*
>
> *"I wrote a letter to the Sun newspaper; within a couple of weeks they'd pulled a few strings and got me in at the Charles Clifford Training Hospital...I was to have four titanium implants, which took six to eight hours (of surgery), I stayed in hospital overnight. Remember I had no holes in my gums to implant teeth – this made the operation very difficult and experimental – I agreed to have the whole operation videoed for training purposes. The next day, I looked like I had done twelve rounds with Mohammed Ali! I was really sore, I'd had a mouth full of stitches...*
>
> *"After a few months, a special set of dentures were made...*I AM OVER THE MOON WITH MY NEW DENTURE. *I can eat anything and have the bite of a lion...I am more confident now. I pioneered this very difficult process and hope it will pave the way for others, especially for my family. For us with no teeth, it's a very long process and painful, but the results outweigh that and the pain is soon forgotten. Please do not be put off by my experience. I am unique as are you, and as technology advances the process will become easier."*

Report of the personal difficulties encountered by an individual affected with an oligodontia-associated ectodermal dysplasia, as quoted from EDlines, the British Ectodermal Dysplasia Society Newsletter, Volume 4 Issue 2, April 2004

Most dentists and geneticists have an awareness of the clinical significance of oligodontia, a condition characterised by the congenital absence of multiple teeth. Although not life threatening in isolation, oligodontia can have a severe impact on quality of life. Individuals affected by this dental condition are predisposed to childhood taunting, speech difficulties, low self esteem and a lifetime of complicated and potentially uncomfortable dental treatment (Hobkirk et al., 1994; Nunn et al., 2003). Typically,

oligodontia is associated not only with multiple missing teeth but for the teeth that do form, there may be morphological anomalies such as microdontia (teeth rudimentary in size and complexity). As a result, there may be a lack of vertical dimension between upper and lower jaws, leading to overclosure of the jaws, eversion of the lips, compromised aesthetics, malocclusion and difficulty with mastication.

A significant proportion of oligodontia sufferers have associated with their dental condition a range of other clinical manifestations that can significantly impact on their health (Nordgarden et al., 2001). These manifestations, grouped together, often constitute a recognised hereditary dysplasia or syndrome. In particular, the hypohidrotic ectodermal dysplasias (HED's) have oligodontia as a cardinal associated feature and similarly, up to 57% of patients with oligodontia have been reported to have an ectodermal dysplasia (Nordgarden et al., 2001). Due to the relatively ambiguous clinical features of HED in neonates, delayed diagnosis is not unusual. Unfortunately, this has led to a reported mortality rate of 30% during infancy as a result of inappropriately managed HED associated hyperpyrexia, failure to thrive or chronic lung disease (Atherton, 2004; Clarke et al., 1987; Freire-Maia and Pinheiro, 1990).

During the neonatal period, other potential morbidities can also occur in patients with HED as a result of prolonged hypohidrosis-related hyperthermia, including febrile convulsions and brain damage (Söderholm and Kaitila, 1985; Wasserteil, 1986). In later life, these children are also more likely than non-HED oligodontia sufferers to have a poorer quality of life, as a result of their hair, craniofacial and exocrine glandular anomalies, in addition to their dental anomalies (Hummel and Guddack, 1997; Nowak, 1988; Söderholm and Kaitila, 1985; Wasserteil, 1986). The clinical delineation between certain forms of ectodermal dysplasia and non-syndromic forms of oligodontia (those not

associated with any other syndrome or dysplasia), is not always clear, and there are as yet no reports in the literature describing which clinical characteristics are most useful in differentiating these conditions from one another.

Fortuitously for dentistry, the molecular mechanisms necessary for tooth development are rapidly being elucidated by developmental biologists. This is because dental development is a good model for organogenesis in general, with similar or identical embryological and molecular mechanisms observed as those that occur throughout embryogenesis (Thesleff, 2003; Tucker and Sharpe, 1999). Coupled with this is the immense progress that has occurred over the last few decades in developing tools that enable biologists and geneticists to identify the key developmental processes at the molecular level. This has led to an explosion of knowledge in the biology of odontogenesis, to the point that the prospect of constructing teeth in the laboratory from raw materials is now a real possibility (Cobourne, 1999; Thesleff, 2003).

1.2 Clinical Background

1.2.1 DEFINITION AND DESCRIPTION OF OLIGODONTIA

As yet, there is no universally accepted term that is used to describe a congenital variation in number of teeth. The two terms most commonly used in contemporary dental, genetic and developmental biological research are hypodontia and oligodontia. The origin of the term hypodontia is from the Greek, meaning lack of teeth (hypos=lacking, deficient; dontia=teeth), implying a condition in which there may be one or more teeth that fail to develop. Similarly, the term oligodontia means few teeth (oligos=few) implying a condition in which multiple teeth fail to develop. Other terms used to describe a congenital variation in tooth number include aplasia (=absence or defective development of teeth), tooth agenesis (lack of development) or anodontia (=absence of all teeth).

There is also contention as to whether absence of teeth can be considered a congenital abnormality, given that certain teeth normally develop postnatally. Some geneticists argue that agenesis of any organ must be congenital, even if no observation method currently exists, because the pattern of development has already been determined by birth (Freire-Maia and Pinheiro, 1984). Other authors believe that agenesis of a tooth cannot be considered congenital if the initiation of their development is not normally detected until after birth (Cameron and Widmer, 2003).

The term oligodontia was distinguished as a condition of agenesis of six or more permanent teeth, excluding third molars, in a thesis by Schalk-van der Weide in 1992 (Schalk-van der Weide, 1992). Third molars were excluded because their failure to develop is so common, they can be considered a rudimentary tooth from a biological and

evolutionary perspective. Although arbitrary, many authors now base their definition of oligodontia on this publication. The OMIM internet link to the term oligodontia (Online Mendelian Inheritance in Man, 2002) goes on to further differentiate oligodontia (agenesis of six or more teeth, excluding third molars) from hypodontia (agenesis of less than six teeth, excluding third molars). Whether there is a need to distinguish less severe from more severe forms of tooth agenesis by more specifically defining hypodontia and oligodontia is debatable. If an individual fails to develop five or less teeth and is a member of a pedigree in which other relatives display agenesis of six or more teeth, that individual merely expresses a milder variant of the same condition. Identification of pedigrees in which this very clinical finding has occurred has been reported by several authors (Goldenberg et al., 2000; van den Boogaard et al., 2000; Vastardis et al., 1996).

In this study, the term oligodontia will be used exclusively to describe the agenesis of any number of teeth in pedigrees where at least one member has a severe form of the condition (agenesis of six or more teeth, excluding third molars). Using a single term to describe all tooth agenesis patterns has practical advantages. It avoids confusion and allows for comparisons between all individuals. The term oligodontia can be further arbitrarily categorised as mild, moderate or severe according to how many teeth have failed to form, so that the degree to which that individual has been dentally affected can be measured.

1.2.2 NOSOLOGY, MALFORMATIONS AND DYSPLASIAS

A number of genetic diseases and congenital malformation syndromes have an associated oligodontia pattern. To determine which of these conditions should be viewed as a nosological group has far-reaching clinical implications, in terms of diagnosis, patient counselling and clinical management.

The science of the classification of diseases is called nosology. A nosological group contains two or more conditions sharing one or more clinical manifestations. A given disease, dysplasia, anomaly or syndrome may be clinically and causally delineated from others that are similar if the techniques used for phenotypic and genotypic analysis are sensitive enough (Freire-Maia and Pinheiro, 1984). It is important to note that patients with the same condition rarely exhibit identical clinical manifestations of the disease and similarly, two unrelated patients with apparently identical conditions may have a manifestation of two or more different causes (Freire-Maia and Pinheiro, 1984).

All congenital malformations are a result of intrinsic and/or extrinsic causative agents on a developmental field. As discussed in 1.3.1, embryonic development is controlled in a spatially ordered, temporally synchronised and epimorphically hierarchical manner. To put it another way, embryonic development is a sequential developmental progression from a given stage to a more highly complex and mature stage. Epimorphic development gradually limits and reduces the size of the field on which any intrinsic or extrinsic dysmorphogenic cause can act (Freire-Maia and Pinheiro, 1984).

The consequences of epimorphic field development are that:

1. The same causative agent acting at different times on the same given field can produce anomalies differing in type and extent. For example autosomal

dominant holoprosencephaly manifests as cyclopia when acting early, microcephaly with borderline intelligence and unilateral cleft palate at a later stage and normal head size and intelligence but single solitary upper central incisor at an even later stage (Freire-Maia and Pinheiro, 1984; Nanni et al., 2001).

2. Different extrinsic or intrinsic causes acting on the same field may produce the same malformations.
3. More than one field may be affected by a single cause, producing syndromes representing mosaic pleiotropy (many phenotypic effects).

The result of dysmorphogenesis may be a malformation, disruption, minor anomaly, syndrome or dysplasia, dependent on the timing of action and number of causative agents (see fig 1.1). For convenience, these terms are used discontinuously but it is probably more accurate to consider these disorders along a spectrum, as a result of the variability between aetiological agent, pathogenesis and phenotype (Cohen, 1989). A malformation is a morphologic defect of an organ, part of an organ or a larger region resulting from the action of a single cause (genetic or extrinsic) on a single developmental field (primary malformation) or from an extrinsic action on originally normal developmental fields (secondary malformation, or disruption) (Freire-Maia and Pinheiro, 1984). Malformations therefore are the end result of a defect in organogenesis.

A primary malformation may be ascertained at birth (eg. cleft lip, polydactyly), or at a later time (eg. pyloric stenosis). Disruptions (secondary malformations) may be produced by teratogens such as radiation, drugs, metabolic disorders, infection, immunological mechanisms, ischaemia, hyperthermia, mechanical forces, trauma etc. (Freire-Maia and Pinheiro, 1984).

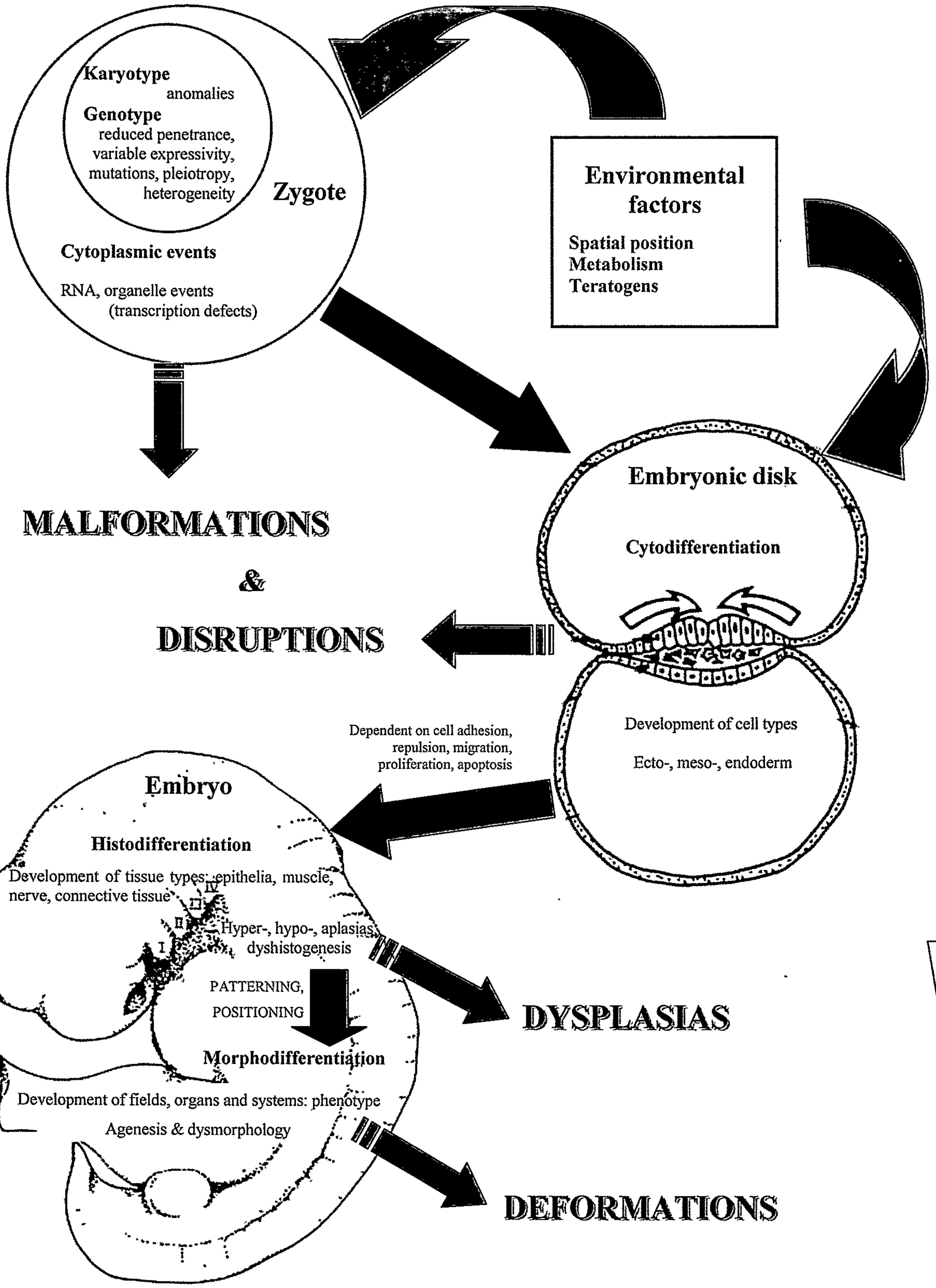


Figure 1.1 General scheme of embryogenesis and the proposed timing of development of various manifestations of dysmorphogenesis. Adapted from Sperber. (Sperber, 1993)

Multiple malformations in an individual may represent:

1. Action of a single cause on a developmental field with multiple locations that may ultimately be widely separated. For example, acrorenal malformation (malformed limbs and kidneys).
2. Action of a single cause on a single developmental field that can trigger a cascade of secondary defects, also known as a sequence. For example, Pierre Robin sequence or holoprosencephaly sequence.
3. Action of a single cause on more than one developmental field resulting in a pattern of malformations. This defines a syndrome.
4. Action of different causes on different developmental fields that have a predisposition to be linked or associated. This is known as an association. For example, VATER association (Vertebral anomalies, Anal atresia, Tracheo-Esophageal fistula, Renal anomalies)
5. Action of different causes on different developmental fields by coincidence, leading to pathogenetically unrelated defects eg. homozygosity for two different autosomal recessive traits. This is also known as a concurrence, random association or syntropy (Freire-Maia and Pinheiro, 1984).

Dysplasia means abnormal growth and is a result of an abnormal organisation of normal cells into tissues. This process and the morphological result are known as dyshistogenesis (dysgenesis means abnormal formation) (Sperber, 1993). In contrast, a primary malformation is a defect in growth, movements of germ layers and differentiation of cells and tissues during development. For example, an ectodermal dysplasia is a result of dyshistogenesis in the ectoderm, one of the three embryonic germ layers that gives rise to the epidermis and epidermal appendages as well as playing a key role in the development

of many other organs, including teeth. Other terms that are synonyms of dysplasia include dysostosis (dysplasia of bone), aplasia (an extreme of dysplasia as a result of agenesis or complete failure to form the tissue or organ), and hypoplasia (a form of dysplasia that results in reduced tissue quantity) (Freire-Maia and Pinheiro, 1984). For example, dental enamel hypoplasia is a form of dysplasia.

In addition to pure dysplasias that are entirely dysplastic in origin, malformation dysplasia syndromes exist that are a constellation of malformations and dysplastic signs originating from the action of a single cause (Freire-Maia and Pinheiro, 1984). An example of a malformation dysplasia syndrome is the EEC syndrome of ectodermal dysplasia, ectrodactyly (congenital absence of all or part of any finger or toe) and cleft lip and/or palate.

Towards the end of morphogenesis malformations can no longer occur but an alteration in morphogenesis can still give rise to a deformation (Sperber, 1993). A deformation is a term used to describe an alteration in morphogenesis that is significantly greater than normal developmental variation. Examples of deformations or minor anomalies include isolated ptosis (usually of the left upper eyelid), short finger, protruding ears, and (often familial) growth retardation (Freire-Maia and Pinheiro, 1984).

1.2.3 EPIDEMIOLOGY OF OLIGODONTIA

Tooth agenesis in some form is a common human anomaly that affects approximately 20% of the population (Online Mendelian Inheritance in Man, 2002) and has been described as one of the most common polymorphisms in man (Nunn et al., 2003). Although tooth agenesis is associated with more than 49 syndromes, several case reports describe nonsyndromic forms that are either sporadic or familial in nature, as reviewed by (Gorlin et al., 2002).

The prevalence of agenesis of one or more teeth in the general population, excluding third molars, is reported to be between 2.3% to 11.7% (O'Dowling and McNamara, 1990; Silverman and Ackerman, 1979), while absence of one or more third molars is seen in 2.5%-35% of examined populations (Burzynski and Escobar, 1983). Because of the extreme variability in and possibly very high prevalence of third molar tooth agenesis, most studies investigating oligodontia do not take this tooth into account.

Recently, a meta-analysis of 33 studies showed that agenesis of one to two teeth (excluding third molars) occurred at a prevalence rate of 4.46%, while agenesis of three to five teeth (excluding third molars) occurred at a prevalence rate of 0.78% (Polder et al., 2004). In contrast, agenesis of six or more teeth (commonly described as non-syndromic oligodontia), is a much rarer condition, with a reported prevalence of 0.08-0.26% (Rolling and Poulsen, 2001). As discussed in 1.2.5, oligodontia is a cardinal feature of the most common form of ectodermal dysplasia, X-linked HED or Christ-Siemens-Touraine syndrome. The reported prevalence of this form of HED is between one and seven per 100,000 population (Buyse, 1990; Clarke et al., 1987).

1.2.4 DIAGNOSIS OF OLIGODONTIA

A comprehensive medical and family history is essential in order to obtain an appropriate diagnosis of oligodontia. The causes of oligodontia may be grouped into two categories: those primarily genetic in origin and those primarily environmental in origin, although some authors also argue that there is an evolutionary trend for agenesis of specific teeth (Weiss et al., 1998). It is important to recognise that there may be multiple aetiologies contributing to a presentation of oligodontia that only a detailed history can identify. It has also been known for some time that precise understanding of many of the aetiological mechanisms is still lacking or uncertain (Burzynski and Escobar, 1983). By far the most commonly reported cause of oligodontia is genetic (either monogenic or polygenic in origin), as is illustrated by the many studies that describe a hereditary pattern to the condition [see 1.5].

Environmental causes may be subdivided into intrauterine and postnatal factors. Intrauterine factors are implicated by association because there are no reported studies that have determined a direct cause-effect relationship with oligodontia. In addition, no intrauterine environmental insult is consistently associated with failure of tooth development. Some of the reported intrauterine factors with a possible association to failed dental development include:

- multiple births
- low birth weight
- increased maternal age
- intrauterine disturbances as a result of maternal anaemia or cardiovascular disease, rubella, syphilis, malnutrition (especially vitamin A deficiency) and even exposure to physical trauma

- prenatal exposure to teratogens such as anticonvulsant medication, serotonin uptake inhibitor antidepressant medication and thalidomide
- although not strictly environmental in origin, many forms of congenital orofacial clefts result in disruption of the presumptive dentition, the consequence of which is commonly hypodontia or even hyperdontia (supernumerary teeth)

(Billings et al., 2004; Cameron and Widmer, 2003; Hall, 1994; Moiseiwitsch, 2000; Olin, 1964; Orup, Jr. et al., 1998; Ranta, 1986; Winter, 1997)

There are few environmental insults that occur postnatally that are severe enough to result in failure of tooth development. Those that have been reported are generally a result of local factors, exposure to early episodes of chemoradiotherapy or severe systemic illness (Burzynski and Escobar, 1983). Local factors imply an insult on a particular tooth bud and this may be a result of trauma or localised infection. High dose radiotherapy has been reported to be associated with agenesis of permanent teeth if treatment is commenced prior to or around the time of their initial calcification. Cases of radiotherapy commencing between the ages of two and three years and resulting in agenesis of all permanent second premolars and second molars (the teeth that develop latest in life, excluding third molars) have been reported (Goho, 1993). There are few reports in the literature describing oligodontia as a direct result of a postnatal systemic illness but rickets, meningococcal and herpes zoster infections have been implicated (Burzynski and Escobar, 1983; Smith et al., 1984; Walton et al., 1998).

Diagnosis of tooth agenesis is made difficult by the fact that there is considerable variability in the timing of tooth development. Within normal populations, the variability in dental maturation is such that there are no reports in the literature of specific ages as the cut off point at which teeth are expected to have erupted or be detected

radiographically. The only exception to this is a reported screening limit for the first primary tooth to erupt in the mouth by 15 months, considered to be +4 standard deviations from the mean emergence time (Pirinen, 1998). There are mean ages at which specific teeth can be expected to be radiographically apparent but these should only be used as a guide. For example, a recent report describes the initial radiographic appearance of developing second premolars and a canine in a female no earlier than 13 years of age, as much as 10 years after the expected age of presentation (Alexander-Abt, 1999). For this reason, it is generally of more value to look for discrepancies in the pattern of tooth development in an individual.

Anecdotally, it is often noted that many cases of "non-syndromic" oligodontia are a source of diagnostic frustration for geneticists and dentists due to the clinical finding of very mild anomalies in hair, nail, sweating or other ectodermally derived tissues, without a clear justification for describing the condition as a true ED. It is one of the objectives of this research to better characterise that pattern of oligodontia that is associated with HED. Because of the many co-morbidities associated with HED that are not a feature of non-syndromic oligodontia, distinguishing between the two conditions becomes extremely important for the purpose of genetic counselling and general management.

1.2.4.1 The Dentition

The absence of teeth has a number of effects on the development of the dental arches and oligodontia is also reported to be associated with a number of coincident dental anomalies. An individual with oligodontia generally displays some form of malocclusion, the most common forms of which relate to increased overbite, problems of spacing or crowding of the teeth, disturbances in tooth eruption and exfoliation, infraocclusion of primary molars with no permanent successor, rotations, impactions or ectopic eruption of

permanent teeth (Baccetti, 1998; Carter et al., 2003; Peck et al., 1996; Pirinen et al., 1996; Symons et al., 1993).

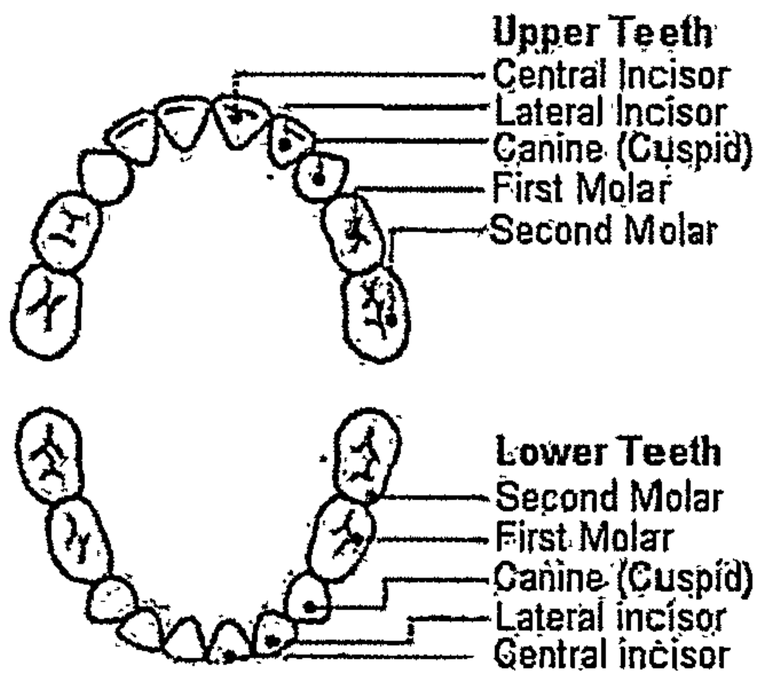
One of the most significant consequences of oligodontia is a progressive increase in freeway space (the space between the occluding surfaces of the teeth when the mandible is in the rest position) with age, resulting in a true overclosure of the jaws. This form of malocclusion is difficult to manage and is one of the reasons why patients with oligodontia often need to endure many hours of dental treatment over a prolonged period.

Dental anomalies commonly associated with oligodontia include microdontia (abnormally small teeth), conical teeth (abnormally tapered crown shape) and taurodontism (abnormally large body of tooth in conjunction with shortened root). These dental features have been documented particularly in patients with ectodermal dysplasias (Pirinen, 1998). Any, all or none of these features may be present in an individual with oligodontia. In addition there is a reported lack of diagnostic standards in determining the presence and extent of these dental anomalies, making the diagnostic process a subjective one (Hall, 1994).

In helping to characterise the phenotype of oligodontia patients, it is important to determine the pattern of missing teeth. In the primary dentition, there are twenty teeth that normally erupt, two incisors, one canine and two molars in each of the four quadrants in the mouth (upper left, upper right, lower left, lower right), as shown in Fig 1.2. In the permanent dentition, eight teeth consisting of two incisors, one canine, two premolars and three molars usually form in each quadrant, giving a total of thirty-two teeth. In this study, tooth type will be identified by a number representing distance from the midline of the jaw and the letter U or L representing the Upper or Lower jaw (eg U1=upper central incisor, L6=lower first molar).

THE HUMAN DENTITION

a). **Primary or deciduous teeth**



b). **Adult or permanent teeth**

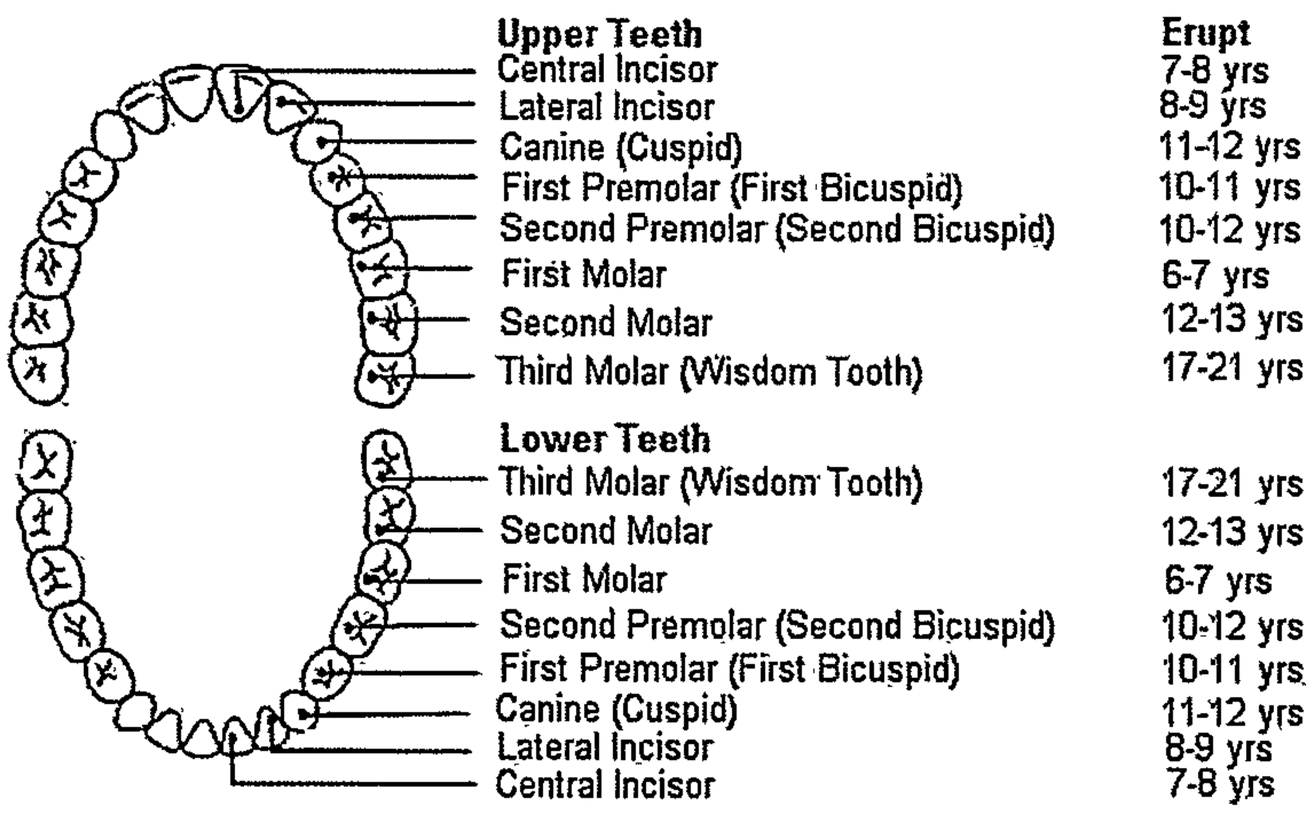


Figure 1.2 Schematic diagram of a). the primary dentition and b). the permanent dentition identifying each tooth and the expected timing of eruption and, for primary teeth, exfoliation (shedding). (Dentistry and Real Life, 2004)

The most accurate diagnosis of a genetic condition is one that is based on the identification of a gene mutation, the consequences of which are understood at a molecular level. Gene mutations that have so far been identified in familial oligodontia include *PAX9* and *MSX1* (Online Mendelian Inheritance in Man, 2002). [See sections 1.3.3 and 1.5.2.1]. In addition, mutations in the ectodysplasin (EDA) gene have been clearly demonstrated in oligodontia-associated X-linked HED (Online Mendelian Inheritance in Man, 2004). [See 1.5.2.2]. A number of studies have now looked at

the dental phenotype-genotype correlation in subjects proven, linked to, or clinically diagnosed (by characteristic pedigree plus clinical features) as having a mutation in the *PAX9*, *MSX1* or *EDA* (X-linked HED) genes. The compilation of this data is graphically demonstrated in fig. 1.3. Note the differences in prevalence of agenesis of various teeth between the three clinical conditions. Although asymmetry of tooth agenesis (i.e. left or right side of the mouth only) has been reported in 57% of *MSX1* and 27% of *PAX9* NSO affected individuals (Line, 2001), there is no significant asymmetry for any tooth type when the data are analysed collectively. For this reason, left and right side is not differentiated in fig 1.3.

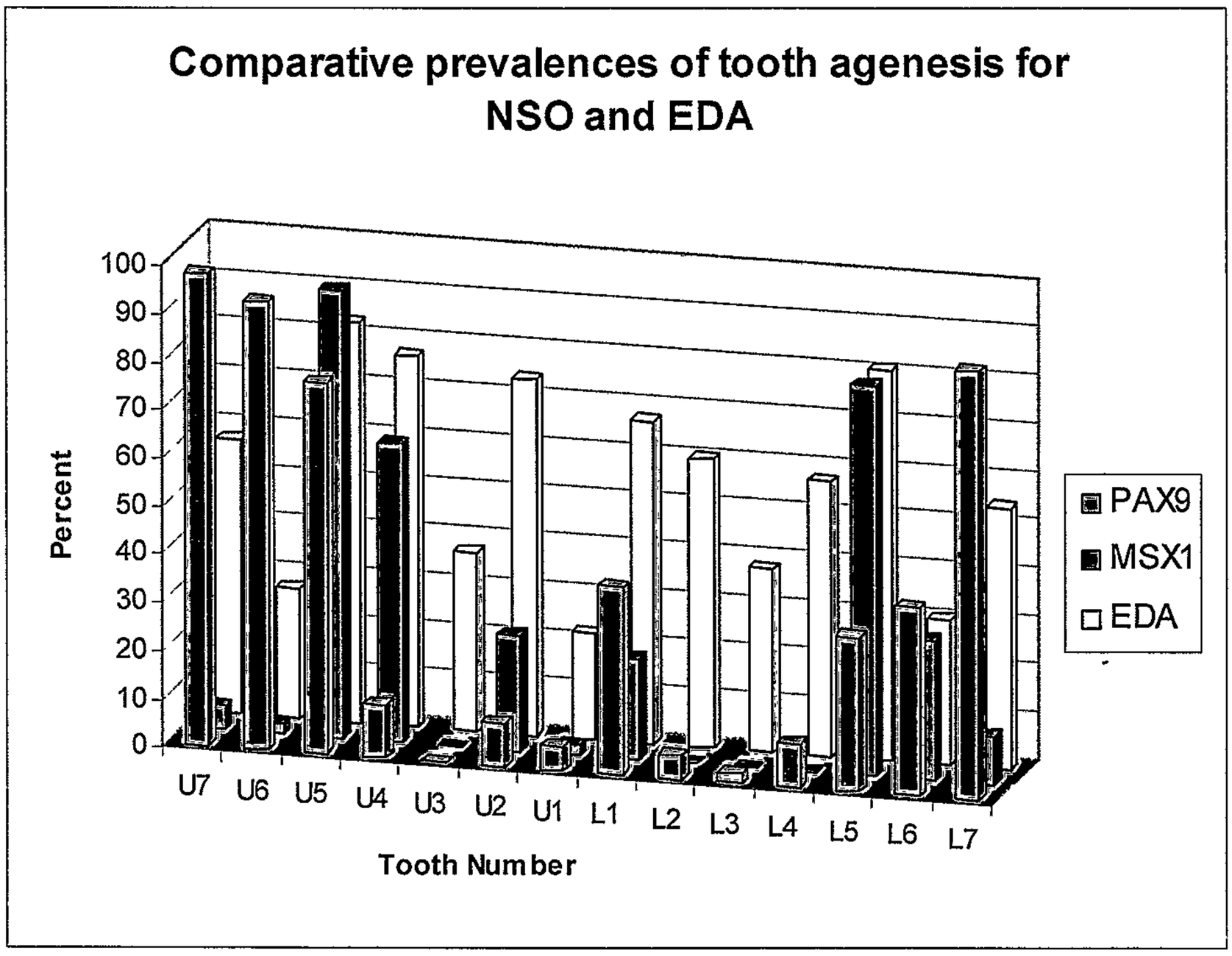


Figure 1.3 Comparative prevalence of tooth agenesis by tooth type and genetic cause (X-linked HED and NSO as a result of *MSX1* and *PAX9* mutations). Data has been tabulated from population (Bergendal et al., 1998) and pedigree (Das et al., 2002; Frazier-Bowers et al., 2002; Lidral and Reising, 2002; Line, 2001; Stockton et al., 2000; van den Boogaard et al., 2000; Vastardis et al., 1996) studies. Total number of subjects represented are 167 for *EDA* data, 21 for *MSX1* data and 22 for *PAX9* data. U=Upper, L=Lower, number represents distance of tooth from midline.

The main findings from fig 1.3 can be summarised as follows:

- much higher prevalence of agenesis of all incisors, canines and L4 for *EDA* compared to *MSX1* and *PAX9*
- in addition, very low prevalence of U4 and L5 agenesis for *PAX9* compared to *MSX1* and *EDA*, and very low prevalence of upper molar and L7 agenesis for *MSX1* compared to *EDA* and *PAX9*
- highest prevalence of molar agenesis is recorded for *PAX9*, and for this reason *PAX9* oligodontia is often described as molar oligodontia
- in hierarchical order, the teeth most likely to be aplastic in X-linked HED for males and females combined are U5, L5, U4, U2, L1, L2, U7, L4, L7, L3, U3 while the remaining tooth types tend to develop greater than 70% of the time
- in hierarchical order, the teeth most likely to be aplastic in *MSX1* NSO are U5, L5, U4, L6, U2 and L1, while in *PAX9* NSO the order is U7, U6, L7, U5, L6, L1 and L5, with the remaining tooth types in each condition present at least 90% of the time

A clearer identification of phenotype can be determined by assessing related family members. For example, in X-linked HED, carrier females (heterozygotes – see 1.2.5.3) often have milder features such as peg or aplastic upper lateral incisors, impacted upper canines and fewer missing teeth, although this may be variable (Söderholm and Kaitila, 1985).

1.2.4.2 Craniofacial and other features

The craniofacial features associated with oligodontia include reduced alveolar ridge height, shallow buccal sulcus depth, reduced facial height and everted lips (as a result of overclosure). Cephalometric analysis reveals reduction in facial height and maxillary

length (retrusion of the maxilla relative to the mandible), obtuse nasolabial angle, increased incisor retroclination and interincisal angle but normal cranial base lengths and angulations, indicating that the altered dentofacial structure is probably due to dental and functional compensation rather than to a different growth pattern (Bixler et al., 1988; Boj et al., 1993; Bondarets and McDonald, 2000; Nomura et al., 1993; Ogaard and Krogstad, 1995; Vierucci et al., 1994).

1.2.5 COMORBIDITIES

There are more than 49 syndromes with which oligodontia is associated (Gorlin et al., 2002). Most commonly, oligodontia is associated with Rieger syndrome, clefts of the lip and/or palate, Down syndrome and Williams-Beuren syndrome (Table 1.1) and with the ectodermal dysplasias (Table 1.2). Syndromes are generally very clearly distinguishable from normal variations in morphogenesis whereas dysplasias are a later dysmorphogenic event and can therefore be more difficult to differentiate from normal morphological variation (fig 1.1).

Because ectodermal dysplasia (ED) is a result of a primary ectodermal defect hair, finger/toenails and/or sweat glands may be malformed or partially absent as well. The non-dental features of ED can manifest in individuals across a wide phenotypic spectrum, from very mild and almost undetectable, to severe and debilitating, and can also be associated with non-ectodermally derived malformations such as immune deficiency or functional abnormalities of the central nervous system (Priolo and Lagana, 2001). The main clinical features of HED are described in 1.2.5.3.

1.2.5.1 Psychosocial Impact

Although no study has addressed the psychosocial impact on patients suffering NSO, there are several reports related to this issue for HED, in which oligodontia is a key debilitating aspect of the condition. Several studies have indicated that patients with HED mostly felt handicapped because of a reported "old man" appearance, and because of their hypotrichosis, oligodontia and eczematous lesions (Hummel and Guddack, 1997; Söderholm and Kaitila, 1985). Nowak (1998) pointed out that children and adolescents with HED are often excluded by their peer group and become the target of criticism and mockery (Nowak, 1988). It has also been suggested that physical fitness may be affected as a result of a lack of sweat glands (Rietschel, 1979).

Conversely, a 1988 study by Tanner found no difference between six boys with HED, their healthy siblings and six other peers (controls) concerning psychological adaptation and intellectual capacity (Tanner, 1988). Hummel and Guddack (1997) conducted a questionnaire study on fourteen children and adolescents with HED of varying severity. They found that adaptive functioning was dependent on the severity of symptoms, the child's intellectual potential and personality, how the disease was dealt with in the family and the reactions of the patients, peers and adults within their immediate social environment (Hummel and Guddack, 1997).

It is interesting to note that earlier studies on HED reported up to half of all patients to have some delayed mental development (Hummel P and Guddack S, 1997). More recent studies have reported a much lower incidence of about 10% (Freire-Maia and Pinheiro, 1990) and rather than being attributed to a genetic cause, it has been suggested that the delay could be a result of uncontrolled febrile episodes during infancy (Söderholm and Kaitila, 1985; Wasserteil, 1986).

1.2.5.2 Speech and Mastication

It has been reported that oligodontia has an impact on mastication (and as a result food intake) and the development of speech, in addition to its aesthetic implications (Coston and Salinas, 1988; Hummel and Guddack, 1997). Specifically, oligodontia is associated with speech misarticulations. Unfortunately, very little research has been conducted on the impact of the dentition on speech (Coston and Salinas, 1988). In 1936, Fymbo described the speech sounds that were most frequently affected by the dentition. In order from the least to most affected, these were /s/ (as in soap), /z/ (as in zebra), /th/ (as in think), "th" (as in there), "sh" as in shoe, "zh" (as in pleasure), "ch" (as in church) and /dz/ (as in jar) (Fymbo, 1936).

In another study assessing the impact of the dentition on speech, Snow (1961) reported that speech sounds most frequently in error were sibilants and linguadentals and, as these are among the most frequently occurring speech sounds in language, the severity of the speech articulation problem is greater in patients with anomalies in the dentition (Coston and Salinas, 1988; Snow, 1961). In a US study, oligodontia associated with HED resulted in distortion of sibilants in two of four individuals (Coston and Salinas, 1988). Of the two affected individuals (ages 6 and 16), one had been wearing dentures for over 10 years and the other had conical shaped and widely spaced teeth. Of the two unaffected individuals (developing speech articulation within normal limits), both were 4 years of age and had been wearing dentures for at least two years.

Table 1.1 Oligodontia Syndromes (Excluding ED)

Syndrome	OMIM	Description of predominant oligodontia pattern (reference)	Other ectodermal involvement	Gene locus	Mode of Inheritance
Rieger syndrome	180500	Incisors (B&E)	No	4q25-q26	AD
	601499		No	13q14	AD
Williams-Beuren syndrome	194050	L5's, also enamel defects (Ha)	Yes	7q11.2	AD
Down syndrome	190685	U2's, 5's & U4's (A; Me)	No		Isolated
Holoprosencephaly	600725	U1 (OMIM)	Not common	7q36	AD
Oligodontia-colorectal cancer syndrome	608615	Severe oligodontia (OMIM)	No	AXIN2	AD
Van der Woude syndrome	119300	Non-specific, CLP (OMIM)	No	1q32-q41	AD
Frontometaphyseal dysplasia					
Coffin Lowry syndrome	305620	Non-specific (B&E)	No		XR
Wolf-Hirschhorn syndrome	303600	Incisors (B&E)	No	Xp22.2-p22.1	X-L
Kabuki syndrome	194190	Non-specific, CLP (OMIM)	Yes	4p16.3	Isolated
Orofaciodigital syndrome I	147920	Incisors, taurodontism, screw driver incisors, CLP (P, Ma)	No		AD
Diastrophic dysplasia	311200	Lower incisors & 3's (B&E)	Yes	Xp22.3-p22.2	XD
Focal dermal hypoplasia	222600	Non-specific, CLP (K)	No	5q32-q33.1	AR
Faciogenital dysplasia	305600	Non-specific, enamel defects (G)	Yes		XD
Mulibrey nanism	305400	Non-specific (OMIM)	No	Xp11.21, XL	XL
Hennekam lymphangiectasia lymphedema syndrome	253250	Non-specific (OMIM)	No	17q22-q23	AR
Hallerman-Streiff syndrome	235510	Non-specific, peg U2's (OMIM)	No		AR
Gorlin-Chaudhry-Moss syndrome	234100	Non-specific, neo. teeth (OMIM)	Yes		Isolated
Carpenter syndrome	233500	D's, E's, 4's, 5's, 7's (B&E)	No		AR
Bardet-Beidl syndrome	201000	Non-specific, micro (Bl)	No		AR
Larsen syndrome	209900	Non-specific, micro, short rt (Bo)	No	Multiple genes	
Cherubism	150250	U2's, 3's, U5's, 7's (T)	No	3p14.3, 3p21.1-p14.1	AD & AR
Oculofaciocardiodental	118400	Non-specific (OMIM)	No	4p16.3	AD
Blepharocheilodontic syndrome	300166	Non-specific (OMIM)	No	Xp11.4	X-L
Alagille syndrome	119580	Non-specific, conical teeth (OMIM) Severe oligodontia (Ho)	No		AD
	118450		No		AD

Table 1.1 List of syndromes commonly reported to be associated with an oligodontia pattern. CLP = cleft lip and/or palate, neo. = neonatal, micro = microdontia, rt = root. OMIM reference numbers and references are provided: (B&E) = (Burzynski and Escobar, 1983), (Ha) = (Hall, 1994), (A) = (Acerbi et al., 2001), (Me) = (Mestrovic et al., 1998), (P) = (Petzold et al., 2003), (Ma) = (Matsune et al., 2001), (K) = (Karlstedt et al., 1996), (G) = (Gunduz et al., 1997), (Bl) = (Blankenstein et al., 2001), (Bo) = (Borgstrom et al., 1996), (T) = (Tsang et al., 1986), (Ho) = (Ho et al., 2000).

1.2.5.3 Hypohidrotic Ectodermal Dysplasia

Ectodermal dysplasias were probably first described in the literature by Danz in 1792, and then later by Thurnam and Charles Darwin (Danz, 1794; Darwin, 1880; Freire-Maia and Pinheiro, 1984; Gorlin et al., 2002; Thurnam, 1848). Weech (1929) was the first to attempt to group the various conditions with defects in hair, teeth, nails and sweat glands (the 4 cardinal signs) into one single entity and postulated that there was a hereditary pattern to many of these conditions. Weech was also responsible for coining the term hereditary ectodermal dysplasia (Weech, 1929).

The most common X-linked form of HED, also known as Christ-Siemens-Touraine syndrome, is considered the classic form of ED and it is pertinent to review all the systems that may be affected in individuals with this condition. X-linked HED is associated with the following features:

1. Oral features

- Oligodontia: the most characteristic feature of X-linked HED (Zonana, 1996). It has been reported that there are generally more teeth present in the maxilla than in the mandible for X-linked HED (Pirinen, 1998).
- Radiographically, the teeth present may have a constriction cervically (at the neck of the tooth) and resemble teeth of dentinogenesis imperfecta (Pirinen, 1998), in that the crowns of the teeth appear bulbous (Winter, 1997). See fig 1.4.a.
- Conical teeth: upper incisors and canines are almost always conical in shape in the X-linked form of HED (Pirinen, 1998). See fig. 1.4.a.

- Microdontia has been reported in apparently healthy mothers of boys diagnosed with X-linked HED (Nakata et al., 1980)
- Hypoplasia or hypomineralisation of the dental enamel has been reported as an associated anomaly in a number of ED syndromes but is not an associated feature of HED (Priolo et al., 2000).
- Taurodontism is more common in the ED syndromes tricho-dento-osseous syndrome and Ellis van Creveld syndrome, but has also been observed in X-linked HED (Vierucci et al., 1994). Taurodontism is a term used to describe teeth in which the body of the tooth is enlarged and the root is reduced in length. The clinical crown usually appears normal but radiographically, the bifurcation or trifurcation (junction at which the roots meet the crown) is displaced apically (see fig 1.4.b). Taurodontism is a result of failure of the Hertwig epithelial root sheath of the tooth germ to invaginate below the crown. Various anatomical ratios have been devised to define taurodontism (Hall, 1994)

Dental Characteristics of Ectodermal Dysplasia

a

b

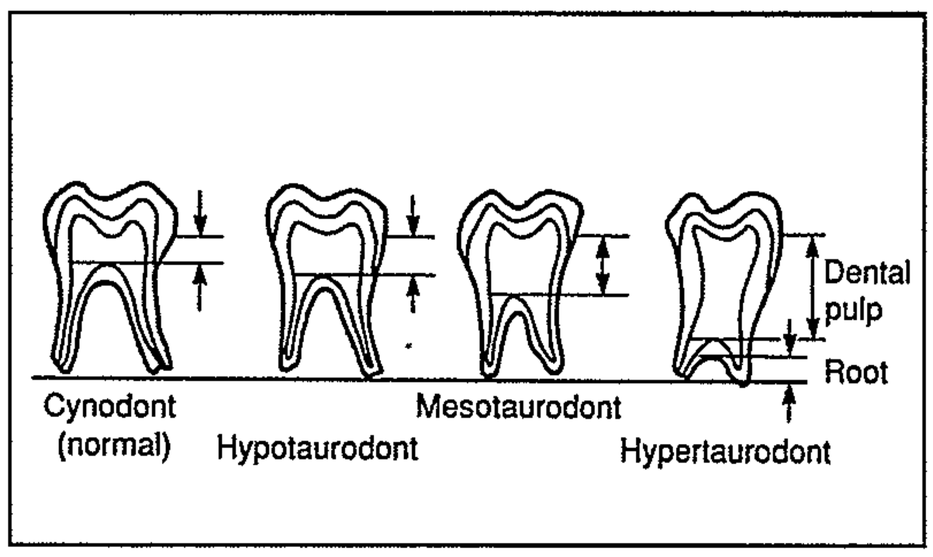


Figure 1.4 a. Panoramic radiograph displaying conical and cervically constricted teeth (with kind permission, Department of Paediatric Dentistry, Westmead); **b.** Diagrammatic representation of the various manifestations of taurodontism (with kind permission, Professor Roger K. Hall).

Table 1.2 Ectodermal Dysplasias and ED Syndromes Associated with Oligodontia

	Syndrome/dysplasia	OMIM	Description of oligodontia pattern (reference)	Gene locus	Mode of inheritance
Predominantly ectodermal involvement ED's	ED1	305100	NS, conical (B)	Xq12-q13.1	XR
	ED3	129490	Incisors, 3's, 5's (A)	2q11-q13	AD
	Hypohidrotic ED, AR	224900	NS (O)	2q11-q13	AR
	Hidrotic ED	602401	NS (O)		AR
	ED with natal teeth, Tumpenny type	601345	NS, severe (O)		AD
	Witkop syndrome	189500	L1&2's,7's,U3's conical (H)	4p16-1	AD
	Taurodontia, congenital absence of teeth & sparse hair	272980	NS, conical, taurodontism (L) NS (O)		AR
	Dermo-odonto-dysplasia	125640	NS (O)		AD
	Trichodental dyplasia	601453			AD
Ectodermal plus haematological and/or CNS involvement ED's	IP	308310	NS, conical (O)	Xq28	XD
	HED-ID	309291	NS (O)		XR
	Cerebellar ataxia & ED	212835	NS (O)		AR
	Ichthyosis, leukocyte vacuoles, alopecia & sclerosing cholangitis	607626	NS, severe, plus enamel defects (O)	3q27-q28	AR
Ectodermal plus major skeletal involvement ED's	EEC1	129900	NS, CLP, micro (O)	7q11.2-q21.3	AD
	EEC3	604292	NS, CLP (O)	3q27	AD
	Limb mammary syndrome	603543	NS, CLP (O)	3q27	AD
	AEC syndrome	106260	NS, CLP (O)	3q27	AD
	ADULT syndrome	103285	NS, CLP (O)	3q27	AD
	Rapp-Hodgkin syndrome	129400	NS, CLP, conical (C)		AD
	Ulnar-mammary syndrome	181450	NS, mild (O)	12q24.1	AD
	LADD	149730	NS, enamel defects (O)		AD
	Ellis van Creveld syndrome	225500	NS, natal teeth, micro, supers	4p16	AR
	Cranioectodermal dysplasia	218330	(L)		AR
	Tricho-odonto-onychial dysplasia	275450	NS, taurodont, enamel defect		AR
	Rothmund-Thomson syndrome	268400	(L)	8q24.3	AR
			NS (O)		
			NS, supers, micro (O)		
Ectodermal plus cytoskeletal or cell stability involvement ED's	CLEPD 1	225000	NS, CLP (O)	11q23-q24	AR
	ED, Margarita Island type	225060	NS, micro (O)	11q23-q24	AR
	Odonto-onychodermal dysplasia	257980	NS, micro (O)		AR
	Pilodental dysplasia with refractive errors	262020	NS (O)		AR
		164200	NS, micro, enamel defects (O)	6q21-q23.2	AD
	Oculodentodigital dysplasia	224750	NS(O)		AR
	Schopf-Schulz-Passarge syndrome	124480	NS, conical (O)		AD
	Deafness-onychodystrophy	129510	NS (O)		AD
	Trichodonto-onychial ED with amnastia	191482	NS (O)		AD
	Bork syndrome				

Table 1.2 List of ED syndromes commonly reported to be associated with oligodontia. Abbreviations: NS = non-specific, CLP = cleft lip and/or palate, micro = microdontia, supers = supernumerary teeth (e.g. hypoplasia) references: (B) = (Bergendal et al., 1998), (A) = (Aswegan et al., 1997), (H) = (Hodges and Harley, 1999), (L) = (Levin, 1988), (C) = (Crawford et al., 1989), (O) = Online Mendelian Inheritance in Man (OMIM)

- Studies on mice with the X-linked HED phenotype ("tabby mice") have demonstrated a number of molar morphotypes including microdont molars and molars with reduced cusp numbers compared to wild type mice (Kristenova et al., 2002; Pispa et al., 1999)
- Reduced alveolar ridge height, shallow sulcus depth and frenal tethering have also been reported (Winter, 1997). Frena (frenula) are bands of connective tissue that attach the labial (lip) or buccal (cheek) mucosa to the alveolar ridge of the maxilla or mandible
- Clefting of the lip and/or palate is not a feature of X-linked HED but is characteristic of other ED syndromes such as the EEC (ectrodactyly, ectodermal dysplasia and cleft lip/palate) or CLPED (cleft lip/palate-ectodermal dysplasia) syndromes
- Radiological demonstration of hypoplasia and aplasia of the major salivary glands and reduced secretion of unstimulated and chewing-stimulated whole saliva have previously been reported in a proportion of HED sufferers, with the submandibular glands possibly more affected than the parotid glands (Nordgarden et al., 1998; Nordgarden et al., 2001; Nordgarden et al., 2003).
- The consequence of reduced salivary flow is a dry mouth (xerostomia). This can be at the very least irritating to patients and as debilitating as predisposing that patient to rampant dental caries, oral infectious diseases such as candidiasis (oral thrush), severe dysphagia and even clinical depression in later adulthood. The increased oral infection and caries risk is a result of a reduced salivary buffering capacity and reduced oral clearance. A predominance of β-haemolytic streptococci in the pharynx has been reported (Söderholm and Kaitila, 1985).

- Aplasia of the mucous glands of the pharynx in addition to decreased salivary flow may also result in dysphagia (mastication or deglutition) while aplasia of mucous glands of the pharynx, larynx, trachea and bronchi can lead to the development of a hoarse voice (Myer III, 1988; Ørstavik, 1998; Peterson-Falzone et al., 1981).

Figure 1.5 Typical facial features of X-linked HED in males. Note sparse scalp hair, frontal bossing, prominent and oblique set ears, periorbital wrinkling and hyperpigmentation, saddle nose deformity, reduced lower face height, everted lower lip and prominent labiomental fold (with kind permission, Department of Paediatric Dentistry, Westmead).

2. Craniofacial features, together with the dental appearance, constitute a facial appearance that has unfortunately been described as "old man" or "vampire" (Tanner, 1988). See fig 1.5. A number of craniofacial features are consistently described in the literature (Hall, 1994; Ørstavik, 1998):

   - Sparse/absent scalp and facial hair, including eyebrows and eyelashes
   - Saddle nose deformity (depressed nasal bridge)
   - Small nasal alar width
   - Depressed midface
   - Reduced lower facial height and overclosure of the jaws, resulting in protruding and everted lips and a prominent mental sulcus and chin point

- Periorbital and perioral wrinkling and hyperpigmentation
- Small, oblique, prominent ears
- Frontal bossing (prominent forehead)

3. The skin features commonly reported (Hall, 1994; Priolo et al., 2000) are:
    - Soft, thin, dry, fine, smooth skin that may be hypopïgmented with patches of hyperkeratosis and/or eczematous as a result of absent sebaceous glands
    - Periorbital wrinkling and hyperpigmentation, also called pseudorhagades
    - Perioral wrinkling and hyperpigmentation associated with jaw overclosure and angular cheilitis, typically a candidal infection of the commissures (corners) of the mouth (Hall, 1994)
    - Subcutaneous fat is often reduced (Ørstavik, 1998)
    - Ozena: atrophic nasal mucosa that may be foul smelling with severe crusting and fetid green secretion due to atrophic rhinitis
    - A very useful dermatological diagnostic sign that can be present at birth is what is known as the "plastic wrap' effect or collodion baby, in which there is shedding or scaling of the skin within the first 24hours of birth (Anderson and Causer, 1989). Although collodion baby is more commonly associated with other conditions such as lamellar ichthyosis, detection of this clinical sign when associated with X-linked HED will help establish a very early clinical diagnosis that could help minimise the risk of some of the neonatal sequelae (see below) as a result of delayed diagnosis

- Other features that may be associated with various forms of ED include palmar–plantar hyperkeratosis, focal hyperkeratosis with fissuring, dysplastic changes to scalp skin, ichthyosiform erythroderma and facial milia (Atherton, 2004; Hall, 1994)

4. Sweating characteristics

   - Sweat gland dysplasia is a characteristic feature of X-linked HED. The hypohidrosis is a result of a reduced number of sweat glands and of a partial/immature development of many of the sweat glands that do form. Several methods have been described to evaluate sweating in HED patients, as shown in Table 1.3. Although these techniques are recommended by some authors, they all have limitations and there is no one ideal sweat test (Berg et al., 1990; Sofaer, 1981; Tso et al., 1985).

Table 1.3 Tests for Hypohidrosis

Visualisation of sweat by dyes	Counting of sweat pores under magnification	Counting of sweat glands in skin biopsy sections	Quantification of sweat output
Bromophenol blue Starch-iodide *o*-phthaldialdehyde Silver nitrate	Direct visual inspection Alginate impression Polyvinylsiloxane impression	Palmar punch biopsies Palmar horizontal cuts Parietal scalp biopsies	Weight of filter paper/gauze Loss of body weight Heat loss

Table 1.3 Previously reported tests for hypohidrosis. No evidence supports the accuracy of one technique over the other. Visualisation of sweat by dyes has been shown to reveal only functional glands and work best when sweating is stimulated by raising the environmental temperature or by local drug injection; counting of sweat pores depends on resolution; biopsy is very invasive; quantification of sweat output gives little information about the number and distribution of sweat glands (Berg et al., 1990; Jorgenson et al., 1988)

- The starch-iodide test has recently been shown to have a sensitivity of only 44% and an imperfect specificity (93%), while scalp biopsy specimens have been shown to be the most accurate way of confirming HED, with 67% sensitivity and 100% specificity (Rouse et al., 2004). In general, patients with X-linked HED tend to have flattened epidermal ridges and a reduced number of sweat pores (Verbov, 1970).
- A history of severe heat intolerance (for example having to frequently soak one's head in water or lie on a tiled floor in warm weather, or having a history of recurrent fainting episodes during sport or exercise) is an important symptom to record, as is a history of possible signs of hypohidrosis during infancy such as recurrent, unexplained episodes of hyperpyrexia or febrile convulsions

5. Hair features (Hall, 1994):
    - Absence of lanugo hair at birth
    - Sparse/absent scalp hair
    - Hair that forms is often blonde, fine, stiff, short or slow growing
    - Eyebrows and eyelashes may be scant, sparse, fine and/or blonde
    - Axillary, body and pubic hair may be scant
    - Beards may be normal
    - Abnormal cuticle patterns have been reported for tooth and nail syndromes, Clouston, Robinson and Rapp-Hodgkin syndrome

- Anomalies in hair shafts observed under light microscopy that have been reported for X-linked HED include variable shaft thickness, pili bifurcati (longitudinal splitting), trichorrhexis nodosa (most common of all hair shaft anomalies, irregularly spaced nodes on the hair shaft that are predisposed to fracture), pili torti (twisting of the hair shaft), bar code anomaly (alternate dark medullated and clear non-medullated bands along the hair shaft) and pseudomonilethrix (Rogers, 1996; Rogers, 2000; Rouse et al., 2004). The term pseudomonilethrix describes an anomaly that gives the appearance of beading (monilethrix) but under higher magnification power is actually a result of deformation causing flattening along segments the hair shaft (see fig 1.6) (Rogers, 1996)
- Other features observed include reduced modulus of elasticity, reduced tensile strength, altered birefringence pattern, higher water content and loss of disulphide bordered protein (Hall, 1994)
- Macroscopically, the scalp hair may be uncombable, extremely brittle or have a steel wool (Rapp-Hodgkin syndrome), dry, lustreless or spangly (sparkly) appearance
- As has been reported for sweat tests, the analysis of hair shafts under microscopy has been shown to be a poor predictor of HED affectation, with the ability of the analysis to support the diagnosis of HED reported to be only between 70-75%. Hair sample analysis does, however, have the advantage over many sweat tests of being a simple and non-invasive examination procedure (Rouse et al., 2004)

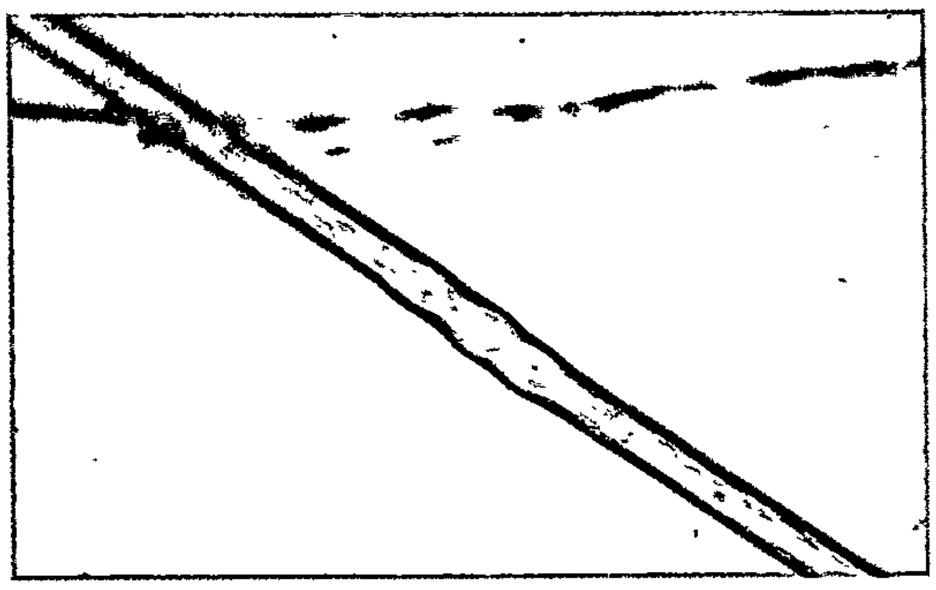


Figure 1.6 Photomicrograph (light microscopy) of hair shaft displaying pseudomonilethrix. Courtesy of Dr Maureen Rogers.

6. Nail features

   - Generally normal in X-linked HED (Ørstavik, 1998) but onycho-dysplasia is frequently associated with other forms of ED's, with features varying from spoon shape to incompletely formed, abnormally keratinised, hypertrophic, discoloured, striated, split or completely absent (Hall, 1994)

7. Other features include dry eyes, photophobia and/or corneal damage as a result of lack of lacrimal glands, ear wax build-up that may lead to hearing problems, nasal obstruction and secondary epistaxis or infections of the nose and paranasal sinuses as a result of reduced nasal secretions and saddle nose deformity, lack of mammary glands and hypoplastic or absent nipples (Dhanrajani and Jiffry, 1998; Myer III, 1988). Asthma tends to be more common with X-linked HED and may be a result of lack of clearance of allergen from the respiratory mucosa by mucous glands.

8. During the neonatal period, it is also important to determine the severity of clinical manifestations of X-linked HED in the carrier mother. In particular, it is important to determine the ability of the mother to breastfeed, as insufficient breast milk may predispose the neonate to malnourishment or failure to thrive (Atherton, 2004). Neonatal death or morbidity is therefore a possibility in X-linked HED for three different reasons:

a) Hyperpyrexia as a result of sweat gland agenesis and/or hypofunction

b) Failure to thrive

- inability of carrier female to breast feed
- dysphagia and obstructed nasal respiration (due to rhinitis) of the neonate

c) Lung disease as a result of recurrent respiratory infections (some patients later require lung transplants) (Atherton, 2004)

Diagnosis during the neonatal and infancy periods can be improved by obtaining an appropriate history (collodion baby, unexplained pyrexia), obtaining an accurate family history, determining maternal carrier characteristics and assessing facial morphology and dental development.

The great variability in the expression of the clinical features from one affected individual to another in ED even applies to affected members of the same family. For X-linked HED, examples of this variable expressivity in heterozygous females may be a manifestation of hypodontia, conical teeth, reduction in scalp/body hair or difficulty breast-feeding. The clinical features of autosomal dominantly inherited HED have also been reported and affected individuals tend to have a mild phenotype comparable with female carriers of X-linked HED, such as variable sparseness of hair, smooth, dry skin,

hypodontia, mild fingernail anomalies and slow nail growth, negligible facial dysmorphia and few other dysplastic features (Aswegan et al., 1997; Jorgenson et al., 1987).

For those ED's with a generally mild phenotype such as autosomal dominantly inherited HED, diagnosis of the least affected family members therefore becomes very difficult and the possibility exists that a diagnosis of NSO is made instead of HED. This could also apply to female carriers of X-linked HED. This can lead to inappropriate genetic counselling, with potentially serious implications for affected individuals, as illustrated by the potential complications that may arise in the neonatal period.

1.2.6 MANAGEMENT OF OLIGODONTIA

The ultimate aim in the dental management of oligodontia is to provide adequate function and restore aesthetic appearance for the patient (Cameron and Widmer, 2003). This treatment ideal is very dynamic as it commences at the earliest point in time at which care is sought (often at the age of 2-3 years) and continues throughout growth and development of the patient over their entire lifetime.

In order to provide the highest quality of care in these patients who often have very complex needs, the dentist has a responsibility to provide access to appropriate counselling for the family and make any affected family members or potential carriers of the condition aware of the treatment limitations and future consequences. This degree of care can only realistically be achieved by a multidisciplinary or interdisciplinary approach, which has been well documented in the literature (Carter et al., 2003; Hobson et al., 2003; Jepson et al., 2003; Meechan et al., 2003; Nunn et al., 2003).

The paediatric dentist has a central role to play during the growth and development phases of a patient with oligodontia. The role of the paediatric dentist is ideally one of a team member in the interdisciplinary management of the paediatric oligodontia patient, with the specific objectives of:

1. Managing behaviour in these children who may be emotionally, psychologically, socially or mentally challenged.
2. Prevent caries and maintain the existing dentition.
3. Intermediate restoration of diminutive teeth with adhesive materials or removable prostheses (dentures) to improve aesthetics.

4. As a team member, aid in helping these patients to be able to eat a wide range of foods, to improve speech, to achieve emotional and psychosocial well being and to improve the acceptance by family and peers of the condition (Nunn et al., 2003). Genetic counselling, psychological or speech therapy may be useful adjuncts at this stage.

As the child grows and develops, the management of spaces in the dentition becomes an important clinical objective. The options available to clinicians is accepting spaces as they exist, closing spaces or redistributing spaces (Jepson et al., 2003). Each of these options in turn will determine the restorative treatment approach.

Redistribution or closure of spaces usually involves orthodontic treatment but may also involve autotransplantation of a tooth from a donor site elsewhere in the mouth (Meechan et al., 2003). Where spaces are left, long term treatment options include fixed prostheses such as bridges or implants, or removable prostheses (dentures). However, the use of fixed prostheses should be restricted or at least very judiciously implemented while the patient is still growing, as continual growth may result in failure of such treatment.

1.3 Embryology and Genetic Background of Odontogenesis

1.3.1 GENERAL AND CRANIOFACIAL EMBRYOLOGY

Prenatal development is divided into three successive phases. The first two combined constitute the embryonic stage and the third is the foetal stage. The first phase spans the first four weeks post-fertilisation and is characterised by early differentiation of the basic tissue types into three layers known as ectoderm, mesoderm and endoderm that then undergo a process of folding and structuring to form a tubular body (Sperber, 1993; Ten Cate, 1998). The last four weeks of the embryonic stage is characterised largely by the differentiation of all major external and internal structures (morphogenesis) and is a particularly vulnerable period of development, with many recognised congenital defects developing during this period (Ten Cate, 1998). The foetal period, from the end of the embryonic stage to term, is largely a period of growth and maturation.

Caudo-Cephalic Folding of a 28 Day Human Embryo

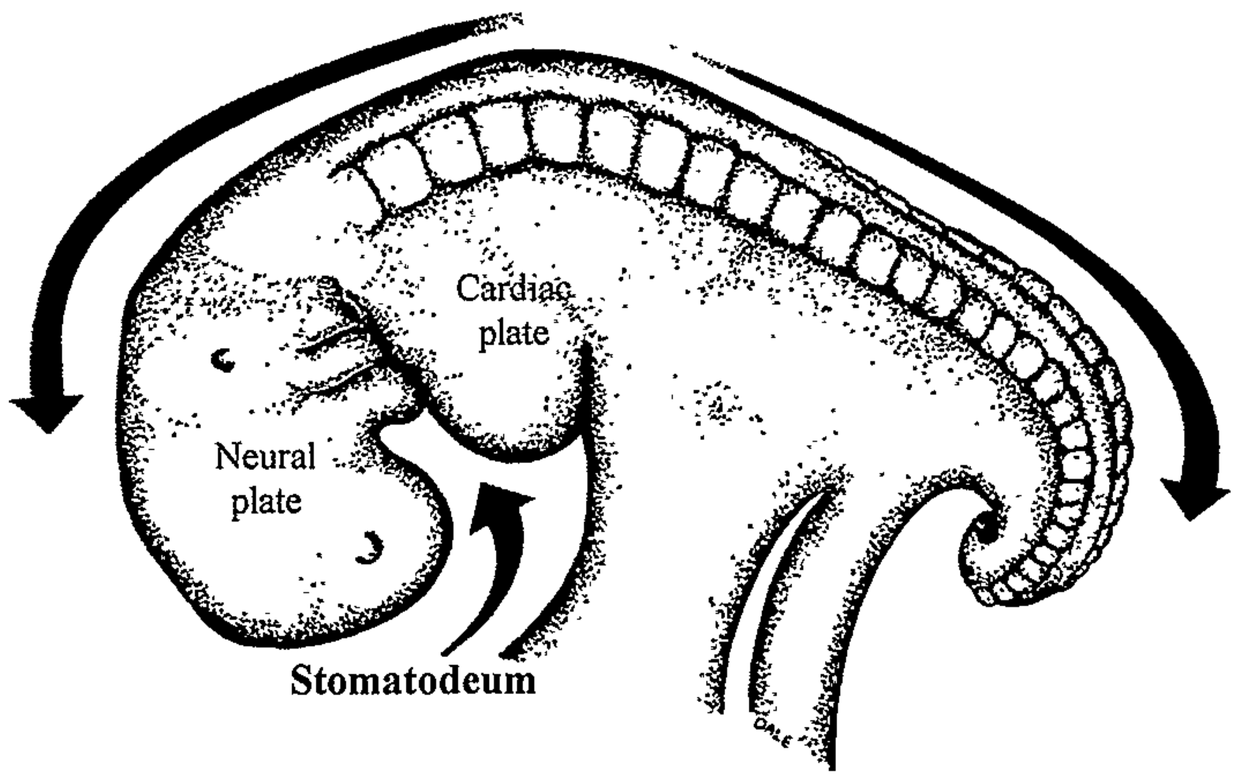


Fig 1.7 28 day human embryo undergoing caudo-cephalic folding, demonstrating how the head fold establishes the primitive stomatodeum (oral cavity), bounded by the neural plate and the developing cardiac plate. Adapted from Ten Cate (Ten Cate, 1998).

During the second embryonic stage, major tissues and organs differentiate from the tripoblastic (three layered) embryo, including the head and face and the tissues contributing to the development of the teeth (Ten Cate, 1998). A key event is the folding of the embryo in two planes along the caudo-cephalic (head-tail) and lateral axes. The caudo-cephalic folding of the embryo becomes critical for the formation of the primitive stomatodeum or oral cavity (see fig 1.7). The head fold allows ectoderm to line the stomatodeum.

Also during this period, the ectodermal layer develops a thickening known as the neural plate at the cephalic end of the embryo from which the future nervous system develops. This neural plate thickening rapidly forms raised margins (neural folds) which eventually fuse to form a neural tube. As the neural tube forms and sinks beneath the surface ectoderm, a group of cells separate from the neuroectoderm and are known as neural crest cells. These cells have the capacity to migrate and differentiate extensively within the developing embryo (Fig 1.8). Due to its neuroectodermal origin, neural crest derived connective tissue is described as ectomesenchyme, in contrast to mesoderm-derived connective tissue, which is known as mesenchyme.

The interaction between epithelium and mesenchyme (or ectomesenchyme) derived tissues is critical for organogenesis throughout the developing embryo. It has been known for over a century that cells signal to each other and thereby direct cell behaviour, thus controlling advancement of development (Thesleff, 2000). The signalling events between epithelial and mesenchymal tissue components has been called embryonic induction and is now believed to be the single most important mechanism of developmental regulation

(Kessler and Melton, 1994), of which dental development is one example (Ruch et al., 1983).

Neural Crest Cell Migration to the Developing Jaws

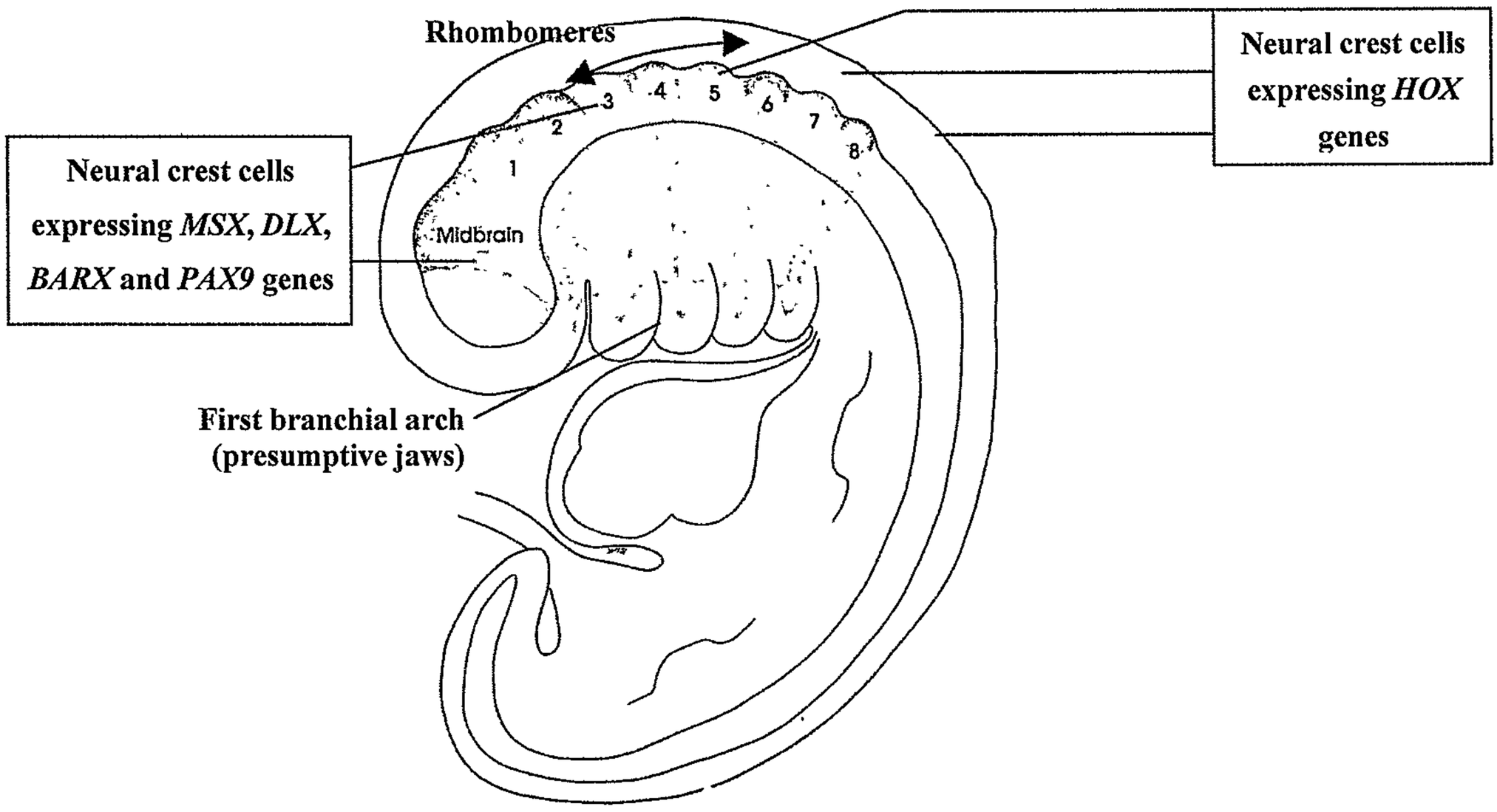


Fig 1.8 The source and pattern of migration of neural crest cells and the expression of homeobox genes from neural crest ectomesenchyme to the developing face and branchial arch system. Note that rhombomeres 1 and 2 express the non-homeotic genes *MSX*, *DLX*, *BARX* and *PAX9*, compared to the *HOX* genes in the lower order rhombomeres. Adapted from Ten Cate (Ten Cate, 1998).

The anterior (rostral) portion of the neural tube expands greatly as the forebrain, midbrain and hindbrain form. The neural crest cells paraxial to the midbrain and in two of the hindbrain rhombomeres (the hindbrain develops as a series of eight bulges known as rhombomeres) migrate anterolaterally to provide the cranial sensory ganglia and most of the embryonic connective tissue needed for craniofacial development (Ten Cate, 1998). See Fig. 1.8. These neural crest cells migrate in two streams, the most rostral to supply much of the connective tissue to the face and the most caudal to supply the first branchial arch. The first branchial arch plays a key role in the development of the face and mouth. At about 24 days, the first branchial arch establishes a second process, the maxillary

process, with the original arch process now know as the mandibular process. The fusion of the mandibular processes gives rise to the mandible.

The anterior portion of the maxilla has a completely separate embryological origin to the branchial arch-derived maxillary process. At about twenty-eight days, localised thickenings develop within the ectoderm just rostral to the opening of the stomatodeum, in the frontal prominence of the neural plate. These horseshoe shaped thickenings known as the olfactory placodes develop into the lateral nasal process and the medial nasal process, with the depressed area between the two medial nasal processes known as the frontonasal process (see fig 1.9). Together with the medial nasal process, this area gives rise to the middle portion of the nose and upper lip, anterior portion of the maxilla and the primary palate.

The maxillary process derived from the first branchial arch then grows medially and approaches both the lateral and medial nasal processes but remains separated from them by distinct grooves. Medial growth of the maxillary process pushes the two medial nasal processes together at the midline where they fuse, eliminating the frontonasal process. The anterior extent of the maxillary processes also eventually fuses with the lateral face of the medial nasal processes, merging that portion of the maxilla carrying the incisors with that carrying all the other teeth. The implications of this developmental process are that all but the anterior maxillary teeth arise from the first branchial arch, whereas the anterior maxillary teeth (upper incisors and canines) arise from the medial nasal process.

The migrating neural crest cells play an important role in the patterning and initiation of dental development by the expression of a number of regulatory and signalling genes (discussed in detail in sections 1.3.3, 1.3.4 and 1.5.2). The neural crest cells express the

same genes as the respective rhombomeres from which they derive. A large group of genes known as homeotic or *Hox* genes are responsible in large part for the segment identity that characterises all vertebrates (Cobourne, 1999; Maas and Bei, 1997).

Fusion of Medial Nasal and Maxillary Processes in 7 Week Old Embryo

Fig 1.9 Scanning electron micrograph of a human embryo at 7 weeks, demonstrating the fusion of the maxillary process (first branchial arch derivative) with the medial nasal process. Adapted from Ten Cate (Ten Cate, 1998).

Homeotic genes are all composed of DNA sequences known as homeobox sequences, that are highly conserved across evolutionary time. However, the neural crest cells that contribute the ectomesenchyme of the first branchial arch and the frontonasal process (i.e. neural crest rostral to rhombomere three) in mammals do not express these particular homeobox genes (Sharpe, 1995). The genes rostral to rhombomere three that do supply the craniofacial complex via the first branchial arch include the homeobox gene families *MSX* and *DLX* and one of the important genes that supplies the craniofacial complex via the medial nasal process is *SHH*, which codes for a particular signalling molecule (see sections 1.3.4 and 1.5.2).

1.3.2 ODONTOGENESIS REVISITED

The first morphological evidence of odontogenesis is the formation of a primary thickening of the oral epithelium, known as the primary epithelial bud. This forms a continuous horseshoe shaped sheet of epithelium around the lateral margins of the developing oral cavity and gives rise to two processes which invaginate into the underlying ectomesenchyme (Cobourne, 1999). The outer process, the vestibular lamina, will form the vestibule that demarcates the cheeks and lips from the tooth-bearing regions. The inner process is the dental lamina from which tooth buds arise. It is remarkable that no teeth develop outside the lamina and also that variably present supernumerary teeth always develop within the tooth row (Jernvall and Thesleff, 2000).

Mammalian teeth are initiated from the ectoderm covering the maxillary, medial nasal and mandibular process and they form single rows in the upper and lower jaws. The early stages of tooth development morphologically resemble other epithelial appendages, such as hair and glands. In all such organs, development is regulated by interactions between the epithelial and underlying mesenchymal tissues (Jernvall and Thesleff, 2000). In the case of the mammalian dentition, the underlying mesenchyme is in fact neural crest cell derived ectomesenchyme. Tooth buds form in a row along the dental lamina and begin as discrete swellings which give rise to the enamel organs of the future developing teeth.

1.3.3 DENTAL PATTERNING

Patterning is a key component of embryological development, from the initial axial (rostro-caudal) specification of the embryo through its segmentation and ultimately to the development of the dentition (Ten Cate, 1998). As discussed in section 1.3.1 tooth development is initiated early in embryogenesis and is a result of the interaction between ectoderm and neural crest derived ectomesenchyme. In all mammalian dentitions, the teeth form a meristic series, in which the members of each group (incisors, canines, premolars and molars) have graded characteristic shapes and sizes according to their position along the dental arch (Cobourne, 1999). Like all aspects of embryogenesis, dental patterning is tightly controlled, and although transpositions between teeth of different classes can occur, it usually involves teeth at the border of a particular series (e.g. canines and premolars). More severe anomalies (e.g. molars developing at the front of the arch) have not been reported (Cobourne, 1999).

Classically, two theories have been proposed to account for this tight regulatory control. The field theory (Butler, 1939) suggests that all tooth primordia are initially equivalent and it is the varying concentrations of morphogens in the local environment that subsequently determine the local environment which then determine the individual shapes of teeth. A number of diffusible signaling molecules have been identified that may be involved in concentration dependent, threshold response mechanisms which could produce periodicity along developing dental axes (Cobourne, 1999). However, if these mechanisms are responsible for patterning in both dentitions then they must act very early on in development as the maxillary incisors develop in the medial nasal processes, in contrast to the remainder of the maxillary dentition developing in the maxillary processes of the first arch (Weiss et al., 1998).

The second theory of dental patterning proposed by Osborn is the clonal model, in which the tooth primordia are said to be prespecified, implying that each migrating cell population is equipped with the necessary positional information to produce the different classes of teeth from inception (Osborn, 1978). In this model attention has focused on the migrating neural crest derived ectomesenchyme as the cells with the necessary prespecified induction agents to bring about initiation and patterning of the dentition (Cobourne, 1999).

Ignoring the dilemma posed by the patterning of the dentition in the anterior maxilla (derived from the medial nasal process), most of the current evidence concurs with Butler's field theory. For example, recombination experiments in first branchial arch explants in mice (Lumsden, 1988; Mina and Kollar, 1987) showed that the oral ectoderm was able to induce tooth development in non-dental neural crest derived ectomesenchyme at an early embryonic stage (before E 12.5), whereas later (after E12.5), this potential shifted to the ectomesenchyme, which was able to instruct non-dental ectoderm to form tooth-specific structures. Conversely, dental ectomesenchyme at an early stage (before E12.5) could only induce odontogenesis in oral ectoderm and not in non-oral ectoderm (Lumsden, 1988). More recent evidence in mice points to the control of signaling that determines the oral-aboral (non-oral) polarity commencing in the oral epithelium (*Fgf*8 is thought to be the most likely candidate gene), which in turn restricts the expression pattern of two homeobox genes (*Lhx*6 and *Lhx*7) in the underlying oral-specific branchial arch ectomesenchyme (Grigoriou et al., 1998; Tucker and Sharpe, 1999).

Care should be taken in interpreting these results. The oral ectoderm certainly seems to be responsible for the initiation of tooth development. However, the patterning

(determining location, number and shape of teeth) must depend to a certain extent on signals prespecified in the underlying ectomesenchyme, interacting with the oral ectoderm (Thesleff, 2000; Tucker and Sharpe, 1999). To what extent the expression of signaling molecules in the oral ectoderm is restricted (regulated) by signals from the underlying ectomesenchyme is still not completely understood (Tucker and Sharpe, 1999).

Patterning is both a spatial and temporal event as exemplified by regional development of incisors, canines, premolars and molars, which occurs at different times and involves the classical processes of induction, competence and differentiation (Ten Cate, 1998). The process that initiates differentiation is induction. Furthermore, in order to respond to the induction process, cells must be competent. There is evidence that over time populations of embryonic cells vary their competence, from no response to maximum response and then back to no response, creating windows of competence of varying duration for different populations of cells (Ten Cate, 1998).

Using probes composed of specific nucleic acid sequences, recombinant DNA technology can identify whether specific genes are transcriptionally active (expressing their gene product). By using specific antibodies for specific proteins, immunocytochemistry provides precise identification and localisation of molecules within a cell. These two technologies have led to the recognition of a number of homeobox genes and growth factors that play a crucial role in embryological development. These regulatory molecules act in concert in order to achieve the necessary developmental pattern and subsequent morphogenesis of a particular tissue, organ or organ system, including the dentition.

1.3.4 THE GENETIC BASIS OF ODONTOGENESIS AND DENTAL PATTERNING

The initiation and morphogenesis of teeth, like the development of the whole embryo is under strict genetic control (Thesleff, 2000). The mammalian dentition is a periodically arranged (segmental) organ system whose components are arrayed in specific number and in regionally differentiated locations along the linear axes of the jaws (Weiss et al., 1998). The periodic nature of the location, number and morphological structure of teeth (i.e. dental patterning) suggests that processes involving the quantitative interaction of diffusible signaling factors may be involved (Weiss et al., 1998).

Development of the mammalian tooth has been intensively studied as a model system for epithelial-mesenchymal interactions during organogenesis and progress has been made in identifying key molecules involved in this signaling (Ferguson et al., 1998). As a result, genes that regulate tooth development are being identified with increasing speed. Over 200 genes are now known to have expression patterns during tooth development (Nieminen et al., 2004).

Typically in embryonic development, initiation and control is mediated by interacting pathways of extracellular signaling molecules, their receptors, intracellular signaling proteins and nuclear transcription factors and the same is true of dental development (Sharpe, 2000). Transcription factors are a large class of proteins that regulate gene transcription i.e. the synthesis of RNA, the template from which proteins are synthesised, from DNA. The first family of developmental genes to be discovered was the homeobox gene family, all of which code for a specific type of transcription factor. These genes

have been the subject of intensive investigation to developmental biologists over the last 20 years.

The homeobox is an evolutionarily conserved, 180bp DNA sequence first discovered in Drosophila genes in 1984. The discovery of the homeobox was an important milestone because it demonstrated that genes controlling fly development can also be present in vertebrates. It has become apparent that this same class of homeobox genes regulate the development of all animals, indicating that they have been evolutionarily conserved (Thesleff, 2000). This allowed progress in the understanding of vertebrate development to proceed at a far greater pace than was ever thought possible and was achieved by cloning genes through nucleotide homology with fly genes (Sharpe, 2000). Not only are homeobox genes conserved across species, the same genes have also been found to regulate numerous developmental processes in the same animal (Thesleff, 2000).

The homeobox genes code for DNA binding proteins known as homeoproteins. These homeoproteins exert their effect in many inductive tissues during embryogenesis by regulating gene transcription and therefore exerting a hierarchical control function over the expression of genes required for morphogenesis (Sharpe, 2000). The homeobox itself codes for the homeodomain, a 60 amino acid helix-turn-helix DNA binding motif (Cobourne, 1999). Many vertebrate homeobox genes have been cloned by nucleotide homology but there is an important distinction between homeobox genes that are known to be homeotic and others that are not. Homeotic genes, also known as *HOX* genes, exhibit a feature know as colinearity, in which their spatial arrangement along the chromosome is in the same order as their patterns of expression along the anteroposterior axis, as demonstrated in the fly embryo (Lewis, 1978).

Mutation in homeotic genes thus affects segment identity and results in homeotic transformation, in which one body part is transformed into another (Maas and Bei, 1997). For example, mutation of the antennapedia gene in *Drosophila*, which specifies identity of the second thoracic segment but is also expressed in the head, results in growth of legs from the head sockets (Cobourne, 1999). In mammals, it appears that several classes of homeobox genes, still highly conserved but more divergent than homeotic genes, are also expressed during embryogenesis in patterns which correlate with roles in patterning of the embryo. In other words, these homeobox genes tend to be unclustered within the genome, in contrast to the homeotic genes. Targeted mutation of several of the unclustered (non-*HOX*) homeobox genes has shown that they have an important role to play in craniofacial development (Thomas et al., 1998). As mentioned previously, many of the genes expressed during odontogenesis have now been elucidated from mice studies. However, the mechanism by which these multiple signaling pathways are initiated is still not fully understood. Thesleff provides an excellent schema that relates the timing of expression of these genes to the tissue of expression and stage of odontogenesis (Thesleff, 2000).

A model on how conserved signaling molecules mediate sequential and reciprocal interactions between oral epithelium and ectomesenchyme in the initiation and patterning of the dentition has recently been described (Thesleff, 2003). In this model, signaling molecules are seen as the key regulators of tooth development, and the four most studied families of signals, FGF, BMP, hedgehog (*Hh*) and Wnt, exert their control by regulating the activity of transcription factor genes like the homeobox genes and others.

Some of the key transcription factor genes involved in the initiation and patterning of odontogenesis include the homeobox genes *PITX*2 (pituitary homeobox 2 also known as

Otlx-2 or *RIEG*), *RUNX2* (previously *cbfa*l) and *MSX1* (muscle segment homeobox gene), as well as other transcription factors such as *PAX9* (paired box gene) and p63. These genes all code for proteins that regulate the expression of various genes that are involved in the signal transduction process between ectoderm and ectomesenchyme. Mutations of these genes are responsible for a number of diseases with associated dental effects, as shown in Table 1.4.

Table 1.4 Transcription Factor Gene Mutations Linked to Conditions Associated with Numeric Anomalies of the Dentition

Gene	Locus	Gene Type	Tissue of Origin	Disease	Dental Condition
PITX2	4q25-q26	Homeobox	Oral epithelium	AD Rieger syndrome	Oligodontia, especially upper incisors & 3's
PAX6	11p13	Paired box	Ectomesenchyme	AD Rieger syndrome	Oligodontia, teeth not specified
PAX9	14q13.3	Paired box	Ectomesenchyme	AD NSO	Oligodontia, especially molars
MSX1	4p16.1	Homeobox	Ectomesenchyme	AD NSO, Witkop syndrome	Oligodontia, especially 5's & U4's
RUNX2 (*cbfa*1)	6p21	Homeobox	Ectomesenchyme	AD Cleidocranial dysplasia	Supernumerary teeth
p63	3q27	Tumour protein	Oral epithelium	AD EEC syndromes	Oligodontia, teeth not specified

Table 1.4 Transcription factor genes that have to date been linked to conditions associated with numeric anomalies of the dentition. Sources: (Online Mendelian Inheritance in Man, 2002; Priolo and Lagana, 2001; Riise et al., 2001; Thesleff, 2000)

In addition, knockout experiments in mice have linked mutations in several other homeobox genes, including Dlx (distal-less homeobox), Lhx6 and Lhx7 (Lim-homeobox domain genes) and Barx1 to oligodontia, as illustrated in Table 1.5.

Table 1.5 Transcription Factor Gene Mutations in Knockout Mice

Gene	Locus	Tissue of Origin
*Dlx*1	2q32	Ectomesenchyme
*Dlx*2	2q32	Ectomesenchyme
*Lhx*6	9q34.11	Ectomesenchyme
*Lhx*7	1p31.1	Ectomesenchyme
*Barx*1	9q12	Ectomesenchyme
*Msx*2	5q34-q35	Ectomesenchyme
*Lef*1	4q23-q25	Ectomesenchyme

Table 1.5 Transcription factor gene mutations associated with oligodontia from knockout experiments in mice. Genes and tissue of origin: (Thesleff, 2000), loci: OMIM

Many extracellular signalling molecules that communicate with and help regulate the expression of these transcription factors have also been shown to play an important role in the initiation of dental development. These signals, many of which are growth factors, exert their effects on target cells by interacting with surface receptors. Target cells must therefore express these specific receptors in order for the cell to capture these molecules.

Once captured, the receptor interacts with a cascade of membrane and cytoplasmic-bound signalling molecules until finally a transcription factor is activated to bring about an alteration in gene expression and thus induce specific cell behaviour. Mutations in genes coding for these extracellular molecules have been linked to a number human conditions illustrated in Table 1.6.

Table 1.6 Extracellular signalling ligand and receptor gene mutations in Humans

Gene	Locus	Gene Function	Origin	Disease	Dental Condition
EDA	Xq12-q13.1	TNF signal	Oral epithelium	X-linked HED	Oligodontia, any teeth
EDAR	2q11-q13	TNF receptor	Oral epithelium	AD or AR HED	Oligodontia, teeth not specified
XEDAR	X chromosome	TNF receptor	Unknown	X-linked HED	Oligodontia, teeth not specified
SHH	7q36	Signalling molecule	Oral epithelium	Holoprosencephaly, solitary median maxillary central incisor	Solitary median maxillary central incisor

Table 1.6 Extracellular signalling ligand and receptor gene mutations linked to human conditions associated with oligodontia data obtained from OMIM, Thesleff (Thesleff, 2003) and Mikkola and Thesleff (Mikkola and Thesleff, 2003)

Two of the genes that code for these extracellular signalling molecules are particularly important in the development of ectodermally derived tissues, namely *EDA* and *EDAR* Mutations in either of these genes have been shown to be responsible for HED (*EDA* for the more common X-linked form of the disease and *EDAR* for both autosomal dominant and recessive forms of the disease). In addition, the mouse homolog of both of these genes has been identified (Tabby or *Ta* for *EDA* and Downless, or *Dl* for *EDAR*), which has enabled researchers to carefully characterise the clinical phenotype of both these genetic conditions.

Knockout experiments in mice have identified a number of other growth factors that play an essential role in initiation of dental development. These include fibroblast growth factor (*FGF*) and transforming growth factor (*TGFβ*) isoforms, as seen in Table 1.7.

Table 1.7 Signalling ligand and receptor gene mutations in knockout mice

Gene	Locus	Gene Type	Tissue of Origin
*Fgf*8	10q24	Fibroblast growth factor	Oral ectoderm
*Bmp*4	14q22-q23	Bone morphogenetic protein (member of the *TGFβ* family)	Oral ectoderm
ActivinβA	7p15-p13	Growth factor (member of the *TGFβ* family)	Ectomesenchyme

Table 1.7 Signalling ligand and receptor gene mutations associated with oligodontia from experiments in knockout mice. Data obtained from OMIM and Thesleff (Thesleff, 2003).

Other genes that have been linked to diseases with an associated oligodontia are the intracellular signaling molecules *EDARADD* (*EDAR* associated death domain) and *NEMO* (nuclear factor-κB essential modulator, previously *IKKγ*) and the cell adhesion molecule *PVRL*1 (nectin-1). See Table 1.8.

Table 1.8 Miscellaneous gene mutations associated with oligodontia

Gene	Locus	Gene Function	Disease
EDARADD	1q42.2-q43	Intracellular signaling molecule	AR HED
NEMO	Xq28	Intracellular activator of NF-κB transcription factor	XL Incontinentia pigmenti, EDA ID
*PVRL*1	11q23-q24	Cell adhesion molecule	AR CLPED syndromes
DTDST	5q32-q33.1	Sulfate transporter	Diastrophic dysplasia
He Zhao	10q11.2	Unknown	NSO, any teeth

Table 1.8 Miscellaneous gene mutations linked to diseases associated with oligodontia. Data sources: (Karlstedt et al., 1996; Liu et al., 2001; Mikkola and Thesleff, 2003; Priolo and Lagana, 2001)

There are also a number of intracellular signaling molecules that have been shown to cause oligodontia in knockout mice, and these are shown in Table 1.9.

Table 1.9 Intracellular Molecule Gene Mutations in Knockout Mice

Gene	Locus	Gene Type
*Traf*6	11p13	TNFR associated intracellular signaling molecule
*Traf*3	14q32	TNFR associated intracellular signaling molecule

Table 1.9 Intracellular molecule gene mutations associated with oligodontia from knockout experiments data sources: (Naito et al., 2002; Ohazama et al., 2003).

In addition, the retinoic acid family, a group of molecules with the same structural origin as vitamin A, freely enter the cell to complex with intracellular receptors, which eventually go on to affect gene expression. Retinoic acids have an effect on branchial arch epithelium and other regions of the embryo, including the axial skeleton and limbs. Growth factors and retinoids both regulate the expression of homeobox genes, which in turn regulate the expression of growth factors, an example of a regulatory loop (Ten Cate, 1998).

Of the numerous transcription factor genes described in Table 1.4 and 1.5, the homeoprotein *Pitx*2 is thought to have an important part to play in the determination of regionalization and initiation of dental lamina thickening in the oral ectoderm. *Pitx*2 has been shown to be a positive regulator of *Fgf*8 and a repressor of *BMP*4 signaling that occurs in the presumptive jaws from E9 to E10.5 in the mouse, at the time of the first histological detection of dental lamina thickenings (Liu et al., 2003; Lu et al., 1999; Tucker and Sharpe, 1999).

FGF and *BMP* isoforms subsequently regulate the expression of *PAX* 9 (*FGF*8 stimulates in the molar region, *FGF*9 the incisor region, *BMP*2 and *BMP*4 inhibit *PAX9*) (Mandler and Neubüser, 2001; Neubüser et al., 1997), *Lhx*6 and *Lhx*7 (*FGF* stimulates) (Grigoriou

et al., 1998; Mandler and Neubüser, 2001), *Barx*1 (*FGF* stimulates, *BMP* inhibits) (Mandler and Neubüser, 2001; Tucker et al., 1998) and *MSX1* (*FGF* not essential, *BMP* up-regulates) (Mandler and Neubüser, 2001; Vainio et al., 1993).

1.4 Molecular Genetic Research

1.4.1 INTRODUCTION TO MOLECULAR GENETICS

Genes are the units of transmission of hereditary characteristics among all organisms. They are molecules that code for and thereby specify how each cell within every organism should behave. Medical genetics deals with the diagnosis and management of hereditary disorders and with genetic variation that is of significance in the practice of medicine and medical research. Molecular genetics is a subset of medical genetics that deals with the study of the structure and function of individual genes.

Genetic information is packaged on chromosomes, very long strands of DNA (deoxyribonucleic acid) that reside within the nucleus of each cell. Genes make up only about 5% of the total DNA found on chromosomes (Nussbaum et al., 2001). The DNA strands are composed of a linear sequence of subunits known as nucleotides. The number of nucleotides in a gene or any other DNA sequence is therefore a measure of its length and is described in terms of numbers of base pairs, in reference to the four different bases that make up each of the nucleotides.

The human genome (the complete human DNA sequence) is comprised of approximately three billion base pairs of DNA. With the exception of cells in the germline, all other cells that contribute to the human body (known as somatic cells) have 23 pairs of chromosomes. Excluding mitochondrial DNA, the human genome is derived from these 46 chromosomes that are located in the nucleus of every somatic cell in the body. Of these 23 pairs, 22 are alike in males and females and are called autosomes, while the remaining pair comprises the sex chromosomes XX in females and XY in males (Nussbaum et al., 2001).

Each chromosome pair is a mixture of DNA inherited from both parents that is a result of two mechanisms that occur during meiosis of the germ cells (spermatocytes of males and oocytes of females). See Fig 1.10.The first mechanism is known as independent assortment of paternal and maternal homologs in which, during the first division of meiosis (meiosis I), each of the 23 homologous pairs of chromosomes enters one of two daughter cells (gametes) independently, producing a possible 2^{23} (about 8.4 million) combinations (Strachan and Read, 1999). The second mechanism is recombination in which, during meiosis I, the homologs (after forming a synapsed complex known as a bivalent) exchange chromosomal segments between each other in a random way (Strachan and Read, 1999). Normally, recombination occurs at least once in each chromosome arm during meiosis (Strachan and Read, 1999). The oocyte and spermatocyte gametes that come together during fertilization to form a zygote are therefore one of a virtually unlimited number of possible combinations produced by each parent.

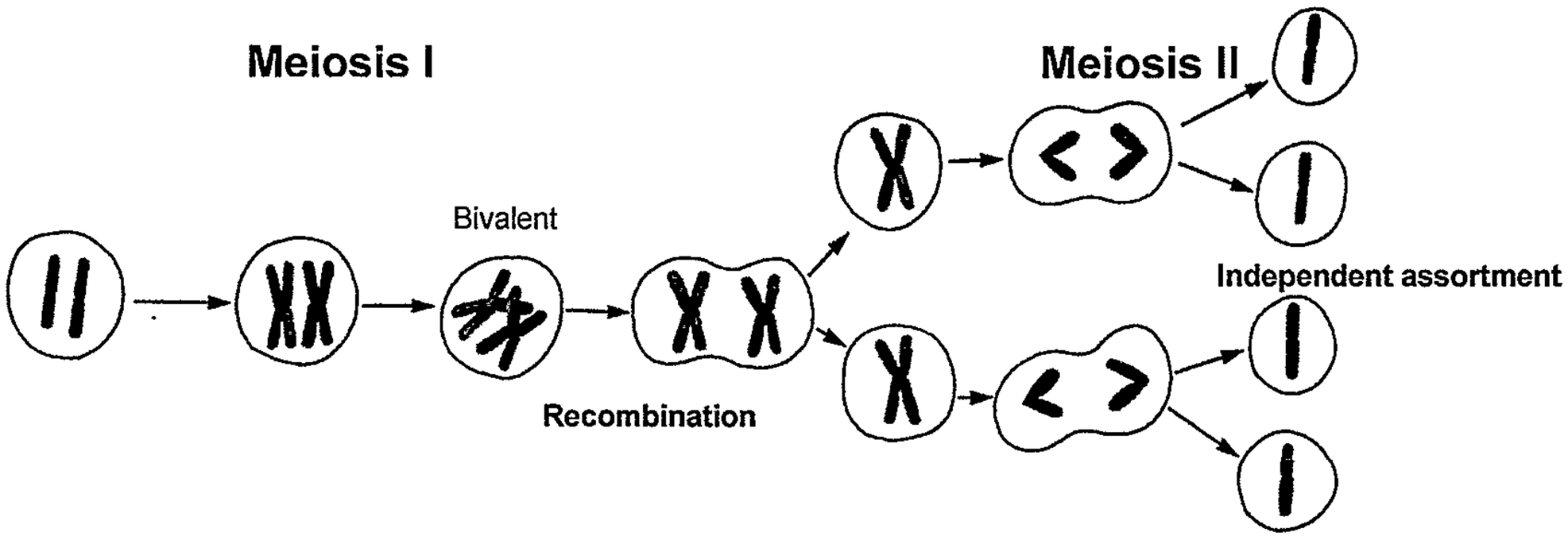


Fig 1.10 Diagrammatic representation of meiosis for a single chromosome pair. The mechanisms of independent assortment and recombination that contribute to genetic variability occur during meiosis of the spermatocyte and oocyte germ cells. Adapted from Kingston (Kingston, 1990).

Spontaneous mutations at a particular locus of the genome can occur either as a result of errors introduced during the normal process of DNA replication, or from a failure to repair DNA damage (Nussbaum et al., 2001). A mutation is any permanent heritable change in the sequence of genomic DNA. If a mutation actually changes the sequence of amino acids in the protein it codes for, it is said to be a missense mutation. Overall, missense mutations account for almost half of all mutations reported to cause genetic disease in humans (Nussbaum et al., 2001).

The same mutation may result in different effects for different individuals. Clinically, this can result in some individuals with the genotype not expressing the disease at all, a phenomenon known as reduced penetrance. In those individuals with the genotype who do show signs of the phenotype, the clinical severity or progression of the disease may be extremely variable and this is known as variable expressivity. For many years, oligodontia has been reported as a genetic condition characterised by reduced penetrance and variable expressivity (Grahnen, 1956). Reduced penetrance of approximately 90% has recently been reported for a unique form of hypodontia not linked to *PAX9* or *MSX1*

(Frazier-Bowers et al., 2003). Evidence for variable expressivity of oligodontia is frequently illustrated by pedigree studies that report different numbers and types of congenitally absent teeth for the same genotype (Das et al., 2002; Frazier-Bowers et al., 2002; Lidral and Reising, 2002; Stockton et al., 2000; Vastardis et al., 1996).

1.4.2 MOLECULAR GENETIC LABORATORY TECHNIQUES

1.4.2.1 DNA Markers: Microsatellites and Single Nucleotide Polymorphisms (SNP's)

Polymorphisms (the occurrence of two or more alleles at a given locus that is not due to mutation alone) can be utilized to great effect in molecular genetics research. The ability to distinguish different inherited forms of a gene or different segments of the genome provides tools that are crucial for a wide array of applications, including mapping a gene to a particular region of a chromosome by linkage analysis, prenatal diagnosis of genetic disease and detection of heterozygous carriers of genetic disease (Nussbaum et al., 2001). Any Mendelian characteristic that can be scored easily and efficiently and that is sufficiently polymorphic that a randomly selected individual has a good chance of being heterozygous can be used for this purpose (Strachan and Read, 1999). These DNA polymorphisms are generally known as genetic "**markers**". An early application of the use of markers to help distinguish different inherited forms of a gene that is still practiced today is the determination of blood groups.

There are two main classes of polymorphisms that are currently being used as "markers" for mapping genes involved in human disease. These are microsatellites (also known as short tandem repeat or STR polymorphisms) and single nucleotide polymorphisms

(SNP's, pronounced snips). Microsatellites are sequences of two, three or four nucleotides repeated many times, that show high levels of allelic variation in the number of repeat units and that are widely spaced across the entire human genome and can be typed using PCR amplification (see 1.4.2.2) (Gray et al., 2000). The combination of a highly polymorphic marker set (microsatellites) and rapid typing technology (via PCR) has led to the development of high throughput semi-automated systems for genotyping. An extensive linkage map of the human genome has been compiled based on highly informative microsatellite loci. This map consists of over two thousand markers that are closely spaced (5-20cM) and cover the entire genome (Weissenbach, 1993).

Because they are highly polymorphic, relatively common and evenly distributed in the human genome, microsatellite markers are ideal for linkage studies of simple Mendelian disorders. However, because these markers often span genetically broad distances, there is an increased risk of recombination events occurring between marker and disease susceptibility gene loci. For this reason, microsatellites only offer a coarse indication for the location of a gene or genes.

SNP's (single nucleotide polymorphisms) offer an alternative to microsatellites. Although there is less allelic variation with single nucleotide polymorphisms, this is more than offset by their much greater abundance in the human genome and by their frequent positioning within coding or regulatory regions of a gene. This increases the possibility of mapping the disease susceptibility gene locus more finely and makes SNP's highly suited to the analysis of polygenetic, complex, multifactorial diseases such as osteoporosis, diabetes, cardiovascular and inflammatory diseases, psychiatric disorders and most cancers (Gray et al., 2000).

1.4.2.2 Polymerase Chain Reaction (PCR) Technique

Polymerase chain reaction (PCR) is a technique for selectively and repeatedly replicating defined sequences of DNA (Russell, 1998). PCR works by enzymatically amplifying a fragment of DNA (the target) located between two oligonucleotide "primers" (single stranded nucleotide sequences) that are complementary to that fragment (Nussbaum et al., 2001). Once amplified by PCR, a DNA fragment of a certain size can be separated using various methods of gel electrophoresis.

1.4.2.3 Gel Electrophoresis

The principle of gel electrophoresis is that an electrical field applied to a porous gel matrix such as agarose or polyacrylamide induces separation of molecules based on their size and electrical charge. DNA molecules have a slightly negative charge so by placing PCR fragments through gel electrophoresis, the DNA fragments will travel through the gel from negative to positive at varying speeds, depending on their size (molecular weight). See fig 3.1.

Following electrophoresis the PCR fragments can be visualized using UV light (fig 3.1), X-ray radiation or fluorescence techniques. Since traditional slab gel electrophoresis techniques are labour intensive they are not suitable for analysis of large numbers of different PCR products but they are a quick way to detect any technical problems with a particular PCR run.

For PCR fragments that are difficult to distinguish on regular slab gels (i.e. dinucleotide microsatellites that differ by just two base pairs), new automated fluorescence-based capillary electrophoresis instrumentation has been developed. These instruments can detect fluorescent dyes attached to specific microsatellite primers that have been PCR

amplified. A laser beam excites the fluorescently labeled PCR products as they pass through the instrument's capillary (Figure 1.11). Modern automated systems are able to quantitate fragments several hundred nucleotides in length even if they differ by only one nucleotide. The particular emission frequency of each dye is transferred to a computer and computer software then determines the size of the fragments by reference to a fragment of known size (size standard) (Ziegle et al., 1992).

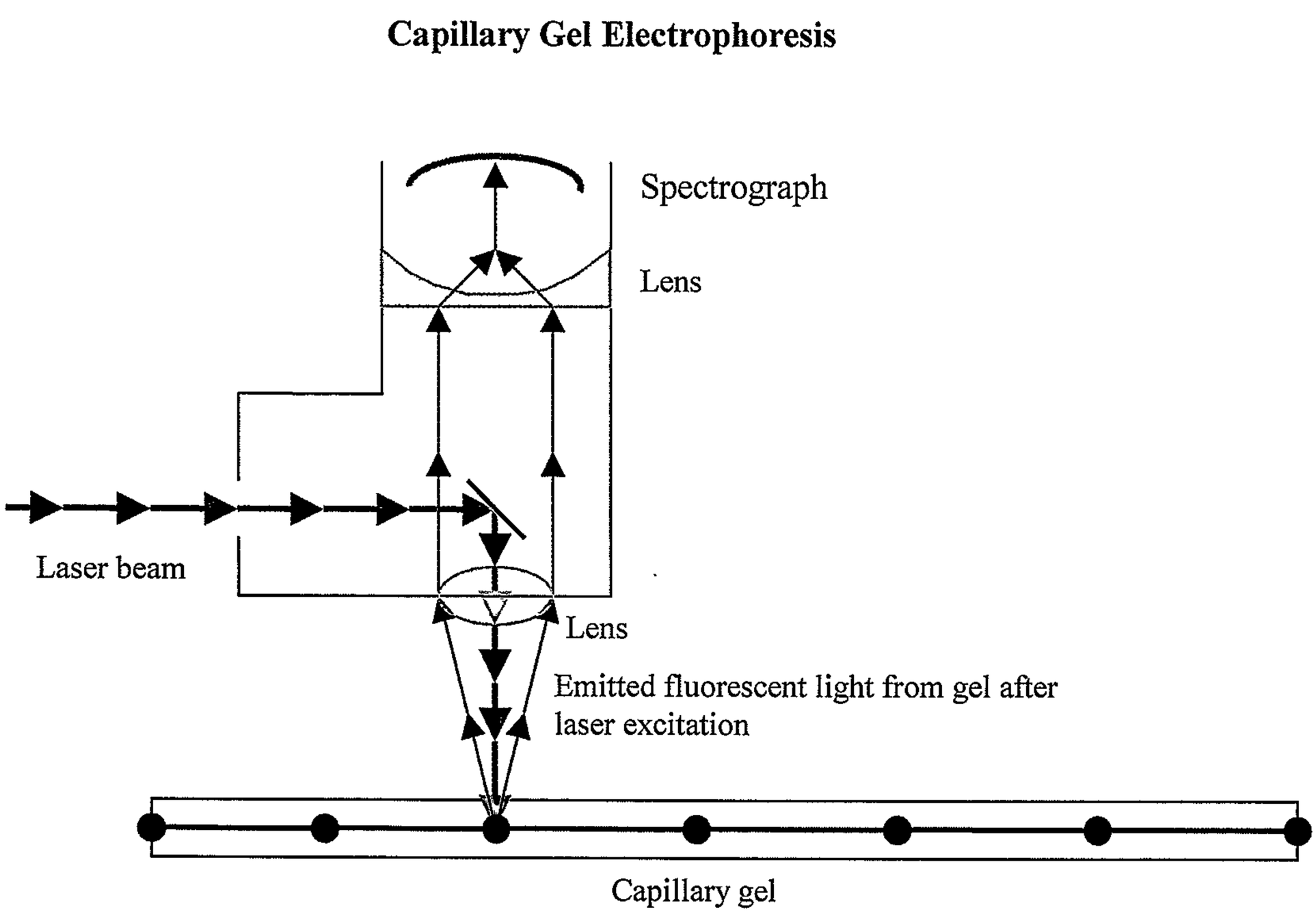


Figure 1.11. Schematic diagram of mechanism of fluorescence excitation of PCR samples traveling electrophoretically through a capillary gel

1.4.3 DATA ANALYSIS

As discussed in 1.4.2.1, microsatellite markers are well suited to mapping Mendelian characteristics in simple (e.g. monogenic) disease models. For a given pedigree with a

known inherited Mendelian condition, mapping the appropriate DNA segment that carries the gene mutation requires a **linkage analysis**. Essentially, linkage analysis compares the likelihood of whether two or more loci are assorting independently or exhibiting linkage during meiosis (segregating with disease) (Nussbaum et al., 2001).

Determining Linkage

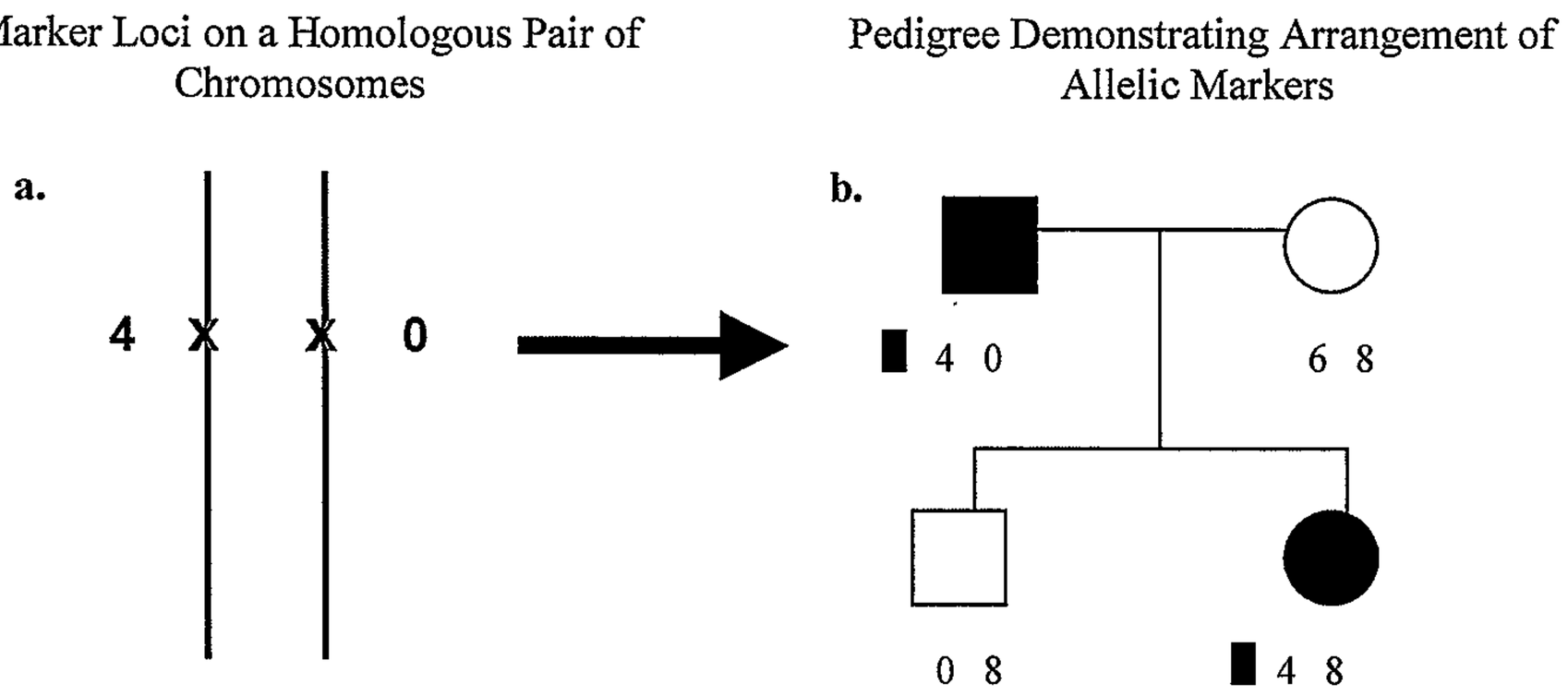


Figure 1.12 illustrates how disease can be mapped to a particular gene locus by the use of a polymorphic marker. a. A homologous pair of chromosomes belonging to the affected (coloured) male (square) in b. The numbers represent the molecular weights of the polymorphic markers that can be differentiated by electrophoresis and represent "allelic" variants. b. The pedigree shown does not demonstrate linkage but is a simplified example of how the linkage analysis technique is conducted for multiple pedigree members. In a small pedigree such as this (described as a phase unknown pedigree) there is insufficient data to assume that locus "4" is segregating with disease.

With familial linkage analysis, it is important to specify that it is loci rather than alleles or phenotypes that are analysed, as linkage is not a mutation screen but rather a rapid and efficient genetic mapping technique, a form of fingerprinting segments of DNA. As such, this implies that linkage analysis does not provide a definitive analysis of genotype but rather a likelihood of whether loci are segregating with disease. Because linkage analysis assesses recombination events, an accurate estimate of this probability is therefore dependent on obtaining DNA from as many affected as well as unaffected members of the pedigree and in as many generations as possible. With very few DNA samples from a pedigree, as in fig 1.12.b, there is not enough information to establish linkage and

mutation analysis would be required in order to make a definitive assessment of the genotype in that particular pedigree.

Another weakness with linkage analysis is that in order to detect the recombination events that allow an assessment of linkage between two loci it is necessary that a parent be heterozygous ("informative") at both loci (Nussbaum et al., 2001). Unfortunately (for linkage analysis) many informative individuals, such as an affected parent, can still be homozygous for a particular polymorphic marker.

It is also important to note that it is possible for an affected member of the pedigree to not show linkage to the suspected locus (the reverse may also occur for unaffected members) even if that segment of DNA carrying the marker does contain the disease gene. This is a result of a recombination event. Ideally, the objective of linkage analysis is to limit the location of the disease gene to a small region of a single chromosome, whereby the markers used are close to the gene so that the risk of recombination is minimized. This is best achieved with a series of closely spaced markers in the region to produce a small map of that genomic segment that is known as a **haplotype**.

1.4.3.1 Linkage by Standard LOD Score Analysis

Standard LOD (logarithm-of-odds) score analysis requires a precise genetic model, including mode of inheritance, penetrance, and disease gene frequencies (Strachan and Read, 1999). For example, a member of the pedigree may be clinically diagnosed as unaffected, but if the disease is associated with a reduced penetrance, there is a possibility that that individual may still be genotypically affected.

The rate at which two alleles cosegregate is related to the distance between them (Ott, 1985). This rate is the probability of a recombination event occurring between the two loci, where a recombination fraction (θ) of 0 equals 0% recombinants and a θ of 0.5 equals 50% recombinants (random assortment).

To determine whether two loci are linked (tending to be transmitted together) a ratio that calculates whether the recombination fraction between them differs significantly from 0.5 (the expected fraction for unlinked loci) is required. This ratio therefore compares the probabilities of two alternatives: (a) the likelihood under the null hypothesis of no linkage and (b) the likelihood of the alternate hypothesis of linkage. The ratio is usually expressed in $\log_{10}$ and is therefore called a logarithm-of-odds (LOD).

The LOD score equation is written as;

$$\mathrm{LOD}(\theta) = \log_{10} \frac{(\theta)^r(1-\theta)^n}{(0.5)^r(1-\theta)^n}$$

r=number of recombinants,
n= number of non-recombinants

(Strachan and Read, 1999)

The LOD score is calculated for several values of θ, for example θ + 0.0, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5. Where the LOD score reaches its maximum, the most likely value for θ is located. This method is called the Maximum Likelihood Estimate (MLE) method. Calculation of this likelihood ratio (or LOD score) can be extremely complicated in some cases and requires the use of computer analysis programs (Terwilliger and Ott, 1994). By convention, a combined LOD score of +3 or greater (equivalent to greater than 1000:1 odds in favor of linkage) is considered definitive evidence that two loci are linked, while a LOD score of -2 or greater excludes linkage (Nussbaum et al., 2001).

Using multiple markers (multipoint analysis) rather than a single marker (two point analysis) for a disease locus can increase the likelihood of detecting linkage to a locus underlying a disease trait, especially if the disorder is genetically heterogeneous (different defective loci causing clinically very similar diseases). Multipoint linkage analysis therefore can provide strong statistical support that a particular order of markers is correct (Nussbaum et al., 2001).

1.4.3.2 Candidate Gene Approach

The candidate gene approach is a powerful method for the molecular genetic study of a disease. Candidate genes are genes of known function (as reported in the literature) that are candidates for disease loci in a particular pedigree, based on whether that gene maps to the same region of the genome as does the disease gene, and whether the function of the gene relates in some way to the pathogenesis of the disease in that pedigree (Nussbaum et al., 2001).

1.5 Genetic Background of Oligodontia

1.5.1 INHERITANCE PATTERNS

A single gene disorder can occur sporadically or it can occur among a number of directly related family members by following one of several characteristic modes of inheritance known as Mendelian inheritance. A sporadic case is an isolated case, in which a new mutation has occurred and as a result has affected only one family member. The inheritance pattern of Mendelian disorders in pedigrees depends on two factors:

1. whether the gene locus is on an autosome or on the X-chromosome (autosomal vs X-linked)
2. whether the phenotype is dominant (expressed in heterozygote carriers to some degree) or recessive (only expressed in homozygote, ie. when both chromosomes of a pair carry the mutual mutant allele). (Nussbaum et al., 2001)

The four basic patterns of Mendelian inheritance are therefore autosomal dominant, autosomal recessive, X-linked dominant and X-linked recessive. In the case of X-linked diseases, males cannot be homozygous or heterozygous because they carry only one X-chromosome and so they are said to be hemizygotes. An example of a pedigree displaying autosomal dominant inheritance is shown in Fig 1.13.

More than half of all known Mendelian phenotypes are autosomal dominant traits, which are typically characterised by:

1. The phenotype usually appearing in every generation (each affected individual having an affected parent). Two exceptions to this rule are for when a new mutation arises (common) or when there is reduced penetrance.

2. Any child of an affected parent having 50% risk of inheriting the trait. (This does not mean that in any one family 50% of the children will be affected).
3. Phenotypically normal family members do not transmit the phenotype to their children (exception to this is for reduced penetrance or for extremely mild phenotype when there is variable expressivity).
4. Male to male transmission (this excludes the possibility of X-linked inheritance).

(Nussbaum et al., 2001)

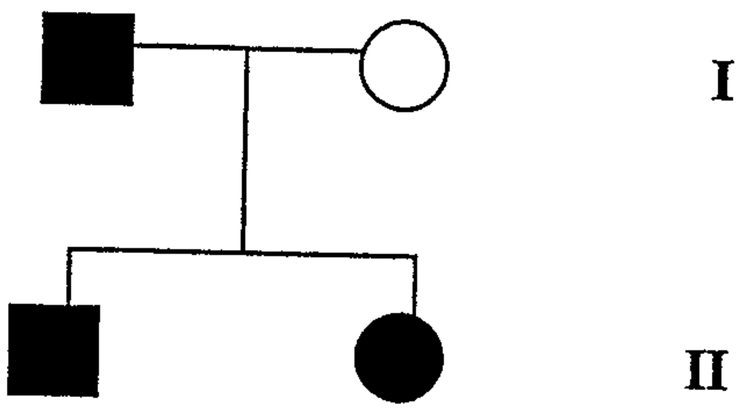


Figure 1.13 Pedigree displaying probable autosomal dominant inheritance.

A number of inheritance patterns have been reported for oligodontia conditions. Oligodontia fulfills the criteria for a genetic trait: in many patients there is no environmental aetiology, it shows a repetitive pattern in affected individuals, it is more common among related than among non-related individuals, it shows frequency differences among races and it shows differences in concordance among twins of differing zygosity (Burzynski and Escobar, 1983). It has been reported that oligodontia can occur as a sporadic case or more commonly can be transmitted as an autosomal dominant trait with incomplete penetrance and variable expressivity, or as an autosomal recessive or X-linked recessive trait (Burzynski and Escobar, 1983). For monogenic autosomal dominant oligodontia, it has been hypothesized that the reduced penetrance of 86% to 90% is a result of interference from epistatic genes (Burzynski and Escobar, 1983). Because mutations in a number of genes have been linked to NSO (see Table 1.10), autosomal dominant NSO is considered to be a genetically heterogeneous condition (Goldenberg et al., 2000).

1.5.2 CANDIDATE GENES

1.5.2.1 Non-Syndromic Oligodontia Candidate Gene Studies

Three NSO Genes have been identified by linkage analysis. See Table 1.10. The link between dental development and the *MSX1* and *PAX9* genes is well established (see 1.3.4). These highly conserved transcription factor genes play an important role on the reiterative signaling mechanisms that occur during odontogenesis.

Table 1.10 Reported Mutations in Non-Syndromic Oligodontia Candidate Genes

Gene	Mutation: Nucleotide / (*Amino Acid*)	Reference
MSX1	**c.587G>C** (*R196P*) **c.314C>A** (*S105X*) **c.605C>A** (*S202X*) **c.182T>A** (*M61K*)	(Mostowska et al., 2003)
PAX9	**c.219_220insG** (*Frameshift at aa 73, premature termination of translation at aa 316*) **PAX 9 deletion** **c.271A>G** (*K91E*) **c.62T>C** (*L21P*) **c.175_176ins288bp** (*Frameshift at aa 58, premature termination of translation at aa 177*) **c.340A>T** (*K114X*) **c.793_794ins C** (*Frameshift at aa 264, premature termination of translation at aa 315*) **c.151G>A** (*G51S*)	(Mostowska et al., 2003)
He Zhao	10q11.2 (Locus)	(Liu et al., 2001)

Table 1.10 lists all reported mutations in the NSO candidate genes *MSX1*, *PAX9* and He Zhao. Note that c.314C>A and c.605C>A mutations in *MSX1* are associated with cleft lip and/or palate and Witkop syndrome, respectively. *R*, *P*, *S*, *M*, *K* etc are the standard abbreviations for the amino acids and X represents termination of the amino acid sequence as a result of the gene mutation.

The only report of linkage to the 10q11.2 region has not yet been associated with mutations in any specific candidate gene. The authors proposed that the genes *Dkk*-1,

PRKG1B or a *KOX* zinc finger cluster are the most likely candidates (Liu et al., 2001). In addition, the oligodontia phenotype of this large Chinese kindred has not been characterised in detail, with agenesis of most permanent teeth being the only previously reported finding (Wang et al., 2000).

1.5.2.2 Autosomal dominant Ectodermal Dysplasia genes

A number of autosomal dominant forms of ED conditions are associated with a pattern of oligodontia (see Table 1.2). Autosomal dominant hypohidrotic ED, otherwise known as ED3 (OMIM 129490), is caused by mutation in the *EDAR* gene and is characterised by a mild HED phenotype (Aswegan et al., 1997; Jorgenson et al., 1987). The mutations associated with the autosomal dominant forms of ED3 are shown in Table 1.11. Autosomal recessive forms of HED exist, and these have also been linked to mutations in *EDAR* as well as to mutations in the *EDARADD* gene (OMIM 224900). The autosomal recessive disorders are known to be phenotypically indistinguishable from the X-linked forms of HED (recessive loss-of-function), whereas the autosomal dominant disorders tend to have more of a dominant-negative affect (Monreal et al., 1999).

Table 1.11 Mutations in *EDAR* Responsible for Autosomal Dominant Hypohidrotic Ectodermal Dysplasia

Gene	Nucleotide (*Amino Acid*)	Reference
EDAR	**c.1072C>T** (*R358X*) **c.1259G>A** (*R420Q*)	(Monreal et al., 1999)

Table 1.11 lists all reported mutations in the ADHED candidate gene *EDAR*. *R* and *Q* are standard amino acid abbreviations while X represents termination of the amino acid sequence as a result of the gene mutation.

Baala et al (1999) provided two main explanations for the localisation of dominant and recessive ED to the same chromosomal region:

1. A cluster of genes with related function may map to chromosome 2q11-q13. These linked genes may encode various subunits of a single multimeric complex.
2. Autosomal dominant and recessive hypo/anhidrotic ED may be allelic disorders.

(Baala et al., 1999)

Mikkola and Thesleff (2003) have recently published a comprehensive review of the function of the protein that is coded for by the *EDAR* gene and many of the other proteins involved in the EDAR and XEDAR signal transduction pathways (including EDA-A1, EDARADD, TRAF3, TRAF6 and NEMO) (Mikkola and Thesleff, 2003). Yan et al found that EDAR protein binds to the EDA-A1 ectodysplasin isoform of EDA, a tumour necrosis factor (TNF) that is coded for by the *EDA* gene (Yan et al., 2000). TNF signaling molecules are important regulators of embryonic organ development (Laurikkala et al., 2001). Mutations in the *EDA* gene have been shown to be responsible for the most common and best known ED, the X-linked form of HED, otherwise known as Christ-Siemens-Touraine syndrome (Kere et al., 1996).

Smahi et al then demonstrated that activation of EDAR triggers NF-kappa-B through the NEMO protein. NF-kappa-B is a transcription factor found to have an important regulatory role during embryogenesis. Mutation in the gene coding for its modulating protein NEMO, results in incontinentia pigmenti, another form of ED (Smahi et al., 2002). Recently, an autosomal recessive form of HED has been linked to the *EDARADD* gene, a gene that codes for another protein in the EDA-EDAR-NEMO signal transduction pathway (Headon et al., 2001).

1.5.2.3 Other Potential Candidate Genes

There are few other genetic conditions with oligodontia as a feature that require careful characterization in order to be clinically differentiable from NSO. Many of the syndromes described in Table 1.1 are associated with one or more very easily identified dysmorphic features.

Very recently, an oligodontia syndrome has been described that is associated with colorectal cancer. This autosomal dominant syndrome, known as oligodontia-colorectal cancer syndrome, has been mapped to the *AXIN2* gene, located at 17q24. The Axin-related protein conductin plays an important role in the regulation of the stability of beta-catenin in the Wnt signaling pathway (OMIM, 2004).

Given the recent elucidation of the EDAR and XEDAR signal transduction pathways and their role in the development of ectodermally derived tissues (1.3.4, 1.5.2.2), it is plausible that potential candidate genes for HED will code for proteins that are an integral component of these pathways. These would include ligands for the EDAR receptor or intracellular signaling molecules that have a direct or indirect effect on NF-kappa-B activation.

1.6 Research Outline and Objectives

1.6.1 OBJECTIVES OF THE RESEARCH

Using modern molecular genetic strategies, it was the ultimate objective of this research to investigate candidate genes for linkage to various forms of oligodontia. Approaching this study from a clinical perspective, it was considered appropriate to focus on several relatively large pedigrees with an autosomal dominant oligodontia trait (the most common inheritance pattern for isolated oligodontia) with no obvious signs of other ectodermally derived dysplasias. Ectodermal dysplasia (ED) was therefore not excluded as a possible diagnosis but rather, the expectation was that the pedigrees examined may display subtle ectodermal dysplastic features and linkage to a candidate gene would be important in confirming whether that pedigree had an isolated oligodontia or one associated with an ED. The objective of this approach was to determine whether non-syndromic oligodontia (NSO) could be differentiated from ED by a purely clinical method, or whether molecular genetic techniques were required for a definitive diagnosis.

The main clinical research hypothesis was that NSO could be differentiated from autosomal dominantly inherited ED of mild phenotype utilising clinical diagnostic methods. Once DNA was extracted from blood collected from these individuals, the main molecular genetic research hypothesis was that pedigrees clinically diagnosed with NSO would show linkage to an appropriate NSO candidate gene, whereas pedigrees clinically diagnosed with autosomal dominant ED would show linkage to an autosomal dominant ED gene.

1.6.2 RESEARCH OUTLINE

This research underwent the following protocol:

- **Clinical component**: after obtaining informed consent, a joint consultation of each subject was jointly undertaken by a paediatric dentist and clinical geneticist. The aims of this consultation were to determine a detailed medical history of each subject; to identify any extra-oral clinical features that may have an association with oligodontia, with particular focus on dysplastic, ectodermally derived structures; to carefully characterise the pattern of oligodontia and associated oral features; to obtain blood samples for extraction of DNA for molecular genetic research; and to offer and, if accepted, to provide genetic counselling. The clinical data obtained from the joint consultation were used to determine a clinical diagnosis for each pedigree and to assign affectation status to each family member within each pedigree.

- **Molecular genetics component**: once a provisional diagnosis was made, the appropriate candidate genes were selected by determining the closest fit to genotype-phenotype correlates reported in the literature. Microsatellite markers near these candidate genes were then used in order to determine whether linkage exists to that candidate gene. When no linkage to candidate genes was established, a genome-wide scan, with microsatellite markers spread 10cM apart, was undertaken in order to determine other possible linked DNA regions.

1.6.3 SIGNIFICANCE OF THE RESEARCH

This research contributes to the clinical characterisation of the non-syndromic forms of oligodontia and provides clinical markers that assist in the differentiation of NSO from hypohidrotic ED. In addition, this research provides phenotypic refinement to what is already known about the phenotype-genotype correlation of the NSO conditions through gene mapping experiments.

2. CLINICAL STUDY OF OLIGODONTIA

2.1 Material and Methods

2.1.1 STUDY DESIGN

The aim of this study was to characterise the clinical features (dental and non-dental) of large, informative families with autosomal dominant oligodontia

Characterisation of the clinical features was achieved by performing:

a) medical and dental history of all available affected and unaffected family members

b) a physical examination by a clinical geneticist

c) a dental examination by a paediatric dentist

d) an analysis of hair samples under light microscopy by a paediatric dermatologist

The methods involved in the molecular genetic component of the study are discussed in Chapter 3.

2.1.2 SUBJECT ASCERTAINMENT

Three families with autosomal dominant oligodontia were selected from a clinical database held at the Department of Paediatric Dentistry, Westmead Centre for Oral Health, New South Wales Australia. These families were initially ascertained through individuals who were referred for assessment of oligodontia. In each family, a minimum three generation pedigree had previously been recorded.

In order to be included for consideration in this study, at least one family member from each pedigree was required to have been seen recently and/or regularly in one of three Departments of Paediatric Dentistry located in the Sydney metropolitan area. These were departments of the Westmead Centre for Oral Health, the Children's Hospital at Westmead or the United Dental Hospital.

Pedigrees were selected based on their likely autosomal dominant inheritance pattern and size (larger pedigrees were desirable, as these were likely to be more informative). Pedigrees could be included if there was (a) apparently isolated oligodontia or (b) oligodontia with a suspected but unconfirmed diagnosis of ED. Where ED was suspected, pedigrees were selected only if there was a clear pattern of autosomal dominant mode of inheritance (with at least one instance of male to male transmission) and there were no other significant malformations. Pedigrees with the possibility of the most common form of ED, the X-linked form of HED, were therefore excluded from the study, as were pedigrees with a previous syndrome diagnosis (e.g. Rieger syndrome) or features suspected to be part of another syndromic form of oligodontia (e.g. cleft lip and palate).

Four suitable pedigrees were identified, on the basis of the inclusion criteria. One family could not be contacted by the commencement date of the study and was therefore excluded. All other families were contacted to determine whether they could be reached at their registered addresses prior to commencement of the study and whether they were willing to be sent further information relating to the study.

Ethics committee approval was sought initially through the Children's Hospital at Westmead from where the study was to be based, and later from the University of

Sydney, and from the Western Sydney Area Health Service, prior to commencement of the study.

The three families were then sent information sheets inviting them to participate in the study and outlining the nature and requirements of the study, as approved by the appropriate Ethics Committees. All three families agreed to participate in the study and the family members that had been contacted were encouraged to recruit as many of their direct relatives as possible, whether affected or unaffected, as this would greatly increase the power for determining linkage (see Chapter 3). In addition, unaffected members would serve as controls for the clinical assessment.

2.1.3 CONSENT

Informed consent was obtained from all participating subjects over the age of 16 years and parental consent for those below 16 years of age, regarding the study requirement.

The main requirements for subjects to participate in the study were:

1. Physical examination and history by clinical geneticist
2. Dental examination by the author
3. Intra-oral and extra-oral photographic records
4. Dental radiographic records, if unavailable or not recent
5. Alginate impressions of the dentition for plaster study model records
6. Saliva function testing, requiring the expectoration of a sample of saliva
7. Hair samples of each subject
8. Venous peripheral blood lymphocytes by venipuncture

2.1.4 CLINICAL GENETICS ASSESSMENT

All study subjects were seen by an experienced clinical geneticist (Dr Meredith Wilson, Western Sydney Genetics Program, Children's Hospital at Westmead; **MW**), who took a detailed family and personal medical history from each subject and performed a general clinical examination. The clinical history included specific questioning about a range of clinical features common in ED. Examination included a general clinical dysmorphology assessment with special attention to abnormalities of the hair, nails and skin, and teeth, and to other abnormalities known to be characteristic of specific oligodontia or ED syndromes. Additional formal dental assessment and special investigations were performed as described in 2.1.5 and 2.1.6. A clinical data sheet was completed for each patient: a copy of the Ectodermal Dysplasia Study Patient Data Sheet is provided in Appendix B.

The objective of the clinical genetic assessment was to:

1. Identify any previously described ED or oligodontia syndrome (see Table 1.1 and Table 1.2)
2. Identify any signs of ED
3. Identify any other features which were likely to be part of a genetic syndrome

The clinical geneticist (MW) initially classified subjects as clinically affected or non-affected with ED +/- oligodontia on the basis of history and clinical features observable on examination. The results of subsequent specialised techniques (microscopic examination of hair, analysis of saliva) were initially excluded by MW in order to replicate what a standard outpatient clinical genetics assessment could provide.

Subjects were then finally classified clinically based on the combined information from the clinical genetics and dental assessments, plus specialised studies of hair and saliva. Pedigrees were then classified as isolated oligodontia or ED. For pedigrees diagnosed with ED, each subject was allocated into one of the following categories:

A: AFFECTED	(1) Definite oligodontia (agenesis of ≥6 teeth, excluding third molars); +/- other signs/symptoms of ED
	(2) Suspected oligodontia (agenesis of 3-5 teeth, excluding third molars); + other signs/symptoms of ED
B: UNCERTAIN	(1) Minimal oligodontia (agenesis of ≤2 teeth, excluding third molars); + other signs/symptoms of ED
	(2) Suspected oligodontia; - other signs/symptoms of ED
C: UNAFFECTED	No oligodontia, no definite signs/symptoms of ED

4. The final assessment for the interpretation of linkage data combined clinical and pedigree data. Where a subject was classified clinically as unaffected or uncertain, but was an obligate "carrier" of AD oligodontia or ED according to the pedigree, they were classified as having inherited the AD gene for the purpose of linkage analysis. The "unaffected" patients could represent examples of decreased penetrance, and the "uncertain" patients could represent variable (decreased) expressivity.

2.1.5 DENTAL ASSESSMENT

A proforma was devised to efficiently obtain the necessary dental data and to avoid overlap with the clinical genetic assessment. This proforma was developed by the author (**MM**) in consultation with the supervising clinical consultants for this study (Dr Meredith Wilson, Western Sydney Genetics Program, Children's Hospital at Westmead; **MW**; and Associate Professor Richard Widmer, Department of Dentistry, Children's Hospital at Westmead; **RW**) and is shown in Appendix A.

Based on a review of the literature on oligodontia discussed in 1.2.1 and 1.2.4, it was clear that diagnosing familial oligodontia would require a careful assessment of all members of the pedigree in terms of:

- whether it was "normal" to have any particular teeth congenitally absent, based on an assessment of truly unaffected members (e.g. spouses of affected members)
- the "normal" range for the timing of tooth formation for each individual and its relevance to younger pedigree members that may not have yet completed dental development
- any other dental anomalies associated with suspected affected members, including conical shaped teeth, microdontia, taurodontism enamel hypoplasia or hypomineralisation, as discussed in 1.2.4 and 1.2.5.3

Because oligodontia is associated with these other dental findings and because tooth agenesis can occur coincidentally in unaffected pedigree members, it was necessary to establish a set of diagnostic criteria for familial oligodontia so that the affectation status of all pedigree members could be more accurately determined. The criteria used in this study to ascribe oligodontia affectation status to pedigree members were:

- Any pedigree member with evidence of agenesis of six or more teeth excluding third molars (8's) in either the primary or permanent dentition was diagnosed with oligodontia and was automatically regarded as affected, irrespective of any other dental findings
- Any pedigree member with evidence of agenesis of three to five teeth excluding 8's in either the primary or permanent dentition was diagnosed with suspected oligodontia but in order to be regarded as definitely affected, evidence of associated dental anomalies typical of other affected pedigree members was essential
- Any pedigree member without any tooth agenesis or with evidence of agenesis of two or less teeth excluding 8's in either the primary or permanent dentition was regarded as unaffected (i.e. diagnosed with coincidental tooth agenesis) unless significant dental anomalies were present that were not inherited from known unaffected pedigree members. Significant dental anomalies included microdontia of at least three teeth and of more than one tooth class, significant taurodontism or cervical constriction as observed in affected subjects
- A diagnosis of HED was suspected for a particular subject when the clinical geneticist (**MW**) in her experience believed that two or more HED features were clinically significant (as noted in 1.2.5.3, there are no known analyses for ectodermal structures that are of sufficient sensitivity or specificity). Any subject with a suspected diagnosis of HED was confirmed affected if oligodontia was confirmed (see 2.1.4).

The objectives of the dental assessment were therefore to determine:

1) As accurately as possible, the number of congenitally absent teeth for each subject. The accuracy would depend to a large extent on the age of the patient, the written and radiographic records available (a radiographic series over a number of years, ideally commencing during the primary dentition stage, will greatly minimize the risk of misdiagnosing tooth agenesis in comparison to dental extraction), and the ability to undertake an accurate dental history (which becomes increasingly unlikely with older subjects).

2) Any discrepancies in the size and appearance of all teeth present in the mouth, such as enamel defects, microdontia or abnormal shaped teeth. Microdontia is a commonly reported associated finding in patients with oligodontia (see 1.2.4, 1.2.5.3). Two simple tests were used to identify microdont teeth from the study models taken (see 2.1.6). For incisors, canines and premolars, a size comparison was made between unaffected and suspected or affected oligodontia patients. If a clinically significant discrepancy was noted by both examiners independently **(MM, RW)**, that tooth was considered microdont. For primary and permanent molars, the test involved determining whether the crown of the tooth was small enough to allow the smallest of a series of prefabricated, stainless steel crown forms (3M^{TM}) to fit over the occlusal surface of the tooth (Fig 2.1).

3) Any radiographic discrepancies of the teeth, such as taurodontism or cervical constriction reported to be associated with HED (see 1.2.5.3).

4) Any significant disturbances in the dental occlusion that may be a result of ectopic eruption of teeth, localised crowding or infra-occlusions (see 1.2.4.1).

5) Any discrepancies in the oral soft tissues such as oral inflammatory lesions, including gingivitis or periodontitis, or any other form of oral pathology.

6) Any jaw bone discrepancies such as marked retrognathia, clefting or narrowing of the palate.

7) Any craniofacial dysmorphic features such as frontal bossing, prominent ears, sparse or fine facial or scalp hair, or periorbital/perioral wrinkling or hyperpigmentation.

Prefabricated Stainless Steel Crown Form

Figure 2.1 Example of a 3M™ prefabricated, stainless steel crown form used to assess tooth size in pedigree members. The crown form sizes developed by the manufacturer range from 9.2 to 11.4mm in width for primary second molars and 10.7 to 12.8mm in width for first and second permanent molars. Up to an additional 2mm of reduction in tooth width is required in order for these crown forms to fit over a tooth. For this study, any primary second molar that permitted the 9.2mm crown form to seat over the entire occlusal surface (without any tooth destruction) was considered microdont. For permanent first and second molars, any tooth that allowed the 10.7mm crown form to seat over the entire occlusal surface was considered microdont.

2.1.6 SPECIAL INVESTIGATIONS

As listed in 2.1.3, a number of special investigations were included in the study to add further clinical characterization of each subject's phenotype. The ability to distinguish ED or other syndromes from NSO is of great clinical importance (see 1.2.5). In particular, the clinical features of ED/HED can be very variable, mild or absent due to variable expressivity and decreased penetrance of the ED phenotype. In this study, clinical analysis was conducted jointly by a paediatric dentist (**MM**) and a clinical geneticist (**MW**). A paediatric dermatologist with a special interest in analysis of hair shaft anomalies (Dr Maureen Rogers, Department of Dermatology, Children's Hospital at Westmead; **MR**) also participated in the study by providing hair shaft analysis reports for all subjects. The purpose and procedure undertaken for each special investigation is listed in Table 2.1.

All special investigations except hair sample analysis were undertaken by the author (**MM**). Clinical photographs, radiographs and study models were very valuable special investigations as part of the dental assessment, as they allowed inter-examiner assessment for concordance between the author and another paediatric dentist (**RW**). For the hair sample analysis a small number of scalp hairs were taken from an unobtrusive site of the head of each subject and sent to a specialist paediatric dermatologist (**MR**) for microscopic analysis.

Because of the reported association between HED and decreased saliva production (see 1.2.5.3), a qualitative and quantitative saliva function test was also performed in order to provide greater detail to the clinical assessment process. The saliva function test was supplied by GC™ (see Fig 2.2) and consisted of 5 separate assessments, namely visual inspection of oral hydration, visual inspection of unstimulated saliva production from the lip mucosa, saliva pH, saliva buffering capacity and stimulated saliva volume over a 5 minute period. See Table 2.1.

Saliva Test Kit

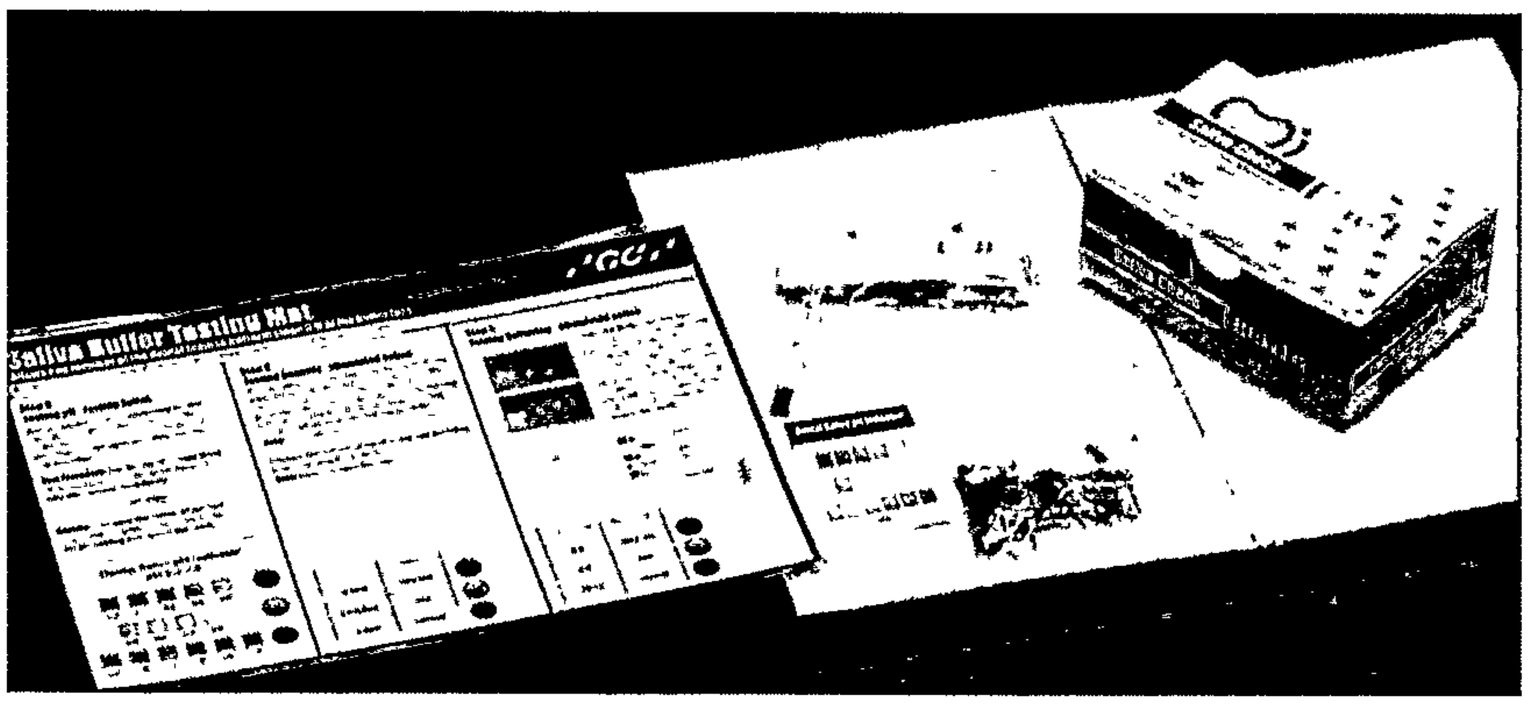


Fig 2.2 GC saliva test kit comprising of pipettes, test tubes, paraffin wax for saliva stimulation, litmus paper, buffer test strips and colour-coded pH and buffer indicators

No formal documentation of sweat gland (eccrine) function was attempted. As discussed in 1.2.5.3, previously described methods for measuring eccrine function in ED include magnification of fingertips or taking silicone palm imprints to highlight eccrine ostia, assessment of sweat gland production by the starch-iodide paper test, and skin or scalp biopsy to document hypoplastic or absent eccrine glands. We considered the possibility of using the starch-iodide test as an additional non-invasive procedure. This technique involves exposing a vinyl-gloved hand to an infrared light source for 5 minutes. The glove is removed, and the palm immediately placed on a piece of specially prepared starch-iodide paper. Purple discoloration marks indicate sweat production. Based on these marks, sweating can be scored as nil, minimal, moderate or intense (Rouse et al, 2004).

Table 2.1 Special Investigations Performed

Investigation	Purpose	Procedure
Intra-oral and extra-oral photographs	Allows inter-examiner reliability testing for diagnosis of any dental or craniofacial dysmorphology and generates a permanent baseline record of each subject.	Standard dental alginate impression followed by pouring of impression with yellow stone to create a cast.
Dental radiography	Allows detection of caries, unerupted teeth, cervical constriction of teeth, taurodontism or any other root anomalies, and any possible jaw bone pathology	Standard intra-oral plane film and extra-oral panoramic radiographs
Study models	Allows inter-examiner reliability testing for diagnosis of any dental or craniofacial dysmorphology and generates a permanent baseline record of each subject. Study models also allow re-assessment of occlusion.	Digital camera (1.0 megapixels, 1:2.8-4.9 zoom) under adequate lighting in a dental surgery.
Saliva function testing	Provides a quantitative and qualitative record of each individual's saliva production capacity and function, allowing cross-comparisons between individuals of the same pedigree and between different pedigrees.	GC™ Saliva Function Test kit used according to manufacturer's instructions. Test kit consists of a scoring method for 1. saliva; 2. unstimulated flow; 3. stimulated saliva production; 4. pH; and 5. buffering capacity.
Hair sample analysis	Provides data on hair structure at a microscopic level that supplements the macroscopic assessment of hair	Investigation under light microscopy.

We decided against using the sweat-iodide paper test because:

(1) none of the investigators had previous experience with this technique, and our expert dermatologist colleague (**MR**) advised that the results were difficult to interpret

(2) no useful literature documenting the sensitivity or specificity of this techniques could be found at the time our study was performed.

Rouse et al (2004) subsequently published a comparative study of techniques to document eccrine function in ED. The starch-iodide test had a sensitivity of 44%, a specificity of 93%, a positive predictive value of 92%, and a negative predictive value of 50%. There was a wide standard deviation in results of the starch-iodide paper sweat test, reflecting the poor predictive value with regard to ED, and poor correlation with other methods. They concluded that non-invasive methods such as hair analysis and sweat testing are not sensitive or highly specific, so obvious abnormality can only support the diagnosis of HED, not confirm it.

2.1.7 BLOOD SAMPLE COLLECTION

At the completion of the clinical genetic and dental consultation, each subject was required to have approximately 10mL of peripheral blood taken from which DNA could be extracted. The blood samples were stored in EDTA and then transferred to the molecular genetics laboratory so that the DNA extraction process could commence.

2.2 Results

2.2.1 THREE AUTOSOMAL DOMINANT OLIGODONTIA PEDIGREES

A total of 26 subjects from three different pedigrees participated in this study. All subjects were Caucasian and of European descent. Pedigree maps for each of these families are displayed in fig 2.3, 2.4 and 2.5. Ascertainment of pedigree members was generally successful but some family members did decline or were unavailable to participate, as summarised below:

Pedigree 1: Father of III.7 (divorced from mother)

Pedigree 2: Mother of II.2 (unwell, in nursing home), brothers and sister of II.2 (live in Ireland and UK)

Pedigree 3: Brothers and sisters of II.6 and their respective children declined to participate

The data collected from all subjects, including the results of the special investigations, was used to make a classification of each pedigree's type of oligodontia (NSO or syndrome/ED-associated). For several pedigree members, serial radiographs (a series of radiographs taken over several years) were available which facilitated a more comprehensive dental assessment. Other radiographs were taken on the day of examination. Panoramic, bitewing and periapical radiographs were all found to be valuable in this study. Following the joint clinical genetic and dental consultation, a clinical diagnosis of autosomal dominant non-syndromic oligodontia was ascribed to two of the pedigrees and a diagnosis of autosomal dominant hypohidrotic ED was ascribed to the remaining pedigree. See 2.2.2.

2.2.2 GENERAL CLINICAL FEATURES OF THE OLIGODONTIA PEDIGREES

The affectation status of pedigrees 1, 2 and 3 are shown in Fig 2.3, 2.4 and 2.5 respectively and is based on the general (non-dental) clinical features listed in Table 2.2, 2.3, and 2.4 as well as the oral findings described in 2.2.3. Collectively, this information was essential in diagnosing the oligodontia condition for each pedigree and in differentiating affected from unaffected pedigree members. After close collaboration between the paediatric dentist (author) and clinical geneticist (**MW**), the following diagnoses were made:

Pedigree 1	Autosomal Dominant Hypohidrotic Ectodermal Dysplasia
Pedigree 2	Autosomal Dominant Non-Syndromic Oligodontia
Pedigree 3	Autosomal Dominant Non-Syndromic Oligodontia

The general clinical features of affected and unaffected members of Pedigree 1 can be summarised as follows (see Table 2.2, Fig 2.3):

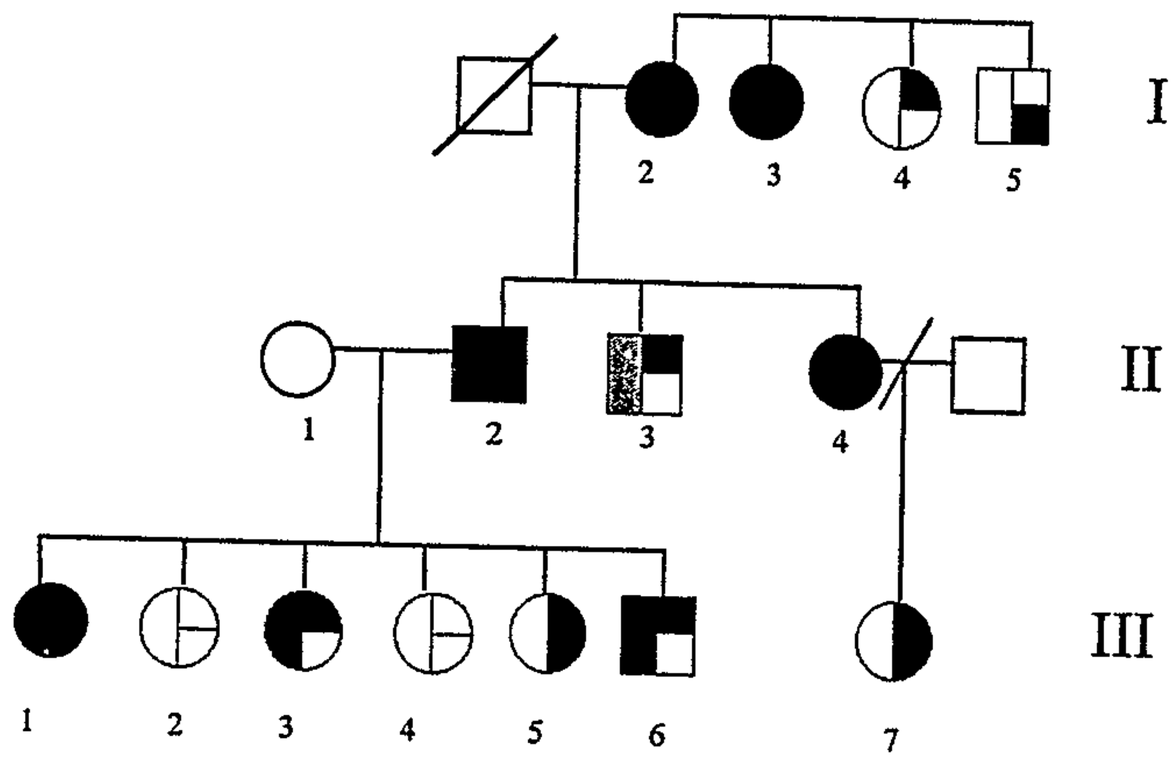
- Moderate to severe symptoms of hypohidrosis were recorded for all 7 affected members, characterised by intolerance to warm weather, history of hyperthermia or fainting during regular exercise, or noticeable absence of sweating during exercise. Two unaffected older members (generation I) also reported moderate to severe heat intolerance

- A particularly unusual feature of a number of affected members was a striking history of dysphagia, often manifesting as choking on certain foods. This condition was so remarkable that one subject (III.1) had undergone barium swallow and upper GI endoscopy investigations in order to determine a gastrointestinal cause to the condition. Considering this individual's other ectodermal dysplastic features (Table

2.2), these symptoms may be a result of a decrease in mucous glands of the oropharynx (see 1.2.5.3)

- Dryness of skin or tear production did not correlate closely with affectation status
- Most affected members (5/7) displayed moderate hair anomalies, including pseudomonilethrix (see 1.2.5.3) and/or mild fingernail anomalies such as thin, pliable nails, convex form or a history of brittleness
- No gross craniofacial dysmorphology was observed, although a lack of posterior teeth in I.2, I.3, II.4 and III.6 resulted in a noticeable reduction in lower facial height
- The association of oligodontia (see 2.2.3) with dysplastic ectodermally derived tissues, along with a lack of association with any medical condition or significant dysmorphia provided the evidence to diagnose Pedigree 1 as demonstrating an autosomal dominantly inherited form of HED

Pedigree 1



Sweat and/or mucous and/or lacrimal gland

Hair and/or nail dysplasia

Suspected oligodontia

Definite oligodontia

Subjects I.2, I.3, II.2, II.4, III.1, III.3 and III.6 believed to be definitely affected
Subjects I.4, I.5, III.2 and III.4 believed to be definitely unaffected
Subjects II.3, III.5 and III.7 remain equivocal after clinical assessment

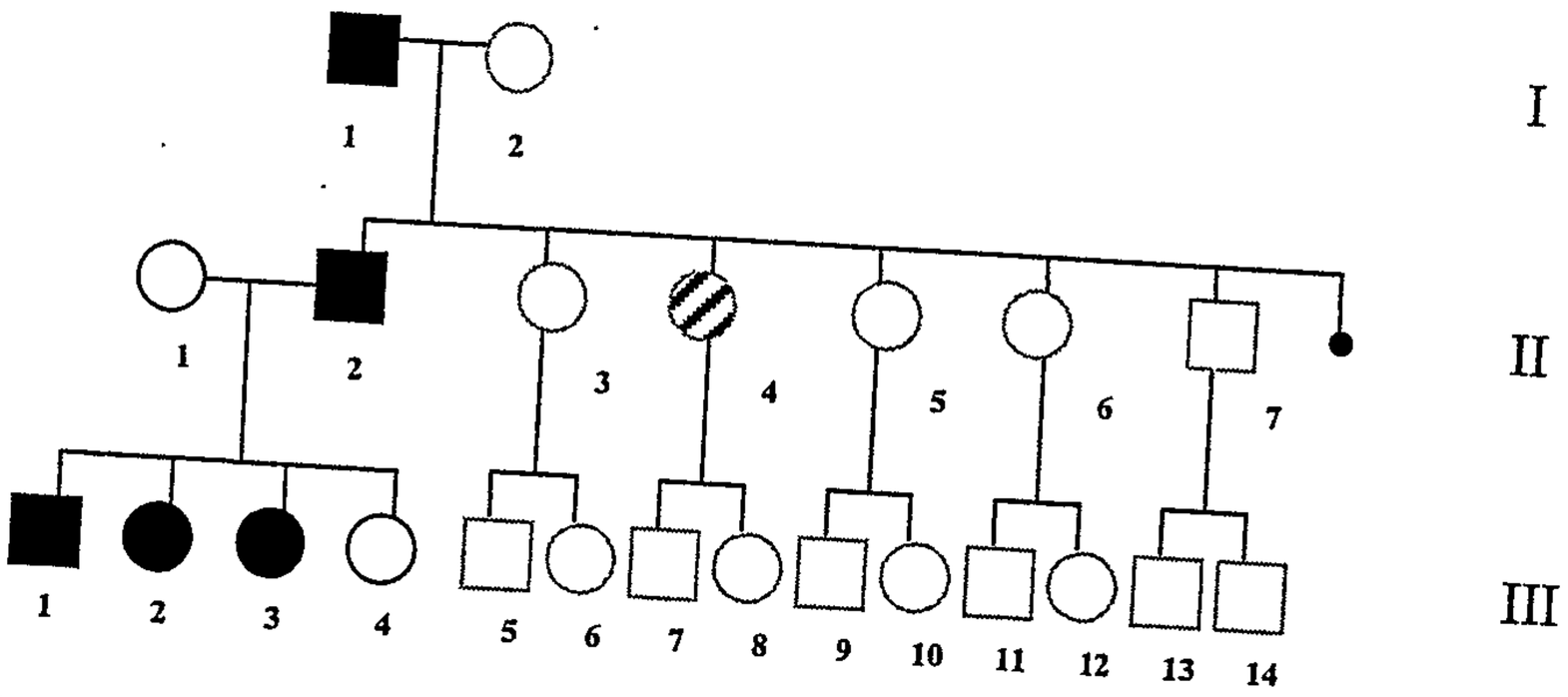
Figure 2.3 Map of pedigree 1 diagnosed with autosomal dominant HED

The general clinical features of affected and unaffected members of Pedigree 2 can be summarised as follows (see Table 2.3, Fig 2.4):

- There was no reported hypohidrosis or heat intolerance
- Some members reported dry skin although this did not correlate well with affectation status
- No significant hair anomalies were consistently observed for affected members
- Fingernails were occasionally reported to be brittle, thin, pliable or slow growing
- No gross craniofacial dysmorphology was observed

Due to a lack of association of the oligodontia trait with significant dysplasia of ectodermally derived tissues and no other significant medical condition or dysmorphia, a diagnosis of non syndromic oligodontia was made for Pedigree 2.

Pedigree 2



Subject II.4 reported to have Charcot Marie Tooth syndrome

Definite oligodontia

Subjects I.1, II.2, III.1, III.2 and III.3 believed to be definitely affected
Subject III.4 believed to be definitely unaffected
Subjects II.3, II.4, II.5, II.6 and II.7 and their offspring unsighted (equivocal)

Figure 2.4 Map of pedigree 2 diagnosed with autosomal dominant NSO

Table 2.2 Clinical Features of Members of Pedigree 1

	I.2	I.3	I.4	I.5	II.2	II.3	II.4
Hair (macro)	fine, sparse, scant eyebrows	normal growing; sparse pubic + axillary	normal	fine, sparse	normal	early frontal balding	sparse scalp hair
(micro)	deformible, some pseudomonilethrix	more deformible, multiple pseudomonilethrix	normal	deformible, some pseudomonilethrix	sightly deformible	slightly deformible	slightly deformible
Nails	slow growing, break easily, convex	normal	normal	normal	thin, ridged, pliable, tendency to split	convex	breaks easily, convex
Sweating	moderate heat intolerance	symptomatic evidence of severe hypohidrosis	symptomatic evidence of severe hypohidrosis	normal	symptomatic evidence of moderate hypohidrosis	symptomatic evidence of mild hypohidrosis	symptomatic evidence of moderate hypohidrosis
Tear Production	reduced	normal	lacrimates easily	normal	normal	reduced	reduced
Saliva Function	test normal, occasional swallowing problems	test normal	test normal, some swallowing problems, dry mouth symptoms	test normal, dry mouth symptoms	normal	test normal	test normal
Skin	dry, severe eczema, thin	dry	dry, itchy, sensitive	dry, severe eczema	fair complexion, sun sensitive, epidermal cysts (scalp), basal cell carcinomas (neck, nose)	bronze pigmentation, moderate wrinkling	thickened skin on palms, prolonged hives
Growth and Development	head circ. 25-50th percentile	normal	normal	normal	head circ. >98th percentile	head circ. 3rd percentile failure to thrive, learning difficulties	head circ. < 3rd percentile
Craniofacial and Skeletal	deep set eyes, short lower face, arthritis in hands and hips, scoliosis, hemivertebrae (L5-6)	short lower face	mild arthritis hands	arthritis hands and knees	prominent ears, length > 97th percentile, mild malar hypoplasia, high nasal bridge, large anterior fontanelle (closed at 3 yrs)	deep set eyes, ? two extra vertebrae (lower lumbar)	short lower face, deep set eyes, high nasal bridge mild scoliosis
Other	severe asthma, allergic rhinitis, ulcerated oesophagus, bowel cancer 2001, UTI's (pyelitis), swallowing problems				chokes on food (dysphagia), hoarse voice	low birth weight recurrent ear infections, rheumatoid arthritis, VSD, hypospadias	moderate asthma

Table 2.2 Pedigree 1 (cont).

	III.1	III.2	III.3	III.4	III.5	III.6	III.7
Hair (macro)	wiry, thin eyebrows	fine, sparse, 1st hair cut age 4 yrs	normal	normal	brittle, white hair until 16mths, spangly	sparse scalp hair laterally, 1st cut age 3	fine, slow growing
(micro)	slightly deformible	almost normal	slightly deformable	slightly deformable	slight deformable,	normal	normal
Nails	flexible, thin	break easily, not curved	normal	normal	normal to thin	thin nails	very soft, thin, break easily, concave
Sweating	symptomatic evidence of severe hypohidrosis	symptomatic evidence of mild hypohidrosis	symptomatic evidence of severe hypohidrosis	normal	symptomatic evidence of moderate hypohidrosis	symptomatic evidence of severe hypohidrosis	symptomatic evidence of moderate hypohidrosis
Tear Production	reduced	normal	normal	normal	reduced	normal	normal
Saliva Function	normal	normal	normal	normal	normal	normal	test normal
Skin	mild keratosis pilaris, severe chicken pox infection age 11	normal, fair complexion, sun sensitive	normal, mild keratosis pilaris	skin allergy to zinc	normal, mild keratosis pilaris	smooth skin, prominent foetal finger pads	thickened skin on palms, occasional hives
Growth and Development	head circ. > 98th percentile	failure to thrive, vomiting, dehydration in infancy	head circ. 50th percentile	normal	head circ. 98th percentile	head circ. 50th percentile	normal
Craniofacial and Skeletal	ear length >90th percentile, short lower face, mild lip eversion, mild malar hypoplasia, mild joint hyper-mobility	normal, thin lips, mild pes planus (flat feet)	deep set eyes, infraorbital wrinkling, everted lower lip, short lower face, malar hypoplasia	deep set eyes	deep set eyes, infraorbital wrinkling joint hypermobility Osgood-Schlatter disease (from 12 yrs), mild scoliosis	prominent ears, length >98th percentile, deep set eyes, short lower face, malar hypoplasia, normal mobility, pes planus	mild scoliosis
Other	ear infections, severe choking (dysphagia)		severe choking (dysphagia)		chokes on some foods (dysphagia)	ear infections, L undescended testis, severe choking (dysphagia)	moderate asthma

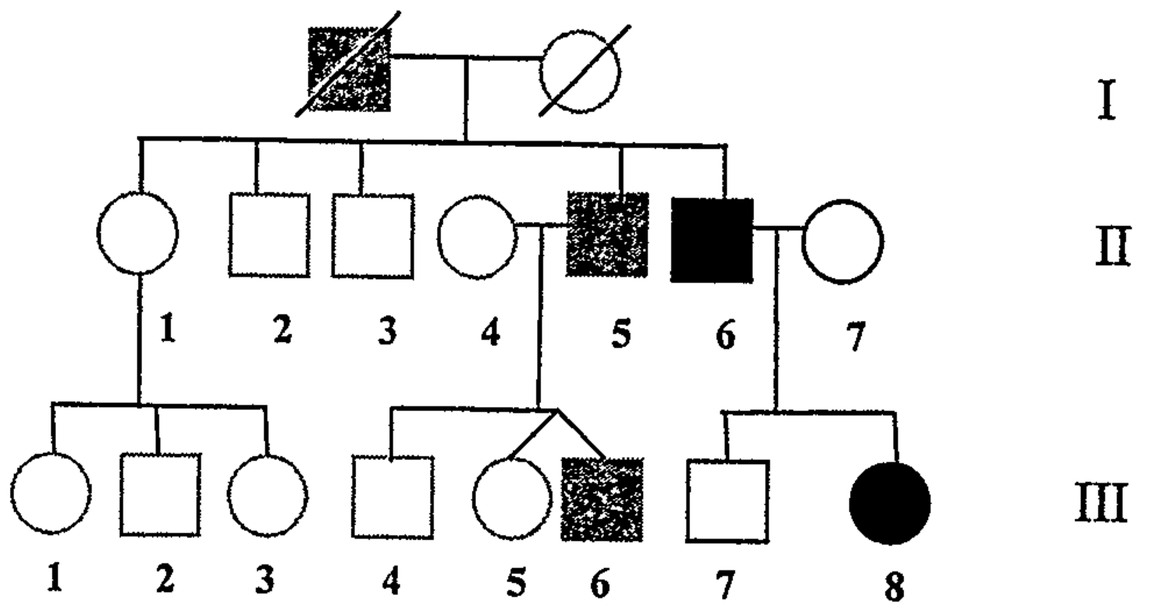
Table 2.3 Clinical Features of Members of Pedigree 2

	I.1	II.1	III.1	III.2	III.3	III.4
Hair (macro)	normal	normal	thick	fine as baby	normal	slow growing as infant
(micro)	normal	normal	slight deformability	normal	very slight deformability	normal
Nails	normal	thin, soft, pliable	normal	thin, slow growing, easily chipped	normal	normal
Sweating	normal	normal	normal	normal	normal	normal
Saliva Function	normal	normal	normal	normal	normal	poor saliva quality
Skin	normal	dry, fair	normal	dry, extensive patchy naevus flammeus philtrum and legs	very dry, fair, keratosis pilaris	dry, fair, keratosis pilaris
Craniofacial and Skeletal	normal	hip replacement (bilateral) from osteoarthritis	clicking hips as neonate (double nappies), mild hypermobility, pes planus	hypermobile thumbs	normal	alveolar cleft, prominent ears, mild pes planus

Table 2.4 Clinical Features of Members of Pedigree 3

	II.6	III.7	III.8
Hair (macro)	normal, bushy	thick, curly, bushy	thick, curly, slightly spongy
(micro)	deformable (pseudomonilethrix)	normal	minor grooving
Nails	normal	normal	normal
Sweating	normal	normal	normal
Saliva function	normal	normal	normal
Skin	normal supernumerary nipple	normal	dry skin acne rosacea
Growth and Development	head circ. 97th percentile	normal	head circ. 90th percentile, low birth weight
Craniofacial and Skeletal	ear length 98th percentile	no earlobes, slight infraorbital wrinkling	infraorbital wrinkling prominent columella
Other	glossopharyngeal neuralgia with occasional choking	mild asthma	single neonatal febrile convulsion

Pedigree 3



Reported to have oligodontia, unsighted

Definite oligodontia

Subjects II.6 and III.8 believed to be definitely affected
Subject III.7 believed to be definitely unaffected
Subjects I.1, II.5and III.6 believed to be affected but unsighted
Subjects II.1, II.2, II.3 and their offspring believed to be unaffected but unsighted

Figure 2.5 Map of pedigree 3 diagnosed with autosomal dominant NSO

The general clinical features of affected and unaffected members of Pedigree 3 can be summarised as follows (see Table 2.4, Fig 2.5):

- Both affected members displayed macroscopic or microscopic evidence of hair deformability
- There were no nail anomalies and no reported hypohidrosis or heat intolerance
- No gross craniofacial dysmorphology was observed

Due to a lack of association of the oligodontia trait with significant dysplasia of ectodermally derived tissues, and no other significant medical condition or dysmorphia, a diagnosis of non-syndromic oligodontia was made for Pedigree 3. The hair anomaly seen in the affected members was considered a coincidental finding and based on the clinical geneticist's experience, was not considered significant enough to be regarded as evidence for an ectodermal dysplasia.

2.2.3 ORAL FEATURES OF THE OLIGODONTIA PEDIGREES

The dental features noted in each of the oligodontia pedigrees are shown in Table 2.5 and 2.6. The main oral features for Pedigree 1 are displayed in Fig 2.6 and can be summarised as follows (see Table 2.5):

- All affected members displayed (or had a history of) moderate oligodontia, with an average of 11 congenitally absent teeth excluding 8's (range 10 to 12). One pedigree member believed to be unaffected (III.2) had agenesis of two L5's. The teeth most frequently undergoing agenesis in affected pedigree members were, in descending order, 8's and 5's (100%), L1's (90%), U2's (80%), L4's (60%) then 7's and U4's (40%). No other tooth types were recorded as congenitally absent.
- Four affected members displayed significant microdontia of several teeth that did develop, particularly the upper incisors, U4's and retained second primary molars (present in members II.4, III.1, III.3 and III.6)
- Cervical constriction of the molars was a commonly occurring radiographic feature in three affected members (III.1, III.3, III.6) as well as one member believed to be unaffected (III.2)

Dentition in Autosomal Dominant HED Pedigree

Figure 2.6 Panoramic radiograph of Pedigree 1, III.1, taken when aged 17 years, displaying evidence of agenesis of U5's, UR4, U2's, L5's, L4's and L1's, as well as cervical constriction of molars

- Other dental anomalies that were noted included tapering or screw driver shaped upper and lower incisors (II.2, II.4) and prominent and/or conical buccal premolar cusps (III.1, III.5, III.6). A reduction in molar cusp number was also observed, as has been reported previously in animal studies (Kristenova et al, 2002; Pispa et al, 1999). In particular, absence of the distopalatal cusp of U6's was noted for II.2, II.4, III.1, III.3 and III.6
- No significant loss in alveolar ridge height was noted except for III.6 or in individuals who had had most if not all remaining teeth extracted, including I.2, I.3, II.3 and II.4
- Periodontal health among affected and unaffected subjects was variable and poor periodontal health, assessed in terms of tooth mobility, plaque levels and bleeding on probing, was not significantly more common in individuals with a spaced dentition
- Oral hydration and saliva production, viscosity, pH and buffering capacity were all within acceptable limits according to the GC™ Saliva Test carried out for each pedigree member

The main oral features for Pedigrees 2 and 3 can be summarised as follows (see Table 2.6):

- All affected members displayed (or had a history of) moderate to severe oligodontia, with an average of 10 congenitally absent teeth (range 5 to 20), excluding 8's. Of the pedigree members classified as unaffected, agenesis of a U4 (Pedigree 2, III.4) and a U5 and L5 (Pedigree 3, III.7) were noted (8's excluded). The teeth most frequently undergoing agenesis in affected pedigree members were, in descending order, 8's and U5's (100%), L5's (85%), U4's (75%), L4's (67%), L7's (40%) and U2's (33%), and finally L1's, L2's, 3's and U7's (15%).

- Microdontia was observed in 5 affected as well as 2 unaffected pedigree members and was usually localised to one or two of the tooth classes (incisors, canines, premolars, molars). According to the diagnostic criteria described in 2.1.5, a subject without clinically evident oligodontia but with other associated dental anomalies such as microdontia could be considered affected but only if those anomalies were inherited from affected parents and not unaffected parents and if there were a significant number of teeth affected. As such, these two individuals (Pedigree 2, III.4 and Pedigree 3, III.7) did not fit these criteria and remained as unaffected.
- There was no radiographic evidence of cervical constriction or significant taurodontism.
- Other oral anomalies that were noted included ectopic eruptions, impactions or transpositions of canines and tapering or screw driver shaped incisors (Pedigree 2, III.2 and III.3, Pedigree 3, III.8). A reduction in molar cusp number was observed for some pedigree members, as has been reported previously for *MSX1* NSO (Lidral and Reising, 2002). In particular absence of the distopalatal cusp of U6's (Pedigree 2, II.2, Pedigree 3, III.7) and absence of the distobuccal cusp (Pedigree 2, II.2, Pedigree 3, all members) was noted
- No significant loss in alveolar ridge height was noted. A cleft of the upper left alveolus (Pedigree 2, III.4)
- Periodontal health among affected and unaffected subjects was variable and poor periodontal health, assessed in terms of tooth mobility, plaque levels and bleeding on probing, was not significantly more common in individuals with a spaced dentition

- Oral hydration and saliva production, viscosity, pH and buffering capacity were all within acceptable limits according to the GC™ Saliva Test carried out for each pedigree member. This included members II.2, III.1, III.3, III.5 and III.6 in Pedigree 1, who all exhibited a history of significant dysphagia.

Table 2.5 Pedigree 1 Dentition, Selected Members

Upper	M			P	M	C	I		I		C	PM		M		
Lower	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
II.2	●	●	○	●	▪	○	▪	▪	▪	▪	○	▪	●	○	○	●
	●	○	○	●	●	○	○	●	●	○	○	●	●	○	●	●
II.4	●	●	○	●	▪	○	●	○	○	●	○	▪	●	○	●	●
	●	●	○	●	▪	○	○	●	●	○	○	▪	●	○	●	●
III.1	●	▼	▼	●	●	○	●	○	○	●	○	▪	●	▼	▼	●
	●	▼	▼	●	●	○	○	●	●	○	○	●	●	▼	▼	●
III.2	▼	▼	○	○	○	○	○	○	○	○	○	○	○	○	▼	▼
	○	○	○	●	○	○	○	○	○	○	○	○	●	○	○	○
III.3	●	▼	▼	●	●	○	●	▪	▪	●	○	●	●	▼	●	●
	●	▼	▼	●	●	○	▼	○	●	▼	○	●	●	▼	▼	●
III.5	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●
	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
III.6	●	▪	▪	●	○	○	●	○	○	●	○	●	●	▪	▪	●
	●	●	▼	●	○	○	○	●	●	○	○	○	●	▼	▼	●
III.7	●	○	○	●	○	○	○	○	○	○	○	○	○	○	○	○
	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	●

Legend: ○ normal teeth, ● absent teeth, ▼ cervical constriction of teeth, ▪ microdontia

Table 2.6 Pedigree 2 and 3 Dentition, Selected Members

	M			PM		C	I		I		C	PM		M		
Upper	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
Lower	8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
Pedigree 2	●	●	○	●	▪	○	▪	○	○	▪	○	▪	●	○	▪	●
II.1	●	●	○	▪	▪	○	○	○	○	○	○	▪	▪	○	●	●
III.1	●	▪	○	●	●	○	▪	○	○	▪	○	●	●	○	▪	●
	●	▪	○	●	●	○	○	○	○	○	○	●	●	○	▪	●
III.2	●	○	○	●	●	▪	●	▪	▪	●	▪	●	●	○	○	●
	●	●	○	●	●	○	○	○	○	○	○	●	●	○	●	●
III.3	●	▪	▪	●	●	○	○	○	○	○	○	○	●	▪	▪	●
	●	▪	▪	●	●	○	○	○	○	○	○	○	●	▪	▪	●
III.4	●	○	▪	○	○	○	○	○	○	○	○	●	○	▪	○	●
	●	○	▪	○	○	○	○	○	○	○	○	○	○	▪	○	●
Pedigree 3	●	○	○	●	●	○	▪	▪	▪	▪	○	●	●	○	○	●
II.6	○	○	○	●	●	○	○	▪	▪	○	○	▪	●	○	○	●
III.7	●	○	○	●	▪	○	○	○	○	○	○	▪	▪	○	○	●
	●	○	○	▪	▪	○	○	○	○	○	○	▪	●	○	○	●
III.8	●	▪	○	●	●	●	●	x	x	●	●	●	●	○	●	●
	●	●	○	●	●	●	●	●	●	●	●	●	●	○	▪	●

Legend: ○ normal teeth, ● absent teeth, ▪ microdont teeth, x extracted teeth

2.3 Discussion

The findings of this clinical investigation (see 2.2) indicate that clinical diagnosis is best achieved by collection of sufficient clinical data and by joint dental, genetic and dermatological collaboration. In order to conduct an accurate dental assessment, a comprehensive understanding of normal and abnormal dental development is necessary. In addition, any previous radiographs or documentation of treatment performed on subjects that had previously attended the department were extremely valuable in determining which teeth were indeed congenitally absent and which teeth may have been extracted.

No one specialist alone was capable of accurately determining each pedigree's oligodontia condition and each subject's affectation status. In particular, subtle hair abnormalities, which could represent ED, are common in the general population, and can be over-interpreted. This can be illustrated best by the clinical findings from Pedigree 3, in which affected members displayed both hair and tooth anomalies but only by consensus could it be decided that an ED could not be clinically confirmed on the available evidence and that the pedigree was best classified as autosomal dominant NSO.

Two different oligodontia conditions were diagnosed for the three pedigrees investigated in this study. One pedigree was diagnosed with autosomal dominant HED and two pedigrees were diagnosed with autosomal dominant NSO. This allowed comparison of the general and dental phenotype between these two clinically similar conditions. A description of the clinical features of autosomal dominant HED associated with oligodontia has only been reported twice previously (Aswegan et al, 1997; Jorgenson et

al, 1987), while a comprehensive assessment of ectodermally derived tissues in NSO has not previously been reported.

Of the two clinical studies that have previously described the autosomal dominant HED phenotype, one pedigree was characterised by hypohidrosis, conical primary teeth and oligodontia (pattern unspecified), hypotrichosis, deficient lactation in females and hyperextensible joints (Jorgenson et al, 1987). The other pedigree was characterised by hypohidrosis, oligodontia (pattern specification incomplete), no obvious craniofacial dysmorphology, sparseness of eyebrows and eyelashes, sparse, fine, slow-growing scalp hair with no structural abnormalities under electron microscopic examination, dry, smooth, thin skin, and mild fingernail abnormalities with slow growing nails (Aswegan et al, 1997).

In the autosomal dominant HED pedigree (Pedigree 1), the general features included hypohidrosis, variable hair sparseness and deformability (examined under light microscopy), mild fingernail deformities, variable decreased tear production, and normal saliva production but a characteristic form of dysphagia implicating a lack of mucous glands in the oropharynx. The oligodontia pattern observed in this pedigree was predominantly one of agenesis of the 5's, L1's, U2's and L4's. Other associated dental anomalies included microdontia, prominent/conical premolar cusps, cervical constriction and tapered incisors.

In contrast, the two NSO pedigrees were characterised by no hypohidrosis, no hypotrichosis but with some members displaying mild structural hair anomalies under light microscopy (Pedigree 3) or mild fingernail deformities (Pedigree 2). The oligodontia pattern observed in these pedigrees were characterised by agenesis of 5's and

4's predominantly. Other associated dental anomalies included microdontia, tapered U1's or L1's or ectopic eruptions but no evidence of cervical constrictions.

Based on these findings, the oligodontia pattern tends not to differ greatly but the associated dental anomalies do tend to differ between the two oligodontia conditions. Characteristic features of autosomal dominant HED that are not observed in NSO include cervical constriction and abnormal premolar cusp form. Both conditions were associated with microdontia. When comparing the oligodontia pattern of X-linked (see Fig 1.3) with autosomal dominant HED, agenesis of the L2's, 3's, 6's and U1's tends to occur much less frequently in autosomal dominant HED.

In terms of oligodontia genotype-phenotype correlations, autosomal dominant HED caused by mutation in *EDAR* is associated with dental agenesis of U2's, L1's and 5's, although to date this pattern has not been characterised in detail (Aswegan et al, 1997). However, the dental features of the autosomal dominant HED pedigree investigated in this study did appear to correlate quite closely with the dental findings reported for the autosomal dominant HED pedigree from the Aswegan study.

In contrast, NSO caused by mutation in *PAX9* is associated with agenesis of molars which does not correlate to any of the pedigrees in this study (see Fig 1.3). NSO caused by mutation in *MSX1* is reportedly associated with agenesis of 5's and U4's (see Fig 1.3), and this does correlate well to the oligodontia pattern observed in the two NSO pedigrees. It is therefore hypothesised that Pedigree 1 could show linkage to the *EDAR* gene and Pedigrees 2 and 3 could show linkage to the *MSX1* gene on completion of the molecular genetic component to the study. This will be further explored in the molecular genetic component of the study.

2.4 Conclusion

Out of 15 subjects examined in the autosomal dominant HED pedigree, oligodontia occurred in 7 of 8 individuals clinically classified as affected, with an average of 11 teeth congenitally absent (range 10-12). Penetrance of oligodontia was therefore estimated at 87.5%. Hypohidrosis, cervical constriction and abnormal premolar cusp morphology were features that helped differentiate HED from NSO.

Out of 11 subjects examined in the two autosomal dominant NSO pedigrees, oligodontia occurred in 7 individuals clinically classified as affected, with an average of 10 teeth congenitally missing (range 5-20). Without genotyping, it was deemed impossible to classify someone as definitely "non-penetrant" or as decreased expressivity if they had <6 teeth missing.

3. MOLECULAR GENETIC STUDY OF OLIGODONTIA

3.1 Materials and Methods

3.1.1 STUDY DESIGN

The aim of this study was to explore the molecular genetic basis of oligodontia clinically assessed in three families

The molecular genetic component of the study can be summarised as follows:

a) DNA samples were obtained from peripheral blood lymphocytes of all affected and unaffected family members who participated;

b) Linkage analysis was undertaken to known loci associated with oligodontia by means of a candidate gene approach, with the candidate genes investigated being *MSX1* and *PAX9* for NSO pedigrees and *EDAR* for the autosomal dominant HED pedigree.

Depending on the likelihood of linkage to one of these candidate gene regions, one of two follow-up strategies would then be employed:

i. Fine mapping followed by direct sequencing of any informative pedigree that showed a strong likelihood of linkage to a particular candidate gene.

ii. Genome wide screening of any potentially informative pedigrees if linkage to all studied genomic regions was excluded, to look for any novel regions showing linkage.

3.1.2 CANDIDATE GENE SELECTION

Following an extensive literature search (see 1.5.2), the candidate genes *MSX1* and *PAX9* were selected for the NSO pedigrees and the *EDAR* gene was selected for the autosomal ED pedigree.

3.1.3 MICROSATELLITE MARKER SELECTION

Microsatellites close to these candidate genes were carefully selected based on their proximity to the gene and to their allelic heterogeneity (see 1.4.2.1). This information was obtained from the Genome Database and Gene Browser websites (2004a; 2004b). The microsatellite markers used for studying the three candidate genes based on this information are listed in Table 3.1.

Table 3.1 Microsatellite Markers used for Linkage Analysis

Candidate Gene	Locus	Microsatellite Marker	Locus
MSX1	4p16.1 (4.85 Mb start)	D4S3023 D4S2925	4.29 Mb start 4.89 Mb start
PAX9	14q13.3 (35.12 Mb start)	D14S69 D14S75 D14S306	35.05 Mb start 35.28 Mb start 37.31 Mb start
EDAR	2q11-q13 (108.97 Mb start)	D21890 D2S1893 D2S1892	107.88 Mb start 109.31 Mb start 111.62 Mb start

Table 3.1 displays the microsatellite markers used during linkage analysis experiments for the three pedigrees and their loci in relation to the candidate gene. The unit Mb refers to the number of megabases (millions of nucleotide base pairs) the locus is from the short arm end of the chromosome

3.1.4 DNA EXTRACTION AND QUANTITATION

DNA was extracted from white blood cells using the following protocol:

1. Approximately 10mL of peripheral vein blood was collected from each subject and stored in EDTA collection tubes before being transported to the laboratory

2. 100μL of the blood volume was pipetted onto blotting paper as a secondary store of each subject's blood sample

3. Remaining blood volume was added to a 50mL Falcon tube and then filled with ELB (erythrocyte lysis buffer), thoroughly vortexed (approximately 20 seconds) and then refrigerated for at least15 minutes to allow time for lysis of the erythrocytes

4. The solution was then vigorously shaken and centrifuged for 10 minutes at 1000rpm and at 4°C

5. The supernatant was then discarded. Care was taken to ensure that none of the white cell pellet was discarded

6. Steps 3 to 5 were then repeated, leaving a WBC pellet

7. The pellet was re-suspended in the remaining 0.5mL ELB, to which 5mL SE (3.75M NaCl salt and 0.5M EDTA), 250μL SDS (20% sodium dodecyl sulphate) and 30μL pronase (20μg/μL protease) was then added, the solution vortexed and then incubated in a water bath at 37°C for at least 24 hours until fully digested

8. Following digestion of cell lysates, 1600μL of sterile saturated NaCl (5M) was added to each sample, vortexed for 20 seconds and then centrifuged at room temperature for 25mins at 3000rpm

9. The supernatant containing the DNA was carefully decanted into a fresh 50mL Falcon tube to which step 8 was repeated

10. The supernatant was again decanted into a fresh 50mL Falcon tube to which ice cold 100% absolute ethanol was added at approximately twice the volume of supernatant, the samples were gently swirled and precipitation of DNA strands observed

11. Using a plastic implement (pipette tip), the DNA was carefully manoeuvred and transferred into a clean 1.5mL screw cap tube and then left for at least one hour to allow the ethanol to evaporate

12. To the DNA strands was added 150-350μL TE buffer (10mM Tris-Cl-EDTA plus 1mM EDTA, pH 8) which was then gently mixed (by slow revolution) for 4-6hrs to ensure the DNA was then dissolved in the TE buffer. The aim was to generate a stock DNA concentration of 100-500μg/mL.

Samples were stored in TE buffer at 4°C until required for study, and access to the samples was restricted according to the guidelines of the relevant Ethics Committees. When required for the study, DNA samples were quantitated by determining the optical density (OD) at 260nm with an OD of 1.0 being equivalent to 50μg/ml of double stranded DNA.

3.1.5 PCR AMPLIFICATION

Oligonucleotide primer sequences (see 3.1.3) for all DNA fragments to be amplified were obtained from Invitrogen™ Life Technologies (www.invitrogen.com). The protocol for PCR amplification is outlined below:

1. Each PCR sample of 20μL was made up using 13.85μL WFI (water for injection), 2μL dNTP's (2mM), 2μL 10x Hot Master Taq (HMT) buffer, 0.15μL HMTaq, 1μL (100ng) DNA and 1μL primer (5μM)

2. PCR amplification was performed using an Eppendorf Master Cycler™ that was programmed to have an initial denaturation step at 94˚C for 2 minutes, which was then followed by 10 cycles of [94˚C (20 seconds) then 65˚C (15 seconds), 64˚C (15 seconds), 63˚C,….56˚C], then 65˚C (45 seconds), then 40 cycles of [94˚C (20 seconds), 55˚C (15 seconds), and 65˚C (45 seconds)], and finally 30 minutes at 65˚C and then holding at 4˚C.

3.1.6 AGAROSE GEL ELECTROPHORESIS

Following PCR, fragments were electophoresed through 2% agarose slab gels to discriminate alleles and thus determine whether these PCR samples were appropriately amplified to undergo quantification via the ABI 310 Genetic Analyser fluorescence detection system (see 3.1.7). Alleles could be discriminated in this way as a result of their weight differentials caused by different numbers of repeat units for each of the microsatellite markers used. For detection of electrophoretic bands, 2µl of loading dye (Ficoll 400, 0.05% bromphenol blue, 50mM EDTA ph 8.0, 20% SDS, Xylene Cyanol) was added. A *Pharmacia* gel electrophoresis apparatus was run at 100 volts, for 30 to 60 minutes at room temperature.

Slab Gel Electrophoresis

Fig 3.1 Differential migration of PCR fragments by electrophoresis

Ethidium bromide stained bands in agarose gels were visualised on a UV transilluminator. They were photographed and scanned to computer using a Kodak™ Electrophoresis Documentation System 120 (DC120) apparatus. See Fig 3.1. Visual verification of the expected PCR products was performed to optimise PCR conditions of all microsatellite and sequencing fragments before genotyping or sequencing via fluorescence-based electrophoresis on the ABI 310 (see 3.1.7).

3.1.7 GENE SCAN ANALYSIS OF MICROSATELLITE MARKERS

The principle of PCR fractionation in capillary electrophoresis is the same as that for gel slab electrophoresis in determining allele sizes. However, capillary electrophoresis was undertaken to quantify the exact size of each allele for each PCR product in an efficient, automated process (see 1.4.2.3). An automated *Applied Biosystems* (ABI) 310 Genetic Analyser fluorescence detection system with GENESCAN® analysis software (Applied Biosystems, Foster City, CA) was used to determine the exact size of these alleles and hence the genotype of the subjects for the particular marker. See Fig 3.2. After PCR thermocycling (see 3.1.5) a one-in-twenty dilution of the PCR samples was performed using ultra pure water (WFI). The diluted samples were then prepared for capillary electrophoresis by mixing 1μl of each PCR sample, 0.12μl of internal size standards (TAMRA GS-500) and 14.88μl of deionised formamide. When multiple (up to 4) samples were pooled (for multiplexing) the amount of formamide was adjusted. Samples were vortexed and then heated to 92°C for 30 seconds only, then immediately placed on ice prior to automated loading of 1.5μl from this mix by the ABI 310.

ABI 310 Genetic Analyser

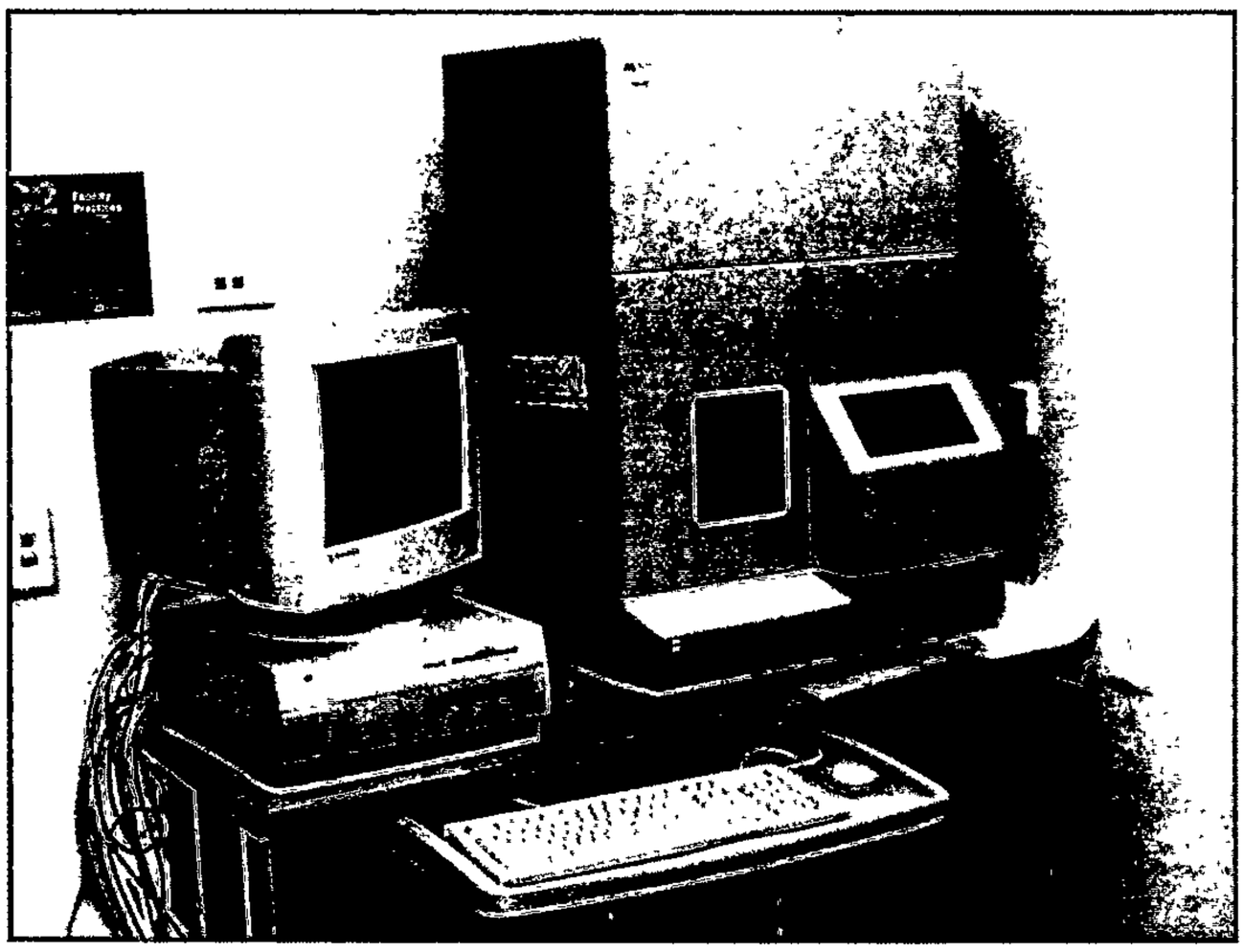
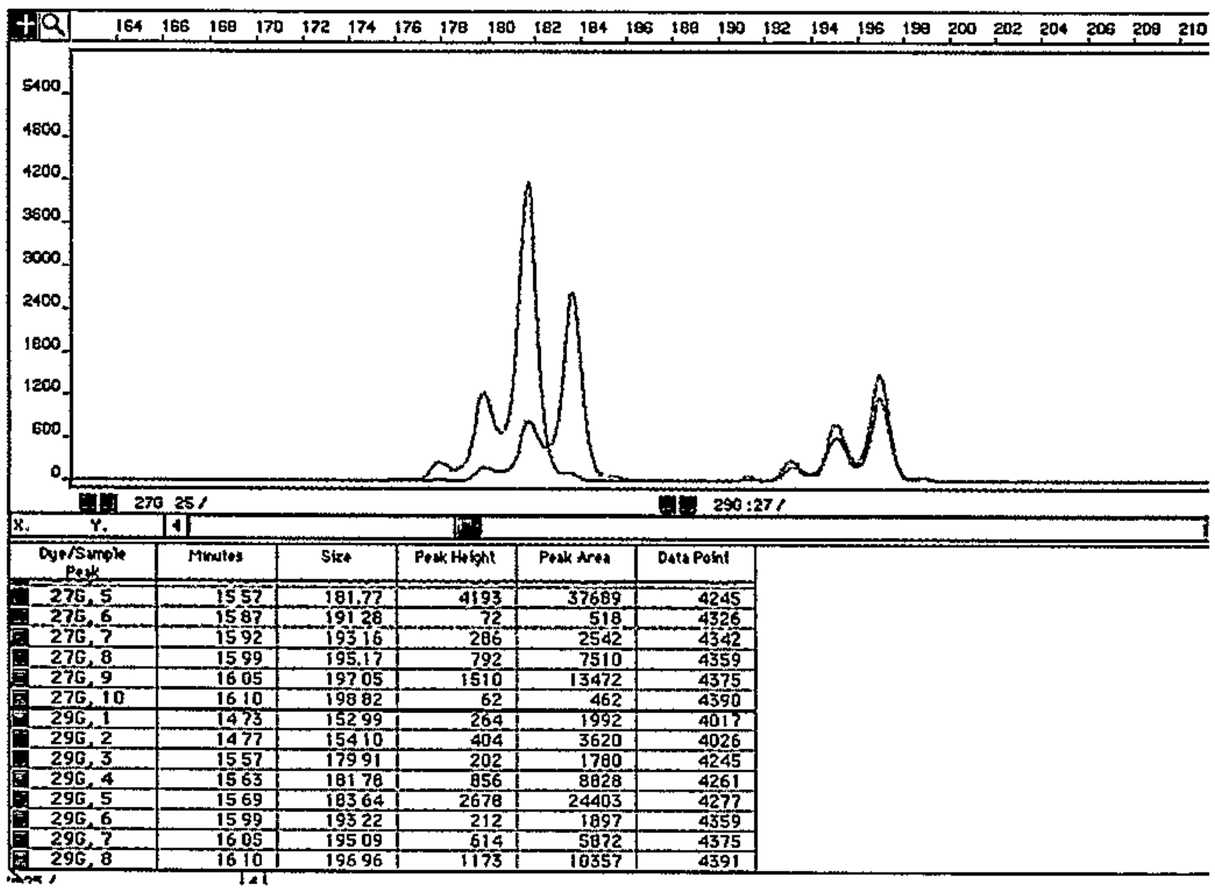


Figure 3.2 ABI 310 genetic analyser incorporating automated capillary electrophoresis technology and GENESCAN® software.

The size of PCR products was determined by reference to internal lane size standards. The GENESCAN® software (*ABI Prism*) automatically recognises the expected fragment size distribution of the size standards that were labelled with the TAMRA (red) dye. A calibration curve based on the standard size fragments was used to estimate the sizes of PCR products labelled with the HEX (yellow) dye, the FAM (blue) dye and TET (green) dye. Results were presented in a spreadsheet format and then converted to a graphical display, known as an electrophoretogram, which shows gel band fluorescence intensities as peaks distributed across a user-selected DNA fragment size range (Fig 3.3). Scores (allele sizes in nucleotide bases) were assigned by the GENOTYPER® software and checked manually by two separate observers to distinguish major peaks from background artefact peaks for alleles. The number of allele peaks depended on whether the individual tested was a heterozygote or homozygote.

Electrophoretogram of Pedigree 1



Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
270, 5	15 57	181.77	4193	37689	4245
270, 6	15 87	191 28	72	518	4326
270, 7	15 92	193 16	286	2542	4342
270, 8	15 99	195.17	792	7510	4359
270, 9	16 05	197 05	1510	13472	4375
270, 10	16 10	198 82	62	462	4390
290, 1	14 73	152 99	264	1992	4017
290, 2	14 77	154 10	404	3620	4026
290, 3	15 57	179 91	202	1780	4245
290, 4	15 63	181 78	856	8828	4261
290, 5	15 69	183 64	2678	24403	4277
290, 6	15 99	193 22	212	1897	4359
290, 7	16 05	195 09	614	5872	4375
290, 8	16 10	196 96	1173	10357	4391

Fig 3.3 High resolution electrophoretogram displaying peaks that relate to the highest intensity of yellow (HEX) fluorescence for each individual PCR fragment that allows quantification of the molecular weight (size) of that PCR fragment. Double peaks indicate heterozygosity, a single peak indicates homozygosity.

3.1.8 LINKAGE ANALYSIS OF CANDIDATE GENE MARKERS

Linkage analysis was performed initially by crude processing of the data produced by the ABI 310 to determine whether any of the microsatellite markers used in the experiments were uninformative and whether any of the markers were probable candidates for linkage. If the marker loci clearly did not segregate with disease, linkage was excluded.

For those markers potentially demonstrating linkage, a more refined analysis was then undertaken by the author and supervising molecular geneticist (Dr Bruce Bennetts, Western Sydney Genetics Program, Children's Hospital at Westmead; **BB**) involving generation of haplotypes. A LOD score analysis using the MLINK package (available at www.angis.org.au) was then conducted for the entire genome of the most informative pedigree by Associate Professor Jenny Donald and Dr Zhixin Liu from the Department of Biological Sciences, Macquarie University.

3.2 Results

3.2.1 UNINFORMATIVE MARKERS

As described in 3.1.3, the microsatellite markers used for the investigation of NSO pedigrees were D4S2925, D4S3023, D14S69, D14S75 and D14S306. Based on the data generated by the ABI 310, a number of microsatellite markers were found to be uninformative for the following NSO pedigrees:

Marker	Reason
D4S2925 for Pedigree 2	Affected parent homozygous
D4S3023 for Pedigree 3	Affected parent homozygous
D14S69 for Pedigree 2	Affected parent homozygous

3.2.2 LINKAGE TO NON-SYNDROMIC OLIGODONTIA CANDIDATE GENES

Pedigrees 2 and 3 were investigated for linkage to the NSO candidate genes *MSX1* and *PAX9*. The results of this investigation are shown in Fig 3.4 and Fig 3.5. Linkage was excluded in Pedigree 2 for *MSX1* and *PAX9* genes. For pedigree 3, linkage to either gene could not be excluded.

MSX1 and *PAX9* Linkage Maps for Pedigree 2

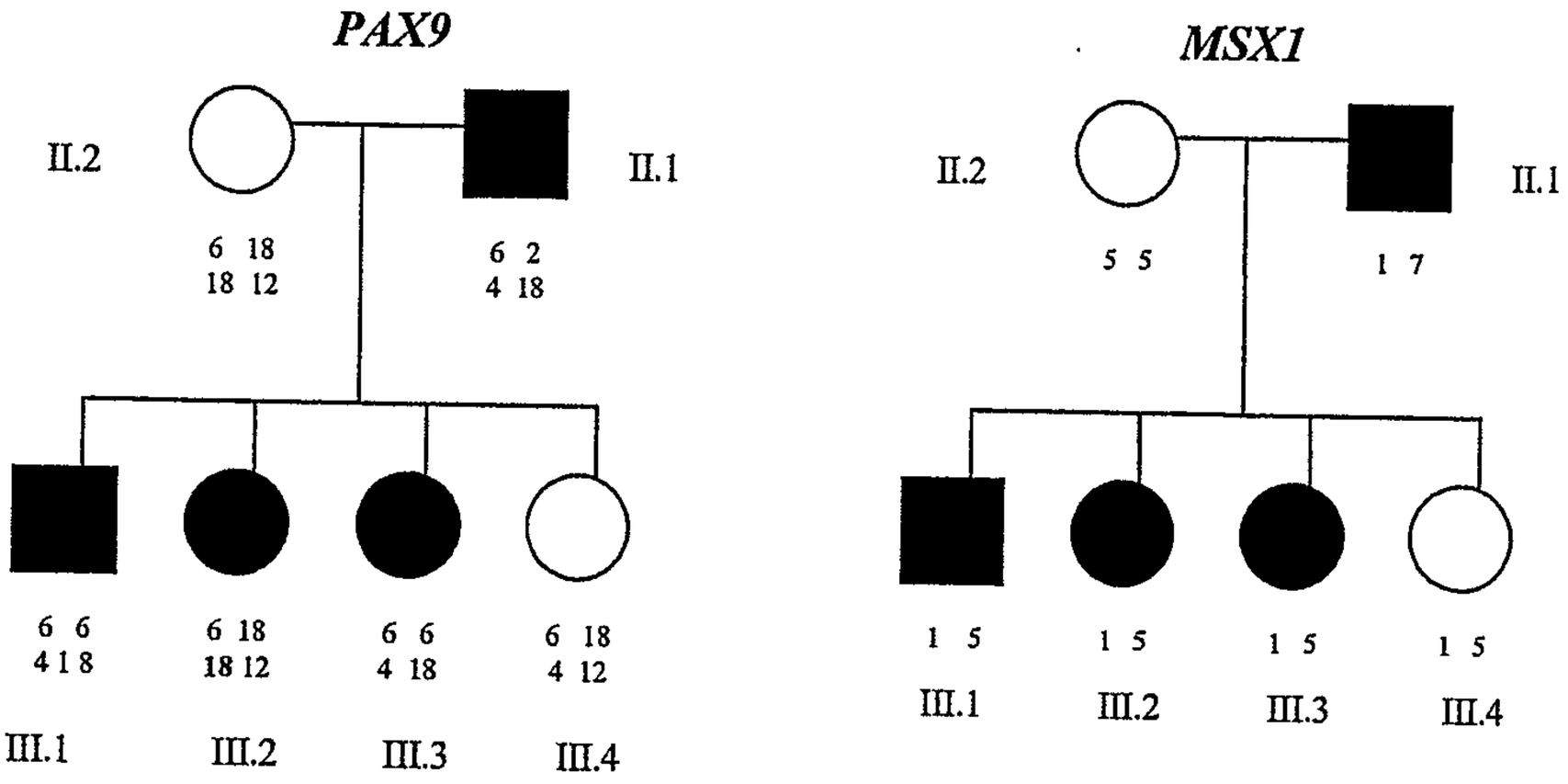


Fig 3.4 Pedigree 2 demonstrates probable exclusion of linkage to *PAX9* (a) and *MSX1* (b) candidate genes, based on ABI 310 electrophoretogram data for microsatellites D14S306, D14S75 (*PAX9*), in descending order (a), and D4S3023 (*MSX1*). Markers in bold type indicate that a recombination event has occurred.

MSX1 and *PAX9* Linkage Maps for Pedigree 3

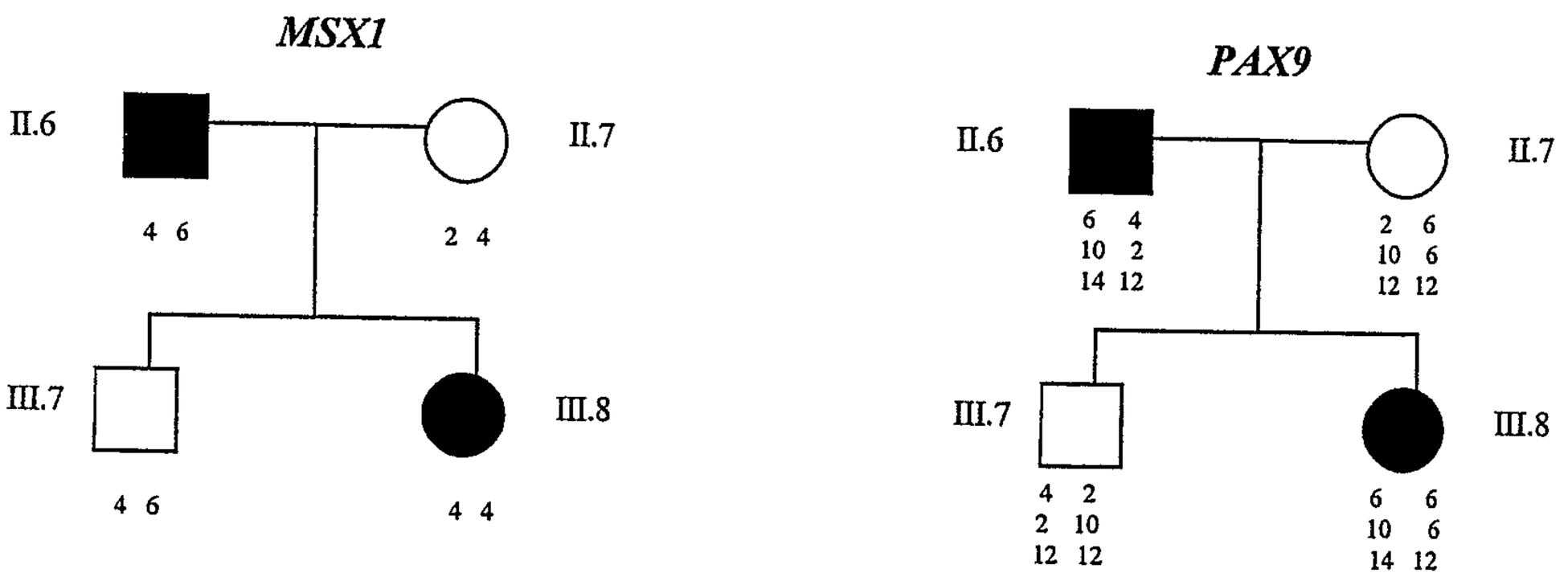


Fig 3.5 Pedigree 3 demonstrates possible segregation of disease with either *MSX1* (a) or *PAX9* (b) candidate genes based on ABI 310 electrophoretogram data for microsatellites D4S2925 (*MSX1*) and D14S69, D14S306 and D14S75 (*PAX9*) in descending order.

3.2.3 LINKAGE TO HYPOHIDROTIC ECTODERMAL DYSPLASIA (*EDAR*) CANDIDATE GENE

Pedigree 1 was investigated for linkage to the *EDAR* candidate gene. The results of this investigation are shown in Fig 3.2.3. Linkage was excluded in Pedigree 1 for *EDAR* based on these findings.

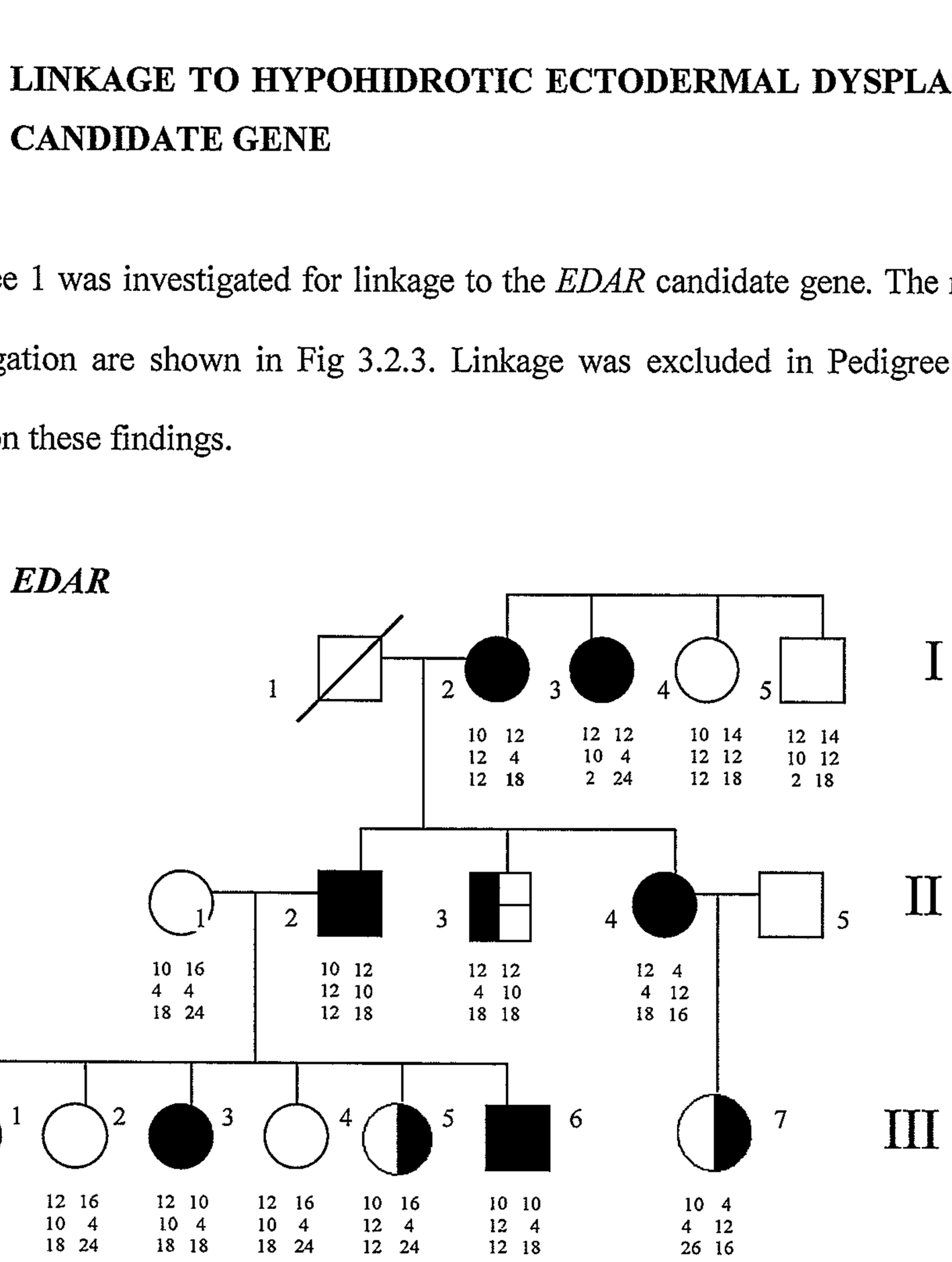


Figure 3.6 Pedigree 1 demonstrates probable exclusion of linkage to the *EDAR* candidate gene, based on ABI 310 electrophoretogram data for microsatellites D2S1890, D2S1893 and D2S1892, in descending order. Markers in bold type indicate that a recombination event has occurred. Note that multiple haplotypes (highlighted in red) are segregating among affected pedigree members.

3.2.4 LINKAGE TO GENOME-WIDE MICROSATELLITE MARKERS

Of the three pedigrees investigated, it was considered feasible to conduct a genome-wide linkage analysis for Pedigree 1, given that linkage to the *EDAR* candidate gene was excluded and that the pedigree was large enough to be informative. PCR samples of all 15 members of Pedigree 1 were processed and sent to the Australian Genome Research

Facility (AGRF) for this procedure. A standard microsatellite set was used, which has markers spaced on average 10cM apart.

In order to process the large amount of data received from AGRF, a hand search was undertaken of 6 affected and 2 unaffected pedigree members initially to determine whether any microsatellite marker segregated with disease. Those markers that showed potential for linkage were then investigated further by undertaking a haplotype construction to determine whether appropriate gene locus segregation had occurred (see Fig 3.7). By this refinement process, the microsatellite marker D17S784 showed potential for linkage. Linkage analysis was then undertaken for this marker located on chromosome 17 at the locus 17q25.3, generating a maximum LOD score of 3.32 at recombination fraction (θ) of 0.

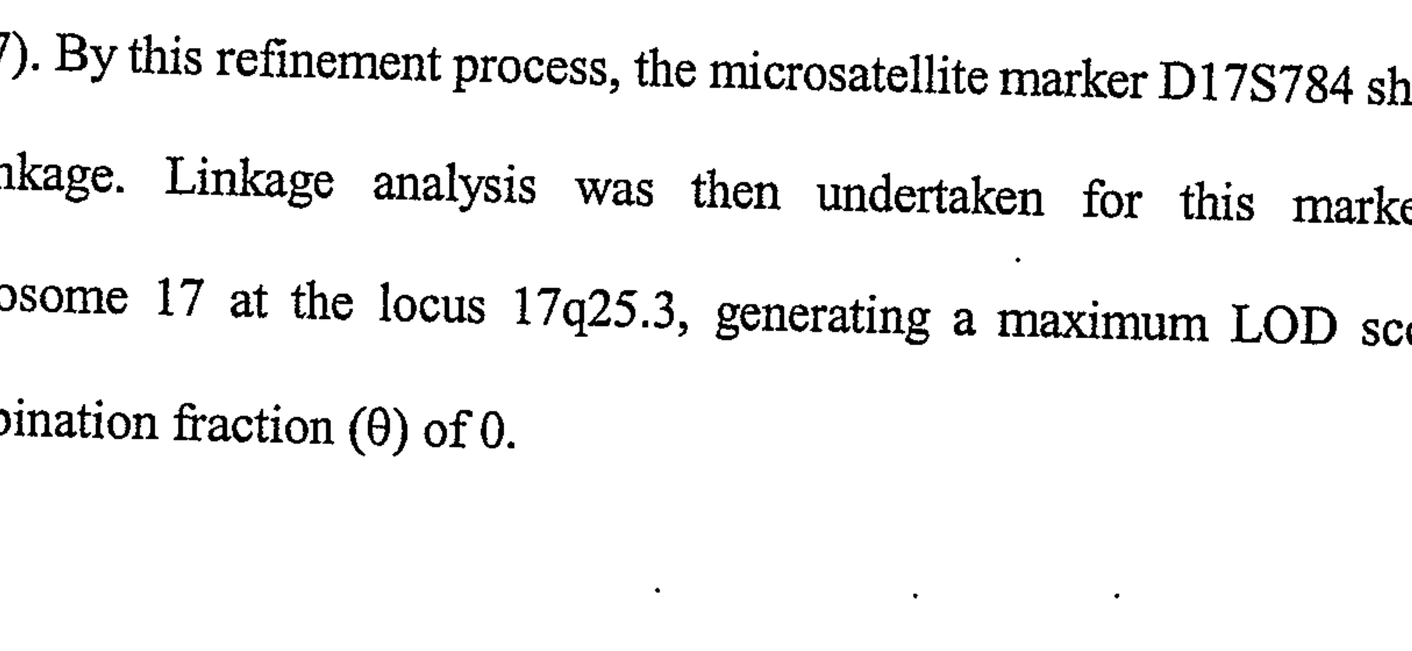


Figure 3.7 Pedigree 1 demonstrating linkage to a genomic segment on chromosome 17. The three adjacent markers in the set are D17S928, D17S784 and D17S785, in descending order. In affected pedigree member I.3, a recombination event has occurred between D17S784 and D17S785, reducing the likelihood that the disease causing gene is located between these two markers.

3.3 Discussion

As discussed in 1.5.1, autosomal dominant NSO is a genetically heterogeneous condition with reduced penetrance (90%). In addition, autosomal dominant HED has been shown to be difficult to clinically differentiate from NSO (1.2.4). One of the objectives of the linkage studies described in 3.1 and 3.2 was to differentiate the three pedigrees based on their genotype, and therefore to confirm or refute the clinical characterization based on each pedigree's phenotype.

Linkage to the NSO candidate genes *MSX1* and *PAX9* appeared to be excluded by linkage analysis for Pedigree 2 (see Fig 3.4). For Pedigree 3, it was not possible to include or exclude *MSX1* and *PAX9* as candidate genes (see Fig 3.5). The weaknesses of this linkage investigation for both these pedigrees were the pedigree size and the limited number of informative microsatellite markers employed. Unfortunately, it is impossible to predict if a pedigree member will be homozygous for a particular marker prior to linkage analysis, which would then render that marker uninformative. This was the case in Pedigree 2 for markers D4S2925 (marker for *MSX1*) and D14S69 (marker for *PAX9*) and in Pedigree 3 for D4S3023 (marker for *MSX1*).

As such, definitive exclusion of linkage of Pedigree 2 and confirmation of linkage of Pedigree 3 to the NSO candidate genes cannot be made. Further investigations to determine whether one of these two genes is the causative gene would involve incorporating more pedigree members into the study or by performing direct sequencing of the most likely candidate gene. See 4.2.1.

For the autosomal dominant HED pedigree (see Fig 3.5), linkage to the *EDAR* candidate gene was also excluded. In Fig 3.5 it is clear that multiple haplotypes are segregating among affected pedigree members, which excludes any one particular haplotype as carrying the disease. This also eliminated the need to perform LOD scores for any one particular marker.

A genome-wide linkage analysis of the autosomal dominant HED pedigree with microsatellite markers spread 10cM apart was then conducted. Three separate LOD score values were generated for all of these markers with the three equivocal pedigree members (II.3, III.5 and III.7) set as affected, unaffected and unknown, in order to eliminate the risk of diagnostic error. From this analysis, microsatellite marker D17S784 provided a positive LOD score greater than 3 when the equivocal members were set as unaffected.

The maximum LOD score for D17S784 was 3.32 at a recombination fraction (θ) of 0. The maximum LOD score dropped to 2.54 when these members were set as unknown. After cross-checking how marker D17S784 segregated with disease using the haplotype construction (see Fig 3.7), the locus of this marker was strongly suggestive of disease susceptibility.

Marker D17S784 mapped to the 17q25.3 locus on chromosome 17. A candidate gene search of this region identified two genes as potential candidates for ED, namely *DDX48* (DEAD box protein) and *CARD14* (caspase recruitment domain protein module).

An additional marker, D13S170 generated a maximum LOD score of 2.32 at θ=0. Although below the threshold score of 3 for statistical significance, this score is sufficiently high to consider further investigation for potential candidate genes.

During the clinical investigation, the affectation status of pedigree members II.3, III.5 and III.7 was considered equivocal (see 2.3). Of interest is the finding that in the haplotype construction using D17S784 from the genome-wide mapping study, none of these three individuals shared the gene locus segregating with the affected pedigree members. Out of these three individuals, two presented with agenesis of only three or less teeth (III.5 and III.7), including third molars, compared to agenesis of an average of 11 teeth for most other affected members (see 2.2.3), it is conceivable that these individuals were phenocopies, characterised by mild hair deformity and dysphagia.

The remaining pedigree member (II.3) was diagnosed with oligodontia based only on a history provided by the patient, as all teeth that had formed had since been extracted more than ten years prior. This individual displayed variable HED characteristics such as poor lacrimation, early frontal balding, but he also had a number of congenital anomalies and chronic illness not present in other family members. In this case, it is not possible to confirm the diagnosis of oligodontia without obtaining dental records or observing clinically acceptable photographs of the subject at a time when all teeth were present. It is conceivable that this individual did not have oligodontia and was not affected with HED.

In addition to obtaining further clinical information or recruiting more informative pedigree members, the only other way to obtain a definitive diagnosis in this family would be to sequence the candidate gene region. If a mutation was identified in the pedigree experiments would then need to be undertaken to confirm whether the gene in question played an important role in ectodermal tissue development.

A literature search was conducted for the two potential candidate genes mapping to the locus on chromosome 17. *DEAD* box genes have recently been shown to be regulators of

transcription and are considered to have an as yet unspecified role to play in embryogenesis (Wilson et al., 2004). There is little information as yet on the specific functions of the protein coded for by *DDX48*. CARD14 is a protein binding module that participates in apoptosis signaling through upstream activation of NFκB signaling (Bertin et al., 2001), a mechanism that is now known to be essential for normal development of ectodermally derived tissues (see 1.2.4).

Based on these findings, *CARD14* appears to be the most likely potential candidate gene of the two genes investigated for the phenotype reported in Pedigree 1. Further investigations are underway to determine whether this may be a causative gene for autosomal dominant HED (see 4.2.1).

3.4 Conclusion

This study excluded *MSX1* and *PAX9* as causative genes for an autosomal dominant NSO pedigree and excluded *EDAR* as a causative gene for an autosomal dominant HED pedigree. The similarity in the phenotype of each of these pedigrees to previously reported pedigrees with mutations in these respective genes indicates that autosomal dominant NSO and HED are both genetically heterogeneous conditions. For the HED pedigree, the gene locus 17q25.3 showed segregation with the disease and a maximum LOD score of 3.32 was determined by linkage analysis. Two potential candidate genes were identified at this gene locus.

4. RESEARCH SUMMARY AND FUTURE DIRECTION

4.1 Summary of Results

The objective of the research presented in this thesis was to investigate the clinical presentation and identify molecular genetic causes of oligodontia. The clinical component to the study achieved the objective of providing a careful characterization of the clinical features in three oligodontia pedigrees investigated. The molecular genetic component to the study achieved the objective in two out of three pedigrees (Pedigree 1 and Pedigree 2) of determining whether oligodontia was linked to previously reported candidate genes.

A careful clinical examination could not provide a definitive diagnosis of the affectation status of a small number of subjects that participated in the study. One of two possible scenarios may be implied by this finding. Firstly, it may be realistic to infer that clinical characterization of individuals with suspected autosomal dominant oligodontia conditions is necessary but may not be sufficient for diagnosis. Alternatively, it may be possible that a clinical investigative technique does exist or could be developed that has a greater predictive value than those implemented in this study or are currently reported in the literature. In either scenario, it is essential that further research is focused on determining the molecular genetic causes of the various oligodontia conditions (see 4.2.1).

As a result of this weakness in the study, two methods were found to be valuable in increasing the likelihood of reaching a definitive diagnosis for future research. Firstly, a comprehensive clinical examination of other family members allowed the clinicians to become aware of the familial characteristics of the condition, thereby facilitating the

differentiation of affected from unaffected individuals in some instances. Secondly and ultimately, it was recognised that the definitive diagnosis relies on identifying a common causative gene among all affected family members. An effective method in achieving this for families large enough to be informative is linkage analysis. However, the possibility of a recombination event occurring or the probability that these genetically heterogeneous conditions are caused by other as yet unidentified candidate genes implies that such an analysis would for now only be a confirmatory test rather than a diagnostic exclusion test (if linkage was identified). Mutation sequencing would be considered the most accurate diagnostic method but is not a viable option unless the phenotype correlates very closely to a known genotype.

In this study, pedigree member III.7 in Pedigree 3 was an example of how a certain diagnosis could not be reached without further investigation into what genotype contributed to the phenotype. In both Pedigree 2 and Pedigree 3, no definite instances of non-penetrance or decreased expressivity could be ascertained by combining the clinical and pedigree data collected, but this did not exclude the possibility of either phenomenon occurring. For pedigree member III.7 in Pedigree 3 it was difficult to conclude whether agenesis of two teeth, excluding 8's was a coincidental finding in an unaffected individual or whether this was an example of decreased expressivity (manifested by agenesis of six teeth, including 8's and several microdont teeth) in an affected individual.

Similarly, and perhaps even more confounding, was the difficulty in differentiating affected from unaffected individuals in the HED pedigree (Pedigree 1), given that diagnosis depended on the presence of a variable set of clinical features ranging from dental, dermatological and glandular disorders. Ranking this group of clinical signs and symptoms became necessary in order to establish affectation status but was done

arbitrarily by the clinicians involved in the study. This was because ascribing relative importance to each of the commonly occurring clinical features has not previously been reported for ED.

The question arises as to whether any clinical features are necessary and whether any are sufficient for establishing a diagnosis of HED. Many individuals in this pedigree provided examples of this diagnostic challenge, including subjects II.3, III.3, III.5, III.6 and III.7. After careful deliberation between the various clinicians involved in this study **(MM, MW, RW, MR)**, confirmed oligodontia was considered the only necessary and sufficient clinical indication for these individuals to be HED affected. Most of the other clinical features were considered collectively in order to determine whether any individuals with suspected or minimal oligodontia should also be considered as affected, but with variable dental expressivity.

The clinical research component to this study identified the need for careful consideration of the dental as well as extra-oral clinical features of each pedigree member by joint dental, clinical genetic and dermatological collaboration in order to establish an appropriate diagnosis. Additionally, it was desirable to obtain an historical radiographic and clinical record that supplemented the data collected on the day of attendance in order to avoid misdiagnosis of tooth agenesis for each affected individual.

The findings of the clinical study showed that autosomal dominant NSO may be associated with mild hair or fingernail deformities, blurring the line that is drawn to differentiate this dental condition from ectodermal dysplasia. In contrast, autosomal dominant HED is associated with sparseness of hair, hypohidrosis, anomalies in premolar

cusp morphology and radiographic evidence of dental cervical constriction, which may help differentiate HED from NSO.

The molecular genetic component of the study was a two-step investigation. Firstly, each pedigree was analysed for linkage to candidate gene loci (candidate gene approach). Genome-wide mapping by linkage analysis was then performed for the most informative pedigree (Pedigree 1) when linkage to the candidate gene was excluded. For both of these investigations, microsatellite DNA markers were used.

The results of the candidate gene approach linkage investigation were:

- Linkage of autosomal dominant HED (Pedigree 1) to the candidate gene *EDAR* was excluded
- Linkage of autosomal dominant NSO to the candidate genes *MSX1* and *PAX9* was excluded for Pedigree 2
- Linkage of autosomal dominant NSO to the candidate genes *MSX1* and *PAX9* could not be excluded for Pedigree 3, based on the size of the pedigree and the number of microsatellite markers used for the investigation

The results of the genome-wide mapping investigation were:

- The autosomal dominant HED pedigree (Pedigree 1) provided a potential disease susceptibility locus on chromosome 17. Microsatellite marker D17S784 at locus 17q25.3 generated a maximum LOD score of 3.32, provided that pedigree members II.3, III.5 and III.7 were considered unaffected. A positive LOD score of 3 or greater is considered statistically significant but further investigation is required to confirm whether a new disease susceptibility locus for autosomal dominant HED has been identified by this research.

- In addition, microsatellite marker D13S170 at locus 13q31.1 on chromosome 13 showed a maximum LOD score of 2.54 and although below the minimum LOD score threshold of 3, should be investigated further to determine whether any other potential candidate genes exist in the region.

In conclusion, the clinical component of the study identified the challenges involved in obtaining a definitive clinical diagnosis of oligodontia. It was recognised that diagnosing a pedigree with a specific oligodontia condition (either autosomal dominant NSO or HED) was possible without molecular genetic input, but necessitated joint dental, dermatological and clinical genetic collaboration. This diagnosis could be made once a thorough family history had been taken and once one or more of the most severely affected pedigree members had been clinically assessed.

In contrast, the diagnosis of members of the family with milder or fewer of the clinical features observed in the affected members was far more difficult and relied heavily on determining the haplotype linked to the condition. What this implies is that oligodontia, as has been previously been reported (Burzynski and Escobar, 1983), is a condition of variable expressivity.

The molecular genetic component of the study excluded several candidate genes as being the genes responsible for the oligodontia condition diagnosed in two out of the three pedigrees investigated. The consequence of this is that there must be as yet unreported genes responsible for autosomal dominant NSO and HED which should now be investigated.

4.2 Future Directions

4.2.1 FURTHER INVESTIGATIONS INTO OLIGODONTIA

Several weaknesses in this clinical and molecular genetic study highlighted some of the directions future research should take in the investigation of oligodontia. Firstly, definitive diagnosis of all subjects cannot be made without initially determining the genetic cause or causes to their oligodontia condition. This assumption is based on the finding that clinical examination alone, even when a multi-disciplinary approach was taken, was insufficient in providing a definitive diagnosis of some pedigree members.

Secondly, once the primary causative genes for oligodontia have been identified, it may be possible to develop a non-invasive, clinical investigative technique that has a high predictive value of affectation status. This will off course depend on identifying a phenotypic trait that correlates consistently with the gene involved in each specific oligodontia condition. No such genotype-phenotype correlations have yet been reported for any of the oligodontia conditions but some associations are known (see 4.2.2).

If it is presumed that the phenotype is a predictor of the genotype, then some propositions can be made about the pedigrees investigated in this study that can be the stimulus for further investigation. For Pedigree 1, if fine mapping and gene sequencing of the 17q25.3 and 13q31.1 loci identify a gene mutation that segregates with disease, a new causative gene mutation for autosomal dominant HED might be isolated. Research is already under way in our laboratory to conduct the fine mapping and candidate gene screening studies.

For Pedigree 2, exclusion of linkage to the candidate genes *MSX1* and *PAX9* is an interesting finding as there is only one other report in the literature of a family with NSO that is not linked to either of these candidate genes (see 1.5.2.1). Unfortunately, due to the unavailability of other family members (see 2.2.1), further investigation into this family would require a collaborative effort with researchers based in either the UK or Ireland. This type of collaborative effort would work best if both centres had access to multiple large pedigrees with NSO not linked to *MSX1* or *PAX9*, so that a large pool of individuals could be characterised both phenotypically and genotypically. Attempts at collaboration for such a multi-centre study have already been initiated.

Finally, Pedigree 3 was characterised by a pattern of oligodontia that correlates best with previously reported pedigrees linked to *MSX1* mutation (see 4.2.2, Fig 1.3). Linkage to this candidate gene could not be confirmed based on the small size of subjects investigated and on an insufficient number of informative markers available (see 3.2.2). Research is already underway in our laboratory to perform direct sequencing of *MSX1* for members of Pedigree 3 for a pathogenic mutation.

4.2.2 GENOTYPE-PHENOTYPE CORRELATIONS FOR A BETTER NOSOLOGY OF OLIGODONTIA CONDITIONS

Much has already been achieved in the understanding of the molecular genetic mechanisms contributing to oligodontia. However, further research is still required in order to determine all of the common genes responsible for the oligodontia conditions observed clinically. The ultimate aim for clinicians and affected individuals is to be able to have a clinical diagnosis confirmed by a simple clinical or molecular genetic test. The likelihood of such a test becoming available depends very much on establishing accurate genotype-phenotype correlations for all the commonly occurring oligodontia conditions.

To date, the best available data on genotype-phenotype correlations amongst autosomal dominant oligodontia conditions is:

MSX1 NSO: Associated with agenesis of 5's, U4's, L6's, U2's and L1's (in addition to 8's), normal primary dentition, teeth that form are mildly microdont and molars that form have a simplified occlusal anatomy, with U7's lacking a distolingual cusp and L6's lacking a distobuccal cusp (Lidral and Reising, 2002; van den Boogaard et al., 2000; Vastardis et al., 1996)

PAX9 NSO: Associated with agenesis of 7's, 6's, 5's and L1's (in addition to 8's), teeth that form are mildly microdont, including peg U2's (Goldenberg et al., 2000)

EDAR HED: Associated with agenesis of U2's, L1's, L2's and 5's (data incomplete), conical primary teeth (consistently reported), other ectodermal dysplastic features include sparse hair, hypohidrosis, dry, smooth, thin skin, mild fingernail abnormalities and deficient lactation, comorbidities include joint hypermobility (Aswegan et al., 1997; Jorgenson et al., 1987).

Non-*EDAR* HED: Associated with agenesis of 5's, L1's, 2's, 4's and 7's (in addition to 8's), teeth that do form are mildly microdont and cervically constricted, other ectodermal dysplastic features include moderate to severe hypohidrosis, dysphagia associated with poor lubrication of the oropharynx, variable dryness of the eyes, variable macroscopic sparseness and microscopic deformability of hair and mild fingernail abnormalities (based on the findings of this research)

It is hoped that with further investigation already commenced in our laboratory, the gene mutation responsible for the new autosomal dominant HED phenotype will be identified.

4.2.3 STEM CELL RESEARCH, PHARMACOGENOMICS AND OLIGODONTIA

Stem cell research offers tremendous potential for treating a variety of diseases by way of regenerating tissues and organs from undifferentiated cells (stem cells) that are capable of extensive proliferation. A dental application of stem cell research may be the manufacture of replacement teeth for individuals who have had a tooth removed or for oligodontia patients.

There are two main factors that have led to the possibility of constructing teeth in the laboratory. Firstly, there has been a rapid increase in understanding of the molecular regulation of tooth development (see 1.4.4). Secondly, the recent breakthroughs in stem cell research have shown that adult stem cells are more plastic (pluripotent) in their behaviour than previously thought, in terms of their ability to change directions of differentiation according to environmental signals (Donovan and Gerhart, 2001; Thesleff, 2003). This implies that there are more opportunities to use adult stem cells for a variety of tissue regeneration projects than was previously expected.

However, in the case of odontogenesis, not all of the molecular mechanisms necessary to replicate a tooth in its entirety have been fully elucidated yet. Nonetheless, dental tissues have been regenerated when transplanted into animals. In addition, there is a need to find new ways to identify and harvest potential stem cells and to induce their differentiation to specific directions; the practical designs for transplantation and culture of cells and tissues present great challenges (Thesleff, 2003).

Recent experiments in gene manipulation on mice may also provide hope to patients affected by HED. As discussed in 1.3.4, the gene responsible for X-linked HED, known as the *EDA* gene, is homologous with the *Tabby* (*Ta*) gene in the mouse. A knockout mouse model (a mouse that has a modified genome by the targeted disruption of a gene) has been developed that lacks this *Ta* gene and is known as the Tabby mouse. The Tabby mouse is an extremely useful model from which to investigate the phenotypic effects of a particular gene mutation and to also determine if by re-inserting the gene, the mouse can revert to a normal phenotype (known as phenotypic rescue).

Two recent experiments have shown that rescue of the Tabby phenotype can be achieved (Gaide and Schneider, 2003; Srivastava et al., 2001). Most impressively, it has now been shown possible that the treatment of pregnant Tabby mice with a recombinant form of EDA-A1, the EDA isoform that is associated with X-linked HED, can permanently rescue the Tabby phenotype in the offspring by being engineered to cross the placental barrier (Gaide and Schneider, 2003). The authors concluded that similar pharmacogenomic approaches may be feasible for the treatment of other developmental diseases caused by deficient expression of a ligand.

The findings of this study also have implications for autosomal dominant HED caused by mutation in the *EDAR* gene, as it is the EDA-A1 isoform that activates the receptor coded for by this gene (see 1.5.2.2). In addition, if this protein can be engineered to cross the placental barrier and activate this pathway in humans, not only could patients with *EDAR*-associated HED benefit from such therapy but also those with *EDARADD*-associated HED and incontinentia pigmenti.

GLOSSARY

Anhidrotic	Deficiency or absence of sweat secretion
Cyclopia	Fusion of the orbits and various degrees of fusion of the eyes, often associated with holoprosencephaly
Dysphasia	Difficulty in speaking or in understanding language
Epistatic	An allele of one gene that can block the phenotypic expression of all alleles of another gene
Haplotype	A group of alleles at closely linked loci usually inherited as a unit
Holoprosencephaly	Developmental disorder of the brain, resulting from defective cleavage of the prosencephalon
Homeobox	A conserved 180 base pair sequence in the coding region of several genes thought critical in specifying segment identity in all vertebrates
Hyperpyrexia	Excessively high fever
Hypohidrotic	Deficient perspiration
Hypotrichosis	Lacking hair
Knockout mice	Mice in which a specific gene has been targeted (disrupted) by recombinant DNA technology in order to investigate gene function
Meristic	Pertaining to, or divided into segments
Microcephaly	Abnormally small head, sometimes due to congenital hypoplasia of the cerebrum
Monogenic	Genetic condition caused by a single gene defect, giving rise to classical Mendelian inheritance patterns
Nosology	Science of the classification of diseases
Onychodysplasia	Distortions of a nail
Polygenic	Inheritance determined by many genes at different loci, with small additive effects
Primordia	First formed, primitive

REFERENCE LIST

Genome Browser. www.genome.ucsc.edu . 2004a.

Ref Type: Electronic Citation

Genome Database. www.gdb.org . 2004b.
Ref Type: Electronic Citation

Acerbi AG, de Freitas C, de Magalhaes MH (2001), Prevalence of numeric anomalies in the permanent dentition of patients with Down syndrome. *Special Care in Dentistry 21(2):75-8,*-Apr

Alexander-Abt J (1999), Apparent hypodontia: a case of misdiagnosis. *American Journal of Orthodontics & Dentofacial Orthopedics* 116:321-323

Anderson R, Causer P (1989), Scaling skin in the neonate: a clue to the early diagnosis of X-linked hypohidrotic ectodermal dysplasia (Christ-Siemens-Touraine syndrome). *Journal of Pediatrics* 114:600-602

Aswegan AL, Josephson KD, Mowbray R, Pauli RM, Spritz RA, Williams MS (1997), Autosomal dominant hypohidrotic ectodermal dysplasia in a large family. *American Journal of Medical Genetics* 72:462-467

Atherton DJ . Dermatological aspects of X-linked hypohidrotic ectodermal dysplasia. 2004. Third international conference on the management of ectodermal dysplasia: ECT04. Eastman Dental Institute, London, UK
Ref Type: Conference Proceeding

Baala L, Hadj Rabia S, Zlotogora J, Kabbaj K, Chhoul H, Munnich A, Lyonnet S, Sefiani A (1999), Both recessive and dominant forms of anhidrotic/hypohidrotic ectodermal dysplasia map to chromosome 2q11-q13. *American Journal of Human Genetics* 64:651-653

Baccetti T (1998), Tooth rotation associated with aplasia of nonadjacent teeth. *Angle Orthodontist* 68:471-474

Berg D, Weingold DH, Abson KG, Olsen EA (1990), Sweating in ectodermal dysplasia syndromes. A review. [Review] [57 refs]. *Archives of Dermatology* 126:1075-1079

Bergendal B, Koch G, Kurol J (1998), On tooth agenesis in patients with anhidrotic ectodermal dysplasia in the Nordic countries - a questionnaire survey. In: *Consensus Conference on Ectodermal Dysplasia with Special Reference to Dental Treatment*, Bergendal B, Koch G, Kurol J, eds. Stockholm: Gothia, pp 54-60

Bertin J, Wang L, Guo Y, Jacobson MD, Poyet JL, Srinivasula SM, Merriam S, DiStefano PS, Alnemri ES (2001), CARD11 and CARD14 are novel caspase recruitment domain (CARD)/membrane-associated guanylate kinase (MAGUK) family members that interact with BCL10 and activate NF-kappa B. *Journal of Biological Chemistry* 276:11877-11882

Billings RJ, Berkowitz RJ, Watson G (2004), Teeth. [Review] [108 refs]. *Pediatrics 113(4 Suppl):1120-7,*

Bixler D, Saksena SS, Ward R (1988), Characterization of the face in hypohidrotic ectodermal dysplasia by cephalometric and anthropometric analysis. *Birth Defects: Original Article Series* 24:197-203

Blankenstein R, Brook AH, Smith RN, Patrick D, Russell JM (2001), Oral findings in Carpenter Syndrome. *International Journal of Paediatric Dentistry* 11:352-360

Boj JR, Duran J, Cortada M, Jimenez A, Golobart J (1993), Cephalometric changes in a patient with ectodermal dysplasia after placement of dentures. *Journal of Clinical Pediatric Dentistry* 17:217-220

Bondarets N, McDonald F (2000), Analysis of the vertical facial form in patients with severe hypodontia. *American Journal of Physical Anthropology* 111:177-184

Borgstrom MK, Riise R, Tornqvist K, Granath L (1996), Anomalies in the permanent dentition and other oral findings in 29 individuals with Laurence-Moon-Bardet-Biedl syndrome. *Journal of Oral Pathology & Medicine 25(2):86-9,*

Burzynski NJ, Escobar V (1983), Classification and genetics of numeric anomalies of dentition. *Birth Defects: Original Article Series* 19:95-106

Butler PM (1939), Studies of the mammalian dentition. Differentiation of the postcanine dentition. *Proceedings of the Zoological Society of London* 109B:329-356

Buyse ML (1990), *Birth Defects Encyclopedia*. Cambridge: Blackwell Scientific

Cameron AC, Widmer RP (2003), *Handbook of Paediatric Dentistry*. Edinburgh: Mosby

Carter NE, Gillgrass TJ, Hobson RS, Jepson N, Eechan JG, Nohl FS, Nunn JH (2003), The interdisciplinary management of hypodontia: orthodontics. *British Dental Journal* 194:361-366

Clarke A, Phillips DI, Brown R, Harper PS (1987), Clinical aspects of X-linked hypohidrotic ectodermal dysplasia. *Archives of Disease in Childhood* 62:989-996

Cobourne MT (1999), The genetic control of early odontogenesis. *British Journal of Orthodontics* 26:21-28

Cohen MM (1989), Syndromology: an updated conceptual overview IV. Perspectives on malformation syndromes. *International Journal of Oral & Maxillofacial Surgery* 18:286-290

Coston GN, Salinas CF (1988), Speech characteristics in patients with hypohidrotic ectodermal dysplasia. *Birth Defects: Original Article Series* 24:229-234

Crawford PJ, Aldred MJ, Clarke A, Tso MS (1989), Rapp-Hodgkin syndrome: an ectodermal dysplasia involving the teeth, hair, nails, and palate. Report of a case and review of the literature. [Review] [23 refs]. *Oral Surgery, Oral Medicine, Oral Pathology* 67:50-62

Danz DFG (1794), Sechste bemerkung. Von menschen ohne haare und zähne. *Stark Arch Geburtsh Frauenz Neugeb Kinderkr* 4:684

Darwin C (1880), *The Variations of Plants and Animals under Domestication*. Paris: C. Reinwald

Das P, Stockton DW, Bauer C, Shaffer LG, D'Souza RN, Wright JT, Patel PI (2002), Haploinsufficiency of PAX9 is associated with autosomal dominant hypodontia. *Human Genetics* 110:371-376

Dentistry and Real Life . Primary and permanent dentition. Internet . 2004: http://users.hol.gr/~jelian/home_en.htm
Ref Type: Electronic Citation

Dhanrajani PJ, Jiffry AO (1998), Management of ectodermal dysplasia: a literature review. *Dental Update* 25:73-75

Donovan PJ, Gerhart J (2001), The end of the beginning for pluripotent stem cells. *Nature* 414:92-97

Ferguson BM, Thomas NS, Munoz F, Morgan D, Clarke A, Zonana J (1998), Scarcity of mutations detected in families with X linked hypohidrotic ectodermal dysplasia: diagnostic implications. *Journal of Medical Genetics* 35:112-115

Frazier-Bowers SA, Guo DC, Cavender A, Xue L, Evans B, King T, Milewicz D, D'Souza RN (2002), A novel mutation in human PAX9 causes molar oligodontia. *Journal of Dental Research* 81:129-133

Frazier-Bowers SA, Pham KY, Le EV, Cavender AC, Kapadia H, King TM, Milewicz DM, D'Souza RN (2003), A unique form of hypodontia seen in Vietnamese patients: clinical and molecular analysis. *Journal of Medical Genetics* 40:e79

Freire-Maia N, Pinheiro M (1984), *Ectodermal Dysplasias: A Clinical and Genetic Study*. New York: Alan R. Liss

Freire-Maia N, Pinheiro M (1990), Precocious mortality in Christ-Siemens-Touraine syndrome. *American Journal of Medical Genetics* 37:299

Fymbo L (1936), The relation of malocclusion of the teeth to defects of speech. *Archives of Speech*216

Gaide O, Schneider P (2003), Permanent correction of an inherited ectodermal dysplasia with recombinant EDA. *Nature Medicine* 9:614-618

Goho C (1993), Chemoradiation therapy: effect on dental development. *Pediatric Dentistry* 15:6-12

Goldenberg M, Das P, Messersmith M, Stockton DW, Patel PI, D'Souza RN (2000), Clinical, radiographic, and genetic evaluation of a novel form of autosomal-dominant oligodontia. *Journal of Dental Research* 79:1469-1475

Gorlin RJ, Cohen MM, Jr., Hennekam RC (2002), *Syndromes of the Head and Neck*. Oxford: Oxford University Press

Grahnen H (1956), Hypodontia in the permanent dentition. A clinical and genetical investigation. *Odontologisk Revy* 7:

Gray IC, Campbell DA, Spurr NK (2000), Single nucleotide polymorphisms as tools in human genetics. *Human Molecular Genetics* 9:2403-2408

Grigoriou M, Tucker AS, Sharpe PT, Pachnis V (1998), Expression and regulation of *Lhx6* and *Lhx7*, a novel subfamily of LIM homeodomain encoding genes, suggests a role in mammalian head development. *Development* 125:2063-2074

Gunduz K, Gunalp I, Erden I (1997), Focal dermal hypoplasia (Goltz's syndrome). *Ophthalmic Genetics 18(3):143-9,*

Hall RK (1994), Abnormalities of tooth number, form and size. In: *Pediatric Orofacial Medicine and Pathology*, London: Chapman & Hall, pp 151-172

Headon DJ, Emmal SA, Ferguson BM, Tucker AS, Justice MJ, Sharpe PT, Zonana J, Overbeek PA (2001), Gene defect in ectodermal dysplasia implicates a death domain adapter in development. *Nature* 414:913-916

Ho NC, Lacbawan F, Francomano CA, Ho V (2000), Severe hypodontia and oral xanthomas in Alagille syndrome. *American Journal of Medical Genetics 93(3):250-2,*

Hobkirk JA, Goodman JR, Jones SP (1994), Presenting complaints and findings in a group of patients attending a hypodontia clinic. *British Dental Journal* 177:337-339

Hobson RS, Carter NE, Gillgrass TJ, Jepson NJ, Meechan JG, Nohl F, Nunn JH (2003), The interdisciplinary management of hypodontia: the relationship between an interdisciplinary team and the general dental practitioner. *British Dental Journal* 194:479-482

Hodges SJ, Harley KE (1999), Witkop tooth and nail syndrome: report of two cases in a family. *International Journal of Paediatric Dentistry* 9:207-211

Hummel P, Guddack S (1997), Psychosocial stress and adaptive functioning in children and adolescents suffering from hypohidrotic ectodermal dysplasia. *Pediatric Dermatology* 14:180-185

Jepson NJ, Nohl FS, Carter NE, Gillgrass TJ, Meechan JG, Hobson RS, Nunn JH (2003), The interdisciplinary management of hypodontia: restorative dentistry. [Review] [21 refs]. *British Dental Journal* 194:299-304

Jernvall J, Thesleff I (2000), Reiterative signaling and patterning during mammalian tooth morphogenesis. [Review] [85 refs]. *Mechanisms of Development* 92:19-29

Jorgenson RJ, Dowben JS, Dowben SL (1987), Autosomal dominant ectodermal dysplasia. *Journal of Craniofacial Genetics & Developmental Biology* 7:403-412

Jorgenson RJ, Salinas CF, Dowben JS, St John DL (1988), A population study on the density of palmar sweat pores. *Birth Defects: Original Article Series* 24:51-63

Karlstedt E, Kaitila I, Pirinen S (1996), Phenotypic features of dentition in diastrophic dysplasia. *Journal of Craniofacial Genetics & Developmental Biology 16(3):164-73,*-Sep

Kere J, Srivastava AK, Montonen O, Zonana J, Thomas N, Ferguson B, Munoz F, Morgan D, Clarke A, Baybayan P, Chen EY, Ezer S, Saarialho-Kere U, de la CA, Schlessinger D (1996), X-linked anhidrotic (hypohidrotic) ectodermal dysplasia is caused by mutation in a novel transmembrane protein. [see comments.]. *Nature Genetics* 13:409-416

Kessler M, Melton DA (1994), Vertebrate embryonic induction: mesodermal and neural patterning. *Science* 266:596-604

Kingston HM (1990), *ABC of Clinical Genetics*. London: British Medical Association

Kristenova P, Peterka M, Lisi S, Gendrault J-L, Lesot H, Peterkova R (2002), Different morphotypes of functional dentition in the lower molar region of tabby (EDA) mice. *Orthodontics & Craniofacial Research* 5:205-214

Laurikkala J, Mikkola M, Mustonen T, Aberg T, Koppinen P, Pispa J, Nieminen P, Galceran J, Grosschedl R, Thesleff I (2001), TNF signaling via the ligand-receptor pair ectodysplasin and edar controls the function of epithelial signaling centers and is regulated by Wnt and activin during tooth organogenesis. *Developmental Biology* 229:443-455

Levin LS (1988), Dental and oral abnormalities in selected ectodermal dysplasia syndromes. [Review] [58 refs]. *Birth Defects: Original Article Series* 24:205-227

Lewis EB (1978), A gene complex controlling segmentation in *Drosophila*. *Nature* 276:565-570

Lidral AC, Reising BC (2002), The role of MSX1 in human tooth agenesis. *Journal of Dental Research* 81:274-278

Line SR (2001), Molecular morphogenetic fields in the development of human dentition. *Journal of Theoretical Biology* 211:67-75

Liu W, Selever J, Lu MF, Martin JF (2003), Genetic dissection of Pitx2 in craniofacial development uncovers new functions in branchial arch morphogenesis, late aspects of tooth morphogenesis and cell migration. *Development* 130:6375-6385

Liu W, Wang H, Zhao S, Zhao W, Bai S, Zhao Y, Xu S, Wu C, Huang W, Chen Z, Feng G, He L (2001), The novel gene locus for agenesis of permanent teeth (He-Zhao deficiency) maps to chromosome 10q11.2. *Journal of Dental Research* 80:1716-1720

Lu MF, Pressman C, Dyer R, Johnson RL, Martin JF (1999), Function of Rieger syndrome gene in left-right asymmetry and craniofacial development. *Nature* 401:276-278

Lumsden AGS (1988), Spatial organisation of the epithelium and the role of neural crest cells in the initiation of the mammalian tooth germ. *Development* 103 (Suppl):155-169

Maas R, Bei M (1997), The genetic control of early tooth development. *Critical Reviews in Oral Biology & Medicine* 8:4-39

Mandler M, Neubüser A (2001), FGF signaling is necessary for the specification of the odontogenic mesenchyme. *Developmental Biology* 240:548-559

Matsune K, Shimizu T, Tohma T, Asada Y, Ohashi H, Maeda T (2001), Craniofacial and dental characteristics of Kabuki syndrome. *American Journal of Medical Genetics* 98:185-190

Meechan JG, Carter NE, Gillgrass TJ, Hobson RS, Jepson NJ, Nohl FS, Nunn JH (2003), Interdisciplinary management of hypodontia: oral surgery. [Review] [15 refs]. *British Dental Journal* 194:423-427

Mestrovic SR, Rajic Z, Papic JS (1998), Hypodontia in patients with Down's syndrome. *Collegium Antropologicum 22 Suppl:69-72,*

Mikkola M, Thesleff I (2003), Ectodysplasin signaling in development. *Cytokine and Growth Factor Reviews* 14:211-224

Mina M, Kollar EJ (1987), The induction of odontogenesis in non-dental mesenchyme combined with early murine mandibular arch epithelium. *Archives of Oral Biology* 32:123-127

Moiseiwitsch JR (2000), The role of serotonin and neurotransmitters during craniofacial development. [Review] [63 refs]. *Critical Reviews in Oral Biology & Medicine 11(2):230-9,*

Monreal AW, Ferguson BM, Headon DJ, Street SL, Overbeek PA, Zonana J (1999), Mutations in the human homologue of mouse dl cause autosomal recessive and dominant hypohidrotic ectodermal dysplasia. [see comments.]. *Nature Genetics* 22:366-369

Mostowska A, Kobielak A, Trzeciak WH (2003), Molecular basis of non-syndromic tooth agenesis: mutations of MSX1 and PAX9 reflect their role in patterning human dentition. *European Journal of Oral Sciences* 111:365-370

Myer III CM (1988), Otolaryngologic manifestations of the ectodermal dysplasias. *Birth Defects: Original Article Series* 24:253-256

Naito A, Yoshida H, Nishioka E, Satoh M, Azuma S, Yamamoto T, Nishikawa S, Inoue J (2002), TRAF6-deficient mice display hypohidrotic ectodermal dysplasia. *Proceedings of the National Academy of Sciences of the United States America* 99:8766-8771

Nakata M, Koshiba H, Eto K, Nance WE (1980), A genetic study of anodontia in X-linked hypohidrotic ectodermal dysplasia. *American Journal of Human Genetics* 32:908-919

Nanni L, Ming JE, Du Y, Hall RK, Aldred MJ, Bankier A, Muenke M (2001), *SHH* mutation is associated with solitary median maxillary central incisor: a study of 13 patients and review of the literature. *American Journal of Medical Genetics* 102:1-10

Neubüser A, Peters H, Balling R, Martin GR (1997), Antagonistic interactions between FGF and BMP signaling pathways: a mechanism for positioning the sites of tooth formation. *Cell* 90:247-255

Nieminen P, Pekkanen M, Aberg T, Thesleff I . Molecules expressed in early (initiation stage) oral epithelium and dental mesenchyme. www.bite-it.helsinki.fi . 2004.
Ref Type: Electronic Citation

Nomura S, Hasegawa S, Noda T, Ishioka K (1993), Longitudinal study of jaw growth and prosthetic management in a patient with ectodermal dysplasia and anodontia. *International Journal of Paediatric Dentistry* 3:29-38

Nordgarden H, Jensen JL, Storhaug K (2001), Oligodontia is associated with extra-oral ectodermal symptoms and low whole salivary flow rates. *Oral Diseases* 7:226-232

Nordgarden H, Johannessen S, Storhaug K, Jensen JL (1998), Salivary gland involvement in hypohidrotic ectodermal dysplasia. [erratum appears in Oral Dis 1998 Dec;4(4):286.]. *Oral Diseases* 4:152-154

Nordgarden H, Storhaug K, Lyngstadaas SP, Jensen JL (2003), Salivary gland function in persons with ectodermal dysplasias. *European Journal of Oral Sciences* 111:371-376

Nowak AJ (1988), Dental treatment for patients with ectodermal dysplasias. [Review] [32 refs]. *Birth Defects: Original Article Series* 24:243-252

Nunn JH, Carter NE, Gillgrass TJ, Hobson RS, Jepson NJ, Meechan JG, Nohl FS (2003), The interdisciplinary management of hypodontia: background and role of paediatric dentistry. [Review] [52 refs]. *British Dental Journal* 194:245-251

Nussbaum RL, McInnes RR, Willard HF (2001), *Thompson & Thompson Genetics in Medicine*. Philadelphia: W.B. Saunders Company

O'Dowling IB, McNamara TG (1990), Congenital absence of permanent teeth among Irish school-children. [Review] [23 refs]. *Journal of the Irish Dental Association* 36:136-138

Ogaard B, Krogstad O (1995), Craniofacial structure and soft tissue profile in patients with severe hypodontia. *American Journal of Orthodontics & Dentofacial Orthopedics* 108:472-477

Ohazama A, Courtney JM, Sharpe PT (2003), Expression of TNF-receptor-associated factor genes in murine tooth development. *Gene Expression Patterns* 3:127-129

Olin WH (1964), Dental anomalies in cleft lip and palate patients. *Angle Orthodontist* 34:111-124

Online Mendelian Inheritance in Man O . #604625 Oligodontia. Center for Medical Genetics, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD) . 5-10-2002. Website: www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Ref Type: Electronic Citation

Online Mendelian Inheritance in Man O . #305100 X-linked HED. Internet . 2004. Website: www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Ref Type: Electronic Citation

Ørstavik KH (1998), Medical symptoms and genetic counselling in hypohidrotic ectodermal dysplasia. In: *Consensus Conference on Ectodermal Dysplasia with Special Reference to Dental Treatment*, Bergendal B, Koch G, Kurol J, Wanndahl G, eds. Stockholm: Gothia, pp 41-53

Orup HI, Jr., Keith DA, Holmes LB (1998), Prenatal anticonvulsant drug exposure: teratogenic effect on the dentition. *Journal of Craniofacial Genetics & Developmental Biology 18(3):129-37,*-Sep

Osborn JW (1978), Morphogenetic gradients: fields versus clones. In: *Development, Function and Evolution of the Teeth*, Butler PM, Joysey KA, eds. New York: Academic Press, pp 171-201

Ott J (1985), *Analysis of Human Genetic Linkage*. Baltimore: The Johns Hopkins University Press

Peck S, Peck L, Kataja M (1996), Site specificity of tooth agenesis in subjects with maxillary canine malpositions. *Angle Orthodontist* 66:473-476

Peterson-Falzone SJ, Caldarelli DD, Landahl KL (1981), Abnormal laryngeal vocal quality in ectodermal dysplasia. *Archives of Otolaryngology* 107:300-304

Petzold D, Kratzsch E, Opitz C, Tinschert S (2003), The Kabuki syndrome: four patients with oral abnormalities. *European Journal of Orthodontics 25(1):13-9,*

Pirinen S (1998), Dental and oral aspects in anhidrotic ectodermal dysplasia. In: *Consensus Conference on Ectodermal Dysplasia with Special Reference to Dental Treatment*, Bergendal B, Koch G, Kurol J, Wanndahl G, eds. Stockholm: Gothia, pp 13-21

Pirinen S, Arte S, Apajalahti S (1996), Palatal displacement of canine is genetic and related to congenital absence of teeth. *Journal of Dental Research* 75:1742-1746

Pispa J, Jung HS, Jernvall J, Kettunen P, Mustonen T, Tabata MJ, Kere J, Thesleff I (1999), Cusp patterning defect in *tabby* mouse teeth and its partial rescue by FGF. *Developmental Biology* 216:521-534

Polder BJ, Van't Hof MA, Van der Linden FPGM, Kuijpers-Jagtman AM (2004), A meta-analysis of the prevalence of dental agenesis of permanent teeth. *Community Dentistry and Oral Epidemiology* 32:217-226

Priolo M, Lagana C (2001), Ectodermal dysplasias: a new clinical-genetic classification. *Journal of Medical Genetics* 38:579-585

Priolo M, Silengo M, Lerone M, Ravazzolo R (2000), Ectodermal dysplasias: not only 'skin' deep. [Review] [168 refs]. *Clinical Genetics* 58:415-430

Ranta R (1986), A review of tooth formation in children with cleft lip/palate. *American Journal of Orthodontics & Dentofacial Orthopedics* 90:11-18

Rietschel R (1979), Anhidrotic ectodermal dysplasia: management. *International Journal of Dermatology* 18:370-371

Riise R, Storhaug K, Brondum-Nielsen K (2001), Rieger syndrome is associated with PAX6 deletion. *Acta Ophthalmologica Scandinavica* 79:201-203

Rogers M (1996), Hair shaft abnormalities. *Australian Journal of Dermatology* 37:1-11

Rogers M (2000), The "bar code" phenomenon: a microscopic artifact seen in patients with hypohidrotic ectodermal dysplasia. *Pediatric Dermatology* 17:329-330

Rolling S, Poulsen S (2001), Oligodontia in Danish schoolchildren. *Acta Odontologica Scandinavica* 59:111-112

Rouse C, Siegfried E, Breer W, Nahass G (2004), Hair and sweat glands in families with hypohidrotic ectodermal dysplasia. *Archives of Dermatology* 140:850-855

Ruch JV, Lesot H, Karcher-Djuricic V, Meyer JM, Mark M (1983), Epithelial-mesenchymal interactions in tooth germs: mechanisms of differentiation. [Review] [112 refs]. *Journal de Biologie Buccale* 11:173-193

Russell PJ (1998), *Genetics*. San Francisco: Benjamin Cummings

Schalk-van der Weide Y . Oligodontia: a clinical, radiographic and genetic evaluation. 21. 1992. Utrecht: Rijksuniversiteit.
Ref Type: Thesis/Dissertation

Sharpe PT (1995), Homeobox genes and orofacial development. *Connective Tissue Research* 32:17-25

Sharpe PT (2000), Homeobox genes in initiation and shape of teeth during development in mammalian embryos. In: *Development, Function and Evolution of Teeth*, Teaford MF, Smith MM, Ferguson MW, eds. Cambridge: Cambridge University Press, pp 3-12

Silverman NE, Ackerman JL (1979), Oligodontia: a study of its prevalence and variation in 4032 children. *Journal of Dentistry for Children* 46:470-477

Smahi A, Courtois G, Rabia SH, Doffinger R, Bodemer C, Munnich A, Casanova JL, Israel A (2002), The NF-kappaB signalling pathway in human diseases: from incontinentia pigmenti to ectodermal dysplasias and immune-deficiency syndromes. [Review] [37 refs]. *Human Molecular Genetics* 11:2371-2375

Smith S, Ross JW, Scully C (1984), An unusual oral complication of herpes zoster infection. *Oral Surgery Oral Medicine Oral Pathology* 57:388-389

Snow K (1961), Articulation proficiency in relation to certain dental abnormalities. *Journal of Speech & Hearing Disorders* 26:209-212

Söderholm AL, Kaitila I (1985), Expression of X-linked hypohidrotic ectodermal dysplasia in six males and in their mothers. *Clinical Genetics* 28:136-144

Sofaer JA (1981), A dental approach to carrier screening in X linked hypohidrotic ectodermal dysplasia. *Journal of Medical Genetics* 18:459-460

Sperber GH (1993), Early embryonic development. In: *Craniofacial Embryology*, Oxford: Wright, pp 7-30

Srivastava AK, Durmowicz MC, Hartung AJ, Hudson J, Ouzts LV, Donovan DM, Cui CY, Schlessinger D (2001), Ectodysplasin-A1 is sufficient to rescue both hair growth and sweat glands in Tabby mice. *Human Molecular Genetics* 10:2973-2981

Stockton DW, Das P, Goldenberg M, D'Souza RN, Patel PI (2000), Mutation of PAX9 is associated with oligodontia. *Nature Genetics* 24:18-19

Strachan T, Read AP (1999), *Human Molecular Genetics*. Oxford: BIOS Scientific Publishers Ltd

Symons AL, Stritzel F, Stamation J (1993), Anomalies associated with hypodontia of the permanent lateral incisor and second premolar. *Journal of Clinical Pediatric Dentistry* 17:109-111

Tanner BA (1988), Psychological aspects of hypohidrotic ectodermal dysplasia. *Birth Defects: Original Article Series* 24:263-275

Ten Cate R (1998), General embryology. In: *Oral Histology: development, structure and function*, St Louis: Mosby-Year Book, Inc., pp 10-23

Terwilliger JD, Ott J (1994), *Handbook of Human Genetic Linkage*. Baltimore: Johns Hopkins University Press

Thesleff I (2000), Genetic basis of tooth development and dental defects. [Review] [23 refs]. *Acta Odontologica Scandinavica* 58:191-194

Thesleff I (2003), Developmental biology and building a tooth. *Quintessence International* 34:613-620

Thomas BL, Tucker AS, Ferguson C, Qiu M, Rubenstein JL, Sharpe PT (1998), Molecular control of odontogenic patterning: positional dependent initiation and morphogenesis. *European Journal of Oral Sciences* 106:Suppl-7

Thurnam J (1848), Two cases in which the skin, hair and teeth were very imperfectly developed. *Proceedings of the Royal Medical and Chirurgical Society* 31:71-82

Tsang MC, Ling JY, King NM, Chow SK (1986), Oral and craniofacial morphology of a patient with Larsen syndrome. *Journal of Craniofacial Genetics & Developmental Biology 6(4):357-62,*

Tso MS, Crawford PJ, Miller J (1985), Hypodontia, ectodermal dysplasia and sweat pore count. *British Dental Journal* 158:56-60

Tucker AS, Matthews KL, Sharpe PT (1998), Transformation of tooth type induced by inhibition of BMP signaling. *Science* 282:1136-1138

Tucker AS, Sharpe PT (1999), Molecular genetics of tooth morphogenesis and patterning: the right shape in the right place. [Review] [58 refs]. *Journal of Dental Research* 78:826-834

Vainio S, Karavanova I, Jowett A, Thesleff I (1993), Identification of BMP-4 as a signal mediating secondary induction between epithelial and mesenchymal tissues during early tooth development. *Cell* 75:45-58

van den Boogaard MJ, Dorland M, Beemer FA, van Amstel HK (2000), MSX1 mutation is associated with orofacial clefting and tooth agenesis in humans. [erratum appears in Nat Genet 2000 May;25(1):125.]. *Nature Genetics* 24:342-343

Vastardis H, Karimbux N, Guthua SW, Seidman JG, Seidman CE (1996), A human MSX1 homeodomain missense mutation causes selective tooth agenesis. [see comments.]. *Nature Genetics* 13:417-421

Verbov J (1970), Hypohidrotic (or anhidrotic) ectodermal dysplasia-an appraisal of diagnostic methods. *British Journal of Dermatology* 83:341-348

Vierucci S, Baccetti T, Tollaro I (1994), Dental and craniofacial findings in hypohidrotic ectodermal dysplasia during the primary dentition phase. *Journal of Clinical Pediatric Dentistry* 18:291-297

Walton AG, Meechan JG, Welbury RR (1998), Meningococcal septicaemia and disseminated intravascular coagulation affecting the premaxillary permanent tooth germs. *Journal of Dentistry for Children* 65:191-193

Wang H, Zhao S, Zhao W, Feng G, Jiang S, Liu W, Li S, Xue H, He L (2000), Congenital absence of permanent teeth in a six-generation Chinese kindred. *American Journal of Medical Genetics* 90:193-198

Wasserteil V (1986), Fever and hypotrichosis in a newborn. *Archives of Dermatology* 122:1325

Weech AA (1929), Hereditary ectodermal dysplasia (congenital ectodermal defect). A report of two cases. *American Journal of Diseases of Children* 37:766-790

Weiss KM, Stock DW, Zhao Z (1998), Dynamic interactions and the evolutionary genetics of dental patterning. [Review] [203 refs]. *Critical Reviews in Oral Biology & Medicine* 9:369-398

Weissenbach J (1993), Microsatellite polymorphisms and the genetic linkage map of the human genome. [Review] [36 refs]. *Current Opinion in Genetics & Development 3(3):414-7,*

Wilson BJ, Bates GJ, Nicol SM, Gregory DJ, Perkins ND, Fuller-Pace FV (2004), The p68 and p72 DEAD box RNA helicases interact with HDAC1 and repress transcription in a promoter-specific manner. *BMC Molecular Biology* 5:11

Winter GB (1997), Anomalies of tooth formation and eruption. In: *Paediatric Dentistry*, Welbury RR, ed. Oxford: Oxford University Press, pp 256-258

Yan M, Wang LC, Hymowitz SG, Schilbach S, Lee J, Goddard A, de Vos AM, Gao WQ, Dixit VM (2000), Two-amino acid molecular switch in an epithelial morphogen that regulates binding to two distinct receptors. *Science* 290:523-527

Ziegle JS, Su Y, Corcoran KP, Nie L, Mayrand PE, Hoff LB, McBride LJ, Kronick MN, Diehl SR (1992), Application of automated DNA sizing technology for genotyping microsatellite loci. *Genomics 14(4):1026-31,*

Zonana J (1996), Ectodermal dysplasias. In: *Principles and Practice of Medical Genetics*, Rimoin DL, Connor JM, Pyeritz RE, eds. pp 1279-1291

APPENDIX

OLIGODONTIA STUDY: CLINIC DATASHEET

Genetic Consultation

1. History

Family / social history

Pedigree

Previous medical history / Developmental milestones

2. History / Examination Relevant to ED Diagnosis

Hair colour / Appearance:

- Scalp

- Eyebrows / Eyelashes

- Other

Skin - Fragility / Sun sensitivity / Complexion

Finger and toenails - Appearance

- Growth rate

Facial features - Periorbital

- Lips

- Ears / Nose

Secretions - Sweat

- Tears

- Other

Other relevant information (skeletal, neurological etc)

Dental Examination

1. History

Pedigree history relevant to ED/ oligodontia diagnosis

Previous dental history

2. Orofacial and Dental Examination

Extra-oral examination - Frontal view

- Profile view

Intra-oral examination

- Soft tissues:

Palate — Cleft / No cleft
Lips — Cleft / No cleft
Relative dryness of mucosa — Dry / Normal
Plaque levels — Low / Moderate / High
Other

- Saliva:

Hydration / Viscosity (unstimulated, sec) >60 / 30-60 / <30
Stringy/Frothy/Clear
pH (unstimulated) <6.0 / 6.0-6.6 / >6.6
Quantity (stimulated, mL) <3.5 / 3.5-5.0 / >5.0
Buffering capacity (stimulated) 0-5 / 6-9 / 10-12

- Dentition:

Teeth and restorations present

			E	D	C	B	A	A	B	C	D	E			
8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
			E	D	C	B	A	A	B	C	D	E			

Dental anomalies / ectopic eruptions

Anomalies in crown or root size / morphology

Eruption anomalies

Dental occlusion