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A Study of Oral Health and Salivary Function in 22q11 Deletion Syndrome

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

Department of Paediatric Dentistry
University of Sydney
Australia, 2007
ACKNOWLEDGEMENTS

Firstly, I would like to thank my supervisors for all their effort and support during the completion of this thesis. Dr Bradley Curtis, thank you for being so generous with your time and for all your advice, encouragement and assistance throughout the entire project. To Dr Sally Hibbert and A/Prof Richard Widmer, thank you for your dedication and support over the last three years. Your observations in the clinic are the reason this project got started and I hope I’ve done it justice for you.

To A/Prof Angus Cameron, Dr Neeta Prabhu, Dr Sherene Alexander and Dr Simrit Malhi, your help and guidance during the course of this degree is greatly appreciated. To my friends and colleagues Dr Fiona Bell and Dr John Camacho, thank you for being there during both the fun and challenging times. Dr Mary Apps, your help and comments on the manuscript are much appreciated.

A special thank-you must go to the children with 22q11 deletion syndrome and their parents for their willingness to be part of this project. Thank you to Mr David Fitzsimons for introducing me to many of these wonderful families.

This treatise is dedicated to my family – to my brother for his support and ability to make me laugh during the more challenging times in the last three years, and to my parents for their love and encouragement. Thank you for starting me off so well in my education.
ABSTRACT

Introduction: Salivary hypofunction is an important causative factor in the development of dental caries. It has a wide range of implications, including large amounts of dental treatment with both a biological and economic cost. Salivary hypofunction is a rare occurrence in children, therefore when a group of children with 22q11 deletion syndrome were observed to have high decay rates and clinical signs of salivary hypofunction, it was decided that further investigation was warranted. The presence of congenital cardiac anomalies places many of these patients at risk of bacterial endocarditis and therefore prevention of oral disease is of high importance. The aims of this research were to determine the prevalence of dental caries and salivary function in a group of children with 22q11 deletion syndrome, and to examine paediatric salivary flow rate values in the literature in order to establish cut-off values for salivary hypofunction in children.

Methods: Subjects with a confirmed diagnosis of 22q11 deletion syndrome were ascertained from a database held at the Department of Speech Pathology, The Children’s Hospital at Westmead. A dental examination was carried out on all children who agreed to participate. Salivary function testing was undertaken on those patients who were co-operative. This included establishing unstimulated and stimulated flow rates, buffering capacity and pH. Further information on medical and dental history, diet, oral hygiene and fluoride exposure was collected via a questionnaire.
Results: A total of 21 patients (range 5-16 years) participated in the study, with a mean age of 9 years 8 months. The mean dmft/DMFT for the group was 4.8 (SD=3.9) and six children (28.6%) were caries free. Eleven subjects (52.3%) had dmft/DMFT ≥ 6, three subjects (14.3%) had a dmft/DMFT 3-5 and seven subjects 33.3% had a dmft/DMFT score < 3. The subjects had a mean of 1.5 decayed teeth (d+D), 1.0 missing teeth (m+M) and 2.4 filled teeth (f+F) each. Untreated decay (d+D ≥ 1) was present in 52.4% of the study group. Missing teeth due to caries (m+M ≥ 1) was present in 33% of the study group and filled teeth (f+F ≥ 1) in 61.9%.

Eighteen patients were able to provide an unstimulated saliva sample. Unstimulated flow rates ranged from 0.00mL/min to 0.64mL/min, with a mean of 0.21mL/min (SD=0.20). Eighteen patients were tested for unstimulated pH, which ranged from 6.0-7.8, and were all within the normal range. Sixteen patients were able to provide a stimulated saliva sample. The stimulated flow rates ranged from 0.08-0.99mL/min, with a mean of 0.39mL/min (SD=0.31). Stimulated pH was tested in 16 patients. The samples ranged from pH 5.8-7.8, with a mean of pH 7.1. All were within normal range except one subject. Very low buffering capacity occurred in 56% of subjects, low buffering capacity in 31% and normal buffering capacity in 13%.

Conclusion: The mean dmft/DMFT for the group was 4.44-6.26 times the NSW average depending on age. High levels of decay (dmft/DMFT ≥ 6) were present in 53% of the group. Untreated decay was present in 52.4% of the study group, missing teeth due to caries in 33.0%, and filled teeth in 61.9% of the study group. These levels were all higher than NSW averages, indicating both a high level of untreated and
treated decay in the study group. The prevalence of caries in this cohort is similar to the decay scores reported in the most severely affected 30% of the Australian population.

Twelve patients (66.7%) out of those who were able to produce a saliva sample were diagnosed with salivary hypofunction. Three subjects (16.7%) had flow rates that could be considered borderline for a diagnosis of salivary hypofunction.

Unstimulated flow rates of $\leq 0.1$ mL/min as the basis for diagnosing salivary hypofunction can be considered reasonable for subjects over the age of 10 years. The use of adult stimulated flow rate values for the diagnosis of salivary hypofunction in children could be considered inappropriate for patients $\leq 13$ years.
TABLE OF CONTENTS

Acknowledgements ii
Abstract iii
Table of Contents vi
List of Tables xi
List of Figures xii
List of Abbreviations xiii
Declaration xv
Presentations/Abstracts xvi

1. LITERATURE REVIEW 1
   1.1 Introduction 1
   1.2 22q11 Deletion Syndrome 3
       1.2.1 Definition and Aetiology of 22q11 Deletion Syndrome 3
       1.2.2 Epidemiology of 22q11 Deletion Syndrome 5
       1.2.3 Diagnosis of 22q11 Deletion Syndrome 6
       1.2.4 Nosology and History of 22q11 Deletion Syndrome 6
       1.2.5 Features of 22q11 Deletion Syndrome 8
           1.2.5.1 Cardiac defects 10
           1.2.5.2 Immunodeficiency 11
           1.2.5.3 Endocrine disorders 12
           1.2.5.4 Cognitive profile 12
1.2.5.5 Velopharyngeal abnormalities 14
1.2.6 Orofacial Findings in 22q11 Deletion Syndrome 15
  1.2.6.1 Disturbances in enamel formation 15
  1.2.6.2 Abnormalities of tooth number 17
  1.2.6.3 Caries and oral health 18
  1.2.6.4 Salivary dysfunction 18
1.2.7 Oral and Craniofacial Findings from Genetic Models 20
1.3 Saliva 21
  1.3.1 Development of Salivary Glands 21
  1.3.2 Physiology of Saliva 24
  1.3.3 Functions of Saliva 25
    1.3.3.1 Lubrication and mucosal integrity 26
    1.3.3.2 Salivary proteins with antimicrobial functions 27
    1.3.3.3 Pellicle formation 28
    1.3.3.4 Maintenance of mineral content 29
    1.3.3.5 Salivary buffer systems and pH regulation 30
    1.3.3.6 Flow rate and oral clearance 32
  1.3.4 The Role of Saliva in the Caries Process 33
    1.3.4.1 Salivary flow rate in relation to caries 35
    1.3.4.2 Salivary fluoride in relation to caries 36
1.4 Salivary Dysfunction 38
  1.4.1 Salivary Gland Hypofunction 38
1.4.1.1 Medications 40
1.4.1.2 Radiation and surgery to the head and neck 41
1.4.1.3 Sjögren syndrome 42
1.4.1.4 Genetic and developmental disease 42

1.5 **Diagnosis of Salivary Gland Hypofunction** 45
1.5.1 Diagnostic tests 45
1.5.2 Sialometry 46
  1.5.2.1 Collection of unstimulated whole saliva 47
  1.5.2.2 Collection of stimulated whole saliva 49
  1.5.2.3 Measurement of salivary flow rate 50
  1.5.2.4 Measurement of buffering capacity 51
  1.5.2.5 Measurement of pH 52
1.5.3 Normal Salivary Values 52
  1.5.4.1 Normal Flow Rates 53
    1.5.4.1.1 Unstimulated flow rates 53
    1.5.4.1.2 Stimulated flow rates 54
  1.5.4.2 Normal buffering capacity values 56
  1.5.4.3 Normal pH values 56
1.5.4 Salivary Parameters for Hypofunction in Children 57
1.5.5 Salivary Imaging 59

1.6 **Research Outline and Objectives** 61
1.6.1 Research Aims 61
1.6.2 Significance of the Research

2. MATERIALS AND METHODS
   2.1.1 Study Design
   2.1.2 Subject Ascertainment
   2.1.3 Consent
   2.1.4 Medical and Dental History
   2.1.5 Dental Examination
   2.1.6 Salivary Function Assessment
   2.1.7 Questionnaire
   2.1.8 Statistical Analysis

3. RESULTS
   3.1.1 Patient Sample
   3.1.2 Results of Dental Examination
   3.1.3 Results of Saliva Testing
      3.1.3.1 Clinical presentation of saliva
      3.1.3.2 Unstimulated flow rates
      3.1.3.3 Unstimulated pH
      3.1.3.4 Stimulated flow rates
      3.1.3.5 Stimulated pH
      3.1.3.6 Buffering capacity
   3.1.4 Results of Questionnaire
      3.1.4.1 Medical history
      3.1.4.2 Dental history
3.1.4.3 Oral hygiene and preventive history 85
3.1.4.4 Diet history 85
3.1.4.5 Social history 87

4. DISCUSSION 88
4.1.1 Patient Sample 88
4.1.2 Dental Examination 89
4.1.3 Salivary Function Testing 93
  4.1.3.1 Clinical presentation of saliva 94
  4.1.3.2 Unstimulated flow rates 94
  4.1.3.3 Stimulated flow rates 95
  4.1.3.4 Buffering capacity 97
  4.1.3.5 Unstimulated and stimulated pH values 98
  4.1.3.6 Comparison to the literature on 22q11 deletion syndrome 99
4.1.4 Questionnaire 100
4.1.5 Salivary Gland Hypofunction in 22q11 Deletion Syndrome 103

5. CONCLUSION 105
5.1.1 The Prevalence of Dental Caries 105
5.1.2 Salivary Function in 22q11 Deletion Syndrome 105
5.1.3 Parameters for the Diagnosis of Salivary Hypofunction in Children 106
5.1.4 Future Directions 107

REFERENCES 108
APPENDICES 126
LIST OF TABLES

Table 1.1  Structures derived from the pharyngeal arches and pouches
Table 1.2  Findings associated with 22q11 deletion syndrome
Table 1.3  Distribution of congenital cardiac disease
Table 1.4  Distribution of IQ in patients with 22q11 deletion syndrome
Table 1.5  Characteristics of the salivary glands
Table 1.6  Principal functions of saliva
Table 1.7  Common causes of hyposalivation and/or changes in saliva composition
Table 1.8  Oral signs and symptoms related to salivary hypofunction
Table 1.9  Normal pH values of unstimulated and stimulated saliva
Table 3.1  Results of the dental examination
Table 3.2  Mean, minimum and maximum values for decayed, missing and filled teeth
Table 3.3  A comparison of the study group to data from the Child Dental Health Survey NSW, 2000.
Table 3.4  Summary of results of the saliva test
Table 3.5  List of medical conditions present in the study group
Table 3.6  Dental treatment received in the previous 12 months
Table 3.7  Oral hygiene and preventive measures
Table 3.8  Drinks given from a bottle other than breastmilk and formula
Table 3.9  The age at which bottle feeding was stopped
Table 3.10 Consumption of cariogenic foods and drinks
Table 3.11 Education level of parents
LIST OF FIGURES

Figure 1.1  An illustration of factors affecting the development of dental caries
Figure 1.2  Mean unstimulated flow rates in children and adults by age
Figure 1.3  Stimulated flow rates in boys and adult males
Figure 1.4  Stimulated salivary flow rates in girls and adult females
Figure 1.5  Stimulated flow rates in boys and girls as percentile bands
Figure 3.1  Decayed, Missing and Filled Teeth of the study group according to age
Figure 3.2  Unstimulated flow rates of the study group according to age and sex
Figure 3.3  Unstimulated flow rates of the study group (boys) vs. population means
Figure 3.4  Unstimulated flow rates of the study group (girls) vs. population means
Figure 3.5  Stimulated flow rates of the study group according to age and sex
Figure 3.6  Stimulated flow rates of the study group (boys) vs. population flow rates expressed as percentile bands
Figure 3.7  Stimulated flow rates of the study group (girls) vs. population flow rates expressed as percentile bands
Figure 3.8  Buffering capacity according to the GC™ Saliva Check© kits
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>d</td>
<td>Deciduous decayed teeth</td>
</tr>
<tr>
<td>D</td>
<td>Permanent decayed teeth</td>
</tr>
<tr>
<td>m</td>
<td>Deciduous missing teeth</td>
</tr>
<tr>
<td>M</td>
<td>Permanent missing teeth</td>
</tr>
<tr>
<td>f</td>
<td>Deciduous filled teeth</td>
</tr>
<tr>
<td>F</td>
<td>Permanent filled teeth</td>
</tr>
<tr>
<td>Ca₁₀(PO₄)₆(OH)₂</td>
<td>Hydroxyapatite</td>
</tr>
<tr>
<td>CA</td>
<td>Carbonic anhydrase</td>
</tr>
<tr>
<td>CN</td>
<td>Cranial nerve</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>dt</td>
<td>Deciduous decayed teeth</td>
</tr>
<tr>
<td>DT</td>
<td>Permanent decayed teeth</td>
</tr>
<tr>
<td>dft</td>
<td>Deciduous decayed and filled teeth</td>
</tr>
<tr>
<td>DFT</td>
<td>Permanent decayed and filled teeth</td>
</tr>
<tr>
<td>dmfs</td>
<td>Deciduous decayed, missing and filled surfaces</td>
</tr>
<tr>
<td>DMFS</td>
<td>Permanent decayed, missing and filled surfaces</td>
</tr>
<tr>
<td>dmft</td>
<td>Deciduous decayed, missing and filled teeth</td>
</tr>
<tr>
<td>DMFT</td>
<td>Permanent decayed, missing and filled teeth</td>
</tr>
<tr>
<td>FISH</td>
<td>Fluorescence in situ hybridisation</td>
</tr>
<tr>
<td>Fgf8</td>
<td>Fibroblast growth factor 8</td>
</tr>
<tr>
<td>Fgf10</td>
<td>Fibroblast growth factor 10</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>H$_2$CO$_3$</td>
<td>Bicarbonate</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>IgA</td>
<td>Immunoglobulin A</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>IQ</td>
<td>Intelligence quotient</td>
</tr>
<tr>
<td>LADD</td>
<td>Lacrimo-auriculo-dento-digital syndrome</td>
</tr>
<tr>
<td>Mb</td>
<td>Megabase</td>
</tr>
<tr>
<td>MG1</td>
<td>High molecular weight mucin</td>
</tr>
<tr>
<td>MG2</td>
<td>Low molecular weight mucin</td>
</tr>
<tr>
<td>NSW</td>
<td>New South Wales</td>
</tr>
<tr>
<td>Pax9</td>
<td>Paired box gene 9</td>
</tr>
<tr>
<td>pI</td>
<td>Isoelectric point</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SiC</td>
<td>Significant caries index</td>
</tr>
<tr>
<td>SiC$^{10}$</td>
<td>Modified significant caries index</td>
</tr>
<tr>
<td>sIgA</td>
<td>Secretory immunoglobulin A</td>
</tr>
<tr>
<td>SMMCI</td>
<td>Solitary median maxillary central incisor syndrome</td>
</tr>
<tr>
<td>Tbx1</td>
<td>Murine T-box 1</td>
</tr>
<tr>
<td>TBX1</td>
<td>Human T-box 1</td>
</tr>
<tr>
<td>Tc99m</td>
<td>Technetium 99m pertechnetate</td>
</tr>
<tr>
<td>VSD</td>
<td>Ventricular septal defect</td>
</tr>
<tr>
<td>WCOH</td>
<td>Westmead Centre for Oral Health</td>
</tr>
</tbody>
</table>
DECLARATION

This thesis describes the work carried out in The Children’s Hospital at Westmead, and the Faculty of Dentistry, University of Sydney, Australia between March 2005 and August 2007. The research is entirely my own and has not been submitted in whole or in part for a degree at this or any other university. To the best of my knowledge it does not contain any material published or written by another person except where acknowledged in the text.

Rebecca Eggers Date:
2007:

Poster: “Dental caries and salivary function in 22q11 deletion syndrome”, presented at the annual Australian and New Zealand Society of Paediatric Dentistry (ANZSPD) conference, Broome, Australia.

Poster: “Dental caries and salivary function in 22q11 deletion syndrome”, presented at the biannual International Association of Paediatric Dentistry (IAPD) conference, Hong Kong, China.
1. LITERATURE REVIEW

1.1 INTRODUCTION

Most dentists have an awareness of the clinical significance of salivary hypofunction, commonly seen as consequence of head and neck irradiation. Salivary hypofunction can impact on the quality of life of an individual, resulting in discomfort during eating, speaking and swallowing. Importantly, the increased risk of caries can result in increased dental treatment with a significant biological and economic cost.

Salivary hypofunction is a rare occurrence in children, with few reported cases in the literature. Therefore when a number of children with 22q11 deletion syndrome presented to The Children’s Hospital at Westmead with high caries rates in addition to clinical signs of salivary hypofunction, it was decided that further investigation was warranted.

22q11 deletion syndrome (previously known as Velocardiofacial syndrome, Shprintzen’s syndrome and DiGeorge syndrome) is a common genetic defect that presents with a wide range of anomalies. The most common presentation includes cardiac defects, palatal anomalies, typical facies and behavioural problems. At present there is only a small volume of literature pertaining to the orofacial findings in 22q11 deletion syndrome. However, the limited studies available suggest that poor oral health is a common feature and that salivary hypofunction may be a causative factor (Hibbert et al. 2004; Klingberg et al. 2007).
Increased rates of dental caries in such a group of medically compromised children has implications for their general health in regards to the need for general anaesthesia to complete dental treatment. In addition, the presence of congenital cardiac anomalies places many of these patients at risk of bacterial endocarditis. For this reason it is important to review the orofacial findings in patients with 22q11 deletion syndrome so that contributing factors to poor oral health can be identified. In particular, further assessment of the level of dental caries and salivary function in these patients is required, so that appropriate education of medical and dental professionals, carers and patients can be instituted.

The literature review will focus on the medical and orofacial features of 22q11 deletion syndrome, followed by a review of the development of salivary glands, saliva, salivary hypofunction and its relationship to dental caries.
1.2 22q11 DELETION SYNDROME

1.2.1 DEFINITION AND AETIOLOGY OF 22q11 DELETION SYNDROME

22q11 deletion syndrome describes a common genetic defect caused by a microdeletion on chromosome 22 at the q11.2 band (Driscoll et al. 1992).

The syndrome presents with a wide phenotype of structural and functional anomalies involving almost every organ system (Robin et al. 2005). The most common features include conotruncal (outflow tract) heart defects, anatomic and/or neuromuscular abnormalities of the palate, a typical facial appearance, learning difficulties and psychiatric problems (Goldmuntz 2005; Robin et al. 2005). 22q11 deletion syndrome may be inherited in an autosomal dominant manner with variable expressivity, however it has been reported that 85-95% of cases are de novo deletions (Oskarsdottir et al. 2005; Scambler 2000).

In the large majority of cases there is a 3 Mb deletion of chromosome 22 involving about 30 genes (Robin et al. 2005). It is hypothesised that this deletion disrupts either the neural crest cells, or the cells with which they interact, at a critical phase of organogenesis (Scambler 2000). The affected structures are those derived from the branchial arch and pharyngeal pouch system, and include the thymus, parathyroid glands, branchial arch arteries and face (Maynard et al. 2002; Scambler 2000).
Genetic studies, predominantly in murine models, have identified Tbx1 (a T-box transcription factor) as the major gene candidate (Baldini 2005; Ivins et al. 2005; Jerome et al. 2001). Deletion of its human homologue, TBX1, is sufficient to cause most of the abnormalities observed in 22q11 deletion syndrome (Baldini 2004, 2005).

In Tbx1−/− (homozygous) mice, the first pharyngeal arch is abnormally patterned, the second is very hypoplastic and the third, fourth and sixth arches are not identifiable (Baldini 2005). In addition, the pharyngeal pouches are absent, which is postulated to result in disordered migration of neural crest cells into the pharyngeal arches (Baldini 2002). Neural crest cells contribute to many organs and tissues, including facial bones, the peripheral and enteric nervous system, calcitonin-secreting C cells of the parathyroid, and components of the thymus and vasculature (Epstein 2001).

Furthermore, the full spectrum of malformations can be elicited by altering the dose of Tbx1, which may provide a possible explanation for the phenotypic variability of the syndrome (Baldini 2005; Liao et al. 2004). Table 1.1 outlines the main structures derived from the pharyngeal arches and pouches. The derivatives of the pharyngeal system are diverse and include muscle, bone and endocrine organs. Almost no pharyngeal system is spared by the loss of Tbx1, however they exhibit abnormalities of varying severity, from subtle anomalies to lack of formation (Baldini 2005).
<table>
<thead>
<tr>
<th>Structures derived from pharyngeal pouches</th>
<th>Structures derived from pharyngeal arches</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>Tympanic cavity and auditory tube</td>
</tr>
<tr>
<td>2nd</td>
<td>Palatine tonsils</td>
</tr>
<tr>
<td>3rd</td>
<td>Thymus, inferior parathyroids</td>
</tr>
<tr>
<td>4th</td>
<td>Superior parathyroids</td>
</tr>
<tr>
<td>5th</td>
<td>Ultimobranchial body (calcitonin producing parafollicular cells)</td>
</tr>
</tbody>
</table>

1.2.2 EPIDEMIOLOGY OF 22q11 DELETION SYNDROME

22q11 deletion syndrome is the most common microdeletion syndrome and one of the most common multiple anomaly syndromes in humans (Devriendt et al. 1998). Prevalence rates range from 1 in 2000 to 1 in 7700 live births (Oskarsdottir et al. 2004; Robin et al. 2005; Scambler 2000). It is important to note that 22q11 deletion syndrome is now being detected in a number of individuals with a mild phenotype, indicating the syndrome may be more common than previously thought. This is especially the case in patients without cardiac disease, who may remain undiagnosed until adulthood (Devriendt et al. 1998).
1.2.3 DIAGNOSIS OF 22q11 DELETION SYNDROME

The routine diagnostic test is fluorescent in situ hybridisation (FISH) using probes for the critical region of chromosome 22 (Hong 2001). The test is sensitive and can be used as early as the 10th-12th week of gestation (Hong 2001; Oskarsdottir et al. 2005; Scambler 2000).

1.2.4 NOSOLOGY AND HISTORY OF 22q11 DELETION SYNDROME

Prior to advances in genetics, 22q11 deletion syndrome was described by a number of terms depending on its clinical presentation. As a result, it has been described by several diagnostic labels including DiGeorge syndrome, Velocardiofacial syndrome and Conotruncal anomaly face syndrome (Robin et al. 2005). These clinical syndromes were subsequently found to share a common genetic cause, that is, a 22q11 chromosomal deletion (Goldmuntz 2005).

Most likely, the first description of the condition in the literature was in 1955 by Sedláčková, a phoniatrist from Prague. This was followed by a case report of an affected family in 1968 by Robert Strong, a paediatric cardiologist (Robin et al. 2005). Soon after, Angelo DiGeorge, a paediatric endocrinologist, described a group of children with a lethal T-cell immune deficiency, thymus hypoplasia and hypoparathyroidism, which became known as DiGeorge syndrome (DiGeorge 1968). In subsequent cases,
congenital heart disease, facial dysmorphism, cellular immune deficiency, hypocalcaemia and other abnormalities were reported as common features (Hong 2001).

It should be noted that the findings most often associated with DiGeorge sequence (hypoparathyroidism, absent thymus and congenital conotruncal heart disease) are also associated with a number of causes in addition to a 22q11 deletion. These include 10p deletion, 10q13 deletion, 17p deletion, Zellweger syndrome, peroxisomal disorders and non-genetic causes. The presence of the DiGeorge phenotype does not always indicate a 22q11 deletion (Robin et al. 2005), however molecular analysis has since demonstrated that approximately 90% of patients with the clinical features of DiGeorge syndrome have a microdeletion of chromosome 22q11 (Goldmuntz 2005).

Conotruncal anomaly face syndrome was originally described in Japan in 1976 (Robin et al. 2005). Affected children manifested hypernasal speech, conotruncal cardiac anomalies, mild mental retardation, neonatal tetany, aplasia or hypoplasia of the thymus and dysmorphic facial features. Subsequently, 80-90% of patients with Conotruncal anomaly face syndrome have been identified as having chromosome 22q11 deletions (Goldmuntz 2005).

In 1978, Robert Shprintzen recognised a pattern of features in 12 patients, subsequently known as Velocardiofacial syndrome. It was initially delineated on the
basis of characteristic facies, cardiac anomalies, cleft palate and learning disabilities (Shprintzen et al. 1978).

It is important to remember that although the clinical presentation and severity may be variable, they represent points along a continuum of the same genetic disorder (Robin et al. 2005). DiGeorge syndrome is considered the severe end of the spectrum of disorders associated with a 22q11 deletion (Hong 2001). A number of studies included in this literature review refer to 22q11 deletion syndrome by its various historical names. Where the title is not 22q11 deletion syndrome, the syndrome has been referred to as presented in the original reference.

1.2.5 FEATURES OF 22q11 DELETION SYNDROME

22q11 deletion syndrome has an expansive phenotype with over 180 clinical features described in varying combinations and severity (Robin et al. 2005). A comprehensive list of the features of 22q11 deletions syndrome is presented in Table 1.2. Some of the more common features include cardiac defects, immunodeficiency, endocrine disorders, cognitive deficiencies and velopharyngeal abnormalities.
<table>
<thead>
<tr>
<th>Craniofacial findings</th>
<th>Orthopaedic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overt or submucous cleft palate</td>
<td>Scoliosis</td>
</tr>
<tr>
<td>Retrognathia</td>
<td>Rib fusion</td>
</tr>
<tr>
<td>Platysbasia</td>
<td>Osteopenia</td>
</tr>
<tr>
<td>Asymmetric crying facies in infancy</td>
<td>Fused vertebrae</td>
</tr>
<tr>
<td>Structurally asymmetric face</td>
<td>Extra ribs</td>
</tr>
<tr>
<td>Functionally asymmetric face</td>
<td>Butterfly vertebrae</td>
</tr>
<tr>
<td>Vertical maxillary excess</td>
<td>Hemivertebrae</td>
</tr>
<tr>
<td>Straight facial profile</td>
<td>Spina bifida occulta</td>
</tr>
<tr>
<td>Hypotonic facies</td>
<td>Joint dislocations</td>
</tr>
<tr>
<td>Microcephaly</td>
<td></td>
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<tr>
<td>Small posterior cranial fossa</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Ophthalmological</td>
<td></td>
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<tr>
<td>Tortuous retinal vessels</td>
<td></td>
</tr>
<tr>
<td>Strabismus</td>
<td></td>
</tr>
<tr>
<td>Narrow palpebral fissures</td>
<td></td>
</tr>
<tr>
<td>Posterior embryotoxon</td>
<td></td>
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<tr>
<td>Small optic disk</td>
<td></td>
</tr>
<tr>
<td>Cataract</td>
<td></td>
</tr>
<tr>
<td>Mild orbital hypertelorism</td>
<td></td>
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<tr>
<td>Mild vertical orbital dystopia</td>
<td></td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Auditory</td>
<td></td>
</tr>
<tr>
<td>Narrow external ear canals</td>
<td></td>
</tr>
<tr>
<td>Sensorineural hearing loss</td>
<td></td>
</tr>
<tr>
<td>Mild conductive hearing loss</td>
<td></td>
</tr>
<tr>
<td>Frequent otitis media</td>
<td></td>
</tr>
<tr>
<td>Overfolded helix</td>
<td></td>
</tr>
<tr>
<td>Small ears</td>
<td></td>
</tr>
<tr>
<td>Protuberant, cup-shaped ears</td>
<td></td>
</tr>
<tr>
<td>Attached lobules</td>
<td></td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td></td>
</tr>
<tr>
<td>Laryngeal web</td>
<td></td>
</tr>
<tr>
<td>Laryngomalacia</td>
<td></td>
</tr>
<tr>
<td>Pharyngeal hypotonia</td>
<td></td>
</tr>
<tr>
<td>Reactive airway disease</td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td></td>
</tr>
<tr>
<td>Absent or small adenoids</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
</tr>
<tr>
<td>Nasal vomiting</td>
<td></td>
</tr>
<tr>
<td>Gastroesophageal reflux</td>
<td></td>
</tr>
<tr>
<td>Malrotation of bowel</td>
<td></td>
</tr>
<tr>
<td>Chronic constipation</td>
<td></td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Language and speech</td>
<td></td>
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<tr>
<td>Severe hypermasality</td>
<td></td>
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<tr>
<td>Severe articulation impairment</td>
<td></td>
</tr>
<tr>
<td>Language impairment</td>
<td></td>
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<tr>
<td>Velopharyngeal insufficiency</td>
<td></td>
</tr>
<tr>
<td>High-pitched voice</td>
<td></td>
</tr>
<tr>
<td>Hoarseness</td>
<td></td>
</tr>
<tr>
<td>Cognitive</td>
<td></td>
</tr>
<tr>
<td>Learning disabilities</td>
<td></td>
</tr>
<tr>
<td>Concrete thinking</td>
<td></td>
</tr>
<tr>
<td>Borderline normal intellect</td>
<td></td>
</tr>
<tr>
<td>Mild mental retardation</td>
<td></td>
</tr>
<tr>
<td>Moderate or severe mental retardation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychiatric/psychological</td>
<td></td>
</tr>
<tr>
<td>Bipolar affective disorder</td>
<td></td>
</tr>
<tr>
<td>Manic-depressive illness and psychosis</td>
<td></td>
</tr>
<tr>
<td>Rapid cycling of mood disorder</td>
<td></td>
</tr>
<tr>
<td>Mood disorder</td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td></td>
</tr>
<tr>
<td>Hypomania</td>
<td></td>
</tr>
<tr>
<td>Schizoaffective disorder</td>
<td></td>
</tr>
<tr>
<td>Schizophrenia</td>
<td></td>
</tr>
<tr>
<td>Impulsiveness</td>
<td></td>
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<tr>
<td>Flat affect</td>
<td></td>
</tr>
<tr>
<td>Obsessive compulsive disorder</td>
<td></td>
</tr>
<tr>
<td>Generalised anxiety disorder</td>
<td></td>
</tr>
<tr>
<td>Phobias</td>
<td></td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary sequences/associations</td>
<td></td>
</tr>
<tr>
<td>Robin sequence</td>
<td></td>
</tr>
<tr>
<td>DiGeorge sequence</td>
<td></td>
</tr>
<tr>
<td>CHARGE association</td>
<td></td>
</tr>
<tr>
<td>Potter sequence</td>
<td></td>
</tr>
</tbody>
</table>

*Modified from Robin et al. 2005
1.2.5.1 Cardiac defects

Cardiac anomalies are present in 75-85% of patients, however these figures may overestimate the true frequency as they are derived from hospital based studies which exclude those patients without significant cardiac disease (Goldmuntz 2005). Table 1.3 outlines the results from a multicentre study showing the distribution of cardiac anomalies in 558 patients (Ryan et al. 1997).

<table>
<thead>
<tr>
<th>Cardiac anomaly</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal/non-significant</td>
<td>25%</td>
</tr>
<tr>
<td>Tetralogy of Fallot</td>
<td>17%</td>
</tr>
<tr>
<td>Interrupted aortic arch</td>
<td>14%</td>
</tr>
<tr>
<td>Ventricular septal defect (VSD)</td>
<td>14%</td>
</tr>
<tr>
<td>Pulmonary atresia + VSD</td>
<td>10%</td>
</tr>
<tr>
<td>Truncus arteriosus</td>
<td>9%</td>
</tr>
<tr>
<td>Atrial septal defect</td>
<td>1%</td>
</tr>
<tr>
<td>Atrioventricular septal defect</td>
<td>1%</td>
</tr>
<tr>
<td>Other</td>
<td>9%</td>
</tr>
</tbody>
</table>

*Modified from Ryan et al. (1997)

The largest group of cardiac anomalies include the conotruncal defects and perimembranous ventricular septal defects (Botto et al. 2003; Goldmuntz 2005). The conotruncal defects include interrupted aortic arch type B, truncus arteriosus, tetralogy of Fallot and its variants, and transposition of the great arteries. Vascular anomalies are also common, including aortic arch anomalies, aberrant or isolated subclavian arteries and mirror image of the brachiocephalic vessels (Botto et al. 2003; Goldmuntz 2005;
Less common congenital heart defects include hypoplastic left heart syndrome, heterotaxy syndrome, valvar pulmonary stenosis and bicuspid aortic valve (Goldmuntz 2005).

1.2.5.2 Immunodeficiency

Abnormalities of the immune system are present in nearly 80% of patients, however severe immunodeficiency is rare (Goldmuntz 2005). The most common presentation is a mild to moderate decrease in T-cell numbers as a consequence of thymus hypoplasia (Sullivan et al. 1999). Secondary humoral defects are variable, however IgA deficiencies have been identified in about 13% of the population (Smith et al. 1998). Immune disorders commonly manifest as upper and lower respiratory tract infections. Pneumonia, bronchitis and asthma are relatively common, as are chronic upper respiratory tract infections and acute otitis media. These infections occur frequently during the first 5-6 years of life then decrease to normal levels in later childhood (Shprintzen 2000b).

Patients with 22q11 deletion syndrome also have an increased risk of developing autoimmune diseases. It has been postulated that there is an immunoregulatory gene that is affected in the 22q11 region, and this may predispose to development of autoimmune disease (Di Rocco et al. 1998). Reported autoimmune diseases include juvenile rheumatoid arthritis (Sullivan et al. 1997) and Hashimoto’s thyroiditis (Gorlin 2001).
1.2.5.3 Endocrine disorders

Hypoparathyroidism as a result of hypoplasia or aplasia of the parathyroid glands is present in a significant number of patients. Transient hypocalcaemia has been reported in 49-60% of subjects with 22q11 deletion syndrome, mostly in the neonatal period. Other reported endocrine abnormalities include thyroid disease and growth hormone deficiency (Goldmuntz 2005).

Growth problems are common and whilst cardiac disease may play a role, it appears that it is not the sole contributor. Thirty six percent are less than the 3rd centile and eighty three percent are less than the 50th centile for birth weight (Ryan et al. 1997).

1.2.5.4 Cognitive profile

Children with 22q11 deletion syndrome show a delay in mental and language development as well as gross and fine motor skills (Gerdes et al. 1999). The cognitive and behavioural profile of children with 22q11 deletion syndrome is an integral part of the syndrome and is not secondary to associated anomalies such as cardiac defects or surgery (Goldmuntz 2005).

Virtually all patients have some degree of learning difficulty and most have below average IQ scores. The incidence of mental retardation appears to be increased when the deletion is familial rather than de novo (Gerdes et al. 1999; Swillen et al. 1997). Distribution of IQ scores is shown in Table 1.4.
Table 1.4 Distribution of IQ in patients with 22q11 deletion syndrome*

<table>
<thead>
<tr>
<th>IQ</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>16%</td>
</tr>
<tr>
<td>Low average/delayed</td>
<td>11%</td>
</tr>
<tr>
<td>Borderline</td>
<td>30%</td>
</tr>
<tr>
<td>Mild mental retardation</td>
<td>35%</td>
</tr>
<tr>
<td>Moderate mental retardation</td>
<td>5%</td>
</tr>
<tr>
<td>Severe mental retardation</td>
<td>3%</td>
</tr>
</tbody>
</table>

*Modified from Swillen et al. (1997)

Weaknesses of cognitive function include abstract thought and understanding of concepts, visual perception and visual memory. Relative strengths include rote verbal learning and memory, word reading and simple focused attention (Goldmuntz 2005; Shprintzen 2000a).

Motor development is also delayed, with only 2% functioning in the average range. Most show significant delays in gross and fine motor skills such as crawling, walking and other gross motor skills requiring strength and co-ordination. Hypotonia is present in about half of patients, which may influence the acquisition of gross motor milestones and facial expression (Gerdes et al. 1999).

A wide range of behavioural disorders has been reported amongst this group of patients, however they do have a distinctive temperament that includes extremes of behaviour such as disinhibition, impulsiveness and shyness. They can also be
withdrawn and socially isolated, and their affect has been described as bland, with expressionless facial mannerisms. There is an increased frequency of autistic spectrum disorders, attention deficit and hyperactivity disorders, obsessive-compulsive disorder, depression and anxiety (Goldmuntz 2005). An increased incidence of psychotic illness, in particular schizophrenia and bipolar disorder, has been reported in adolescents and adults with 22q11 deletion syndrome. It has been estimated that 25%-30% of children with 22q11 deletions develop schizophrenic disorders as adults (Goldmuntz 2005).

1.2.5.5 Velopharyngeal abnormalities

Anomalies of the palatal architecture are a common finding, with reports ranging from 75-81% (Goldmuntz 2005; Shprintzen 2000b). The most common findings are submucous cleft palate (44%), followed by occult submucous clefts (38%) and overt clefts of the secondary palate (18%) (Shprintzen 2000b).

An occult submucous cleft palate is normal on visual inspection, however on surgical dissection or nasopharyngoscopy, hypoplasia of musculus uvulae is apparent (Shprintzen 2000b). Cleft lip has also been reported in a few cases, and whilst the incidence is higher than in the general population, it is relatively rare in 22q11 deletion syndrome (Shprintzen 2000b). About 80% of patients present with velopharyngeal insufficiency, which is defined by incomplete closure of the soft palate and pharynx during speech (Goldmuntz 2005). Velopharyngeal insufficiency may be caused by a variety of factors including reduced length of the soft palate, pharyngeal hypotonia,
submucous clefts and adenoid hypoplasia (Goldmuntz 2005).

1.2.6 OROFACIAL FINDINGS IN 22q11 DELETION SYNDROME

Literature on the oral manifestations is limited mainly to case reports on patients with DiGeorge syndrome (Borglum Jensen et al. 1983; Fukui et al. 2000). There are, however, two studies of note that have reviewed a larger cohort of children with 22q11 deletion syndrome (Klingberg et al. 2007; Klingberg et al. 2002). The findings of these studies are outlined below.

1.2.6.1 Disturbances in enamel formation

Disturbances of enamel formation can be caused by a number of prenatal, perinatal and postnatal events, and results in clinically observable hypoplastic or hypomineralised enamel defects in the primary and permanent dentitions (Hall 1994). Over 140 separate forms of insult have been implicated, including congenital heart disease (Hall 1994), infections, disorders of calcium metabolism, and neonatal disturbances, such as birth trauma, low and very low birth weight (Seow et al. 1988).

In a clinical study on the oral manifestations in 22q11 deletion syndrome, Klingberg et al. (2002) found hypomineralised enamel in 43% of patients, half of which occurred in the primary dentition. Enamel hypoplasia was found in 32% of patients with primary teeth and 10% of patients with permanent teeth. The pattern of hypoplasia was found
to be symmetrical and chronological in 10 of the 13 children in the primary dentition. When children with hypoplastic defects in the primary dentition were analysed, 5 of 6 children had medical complications including congenital heart defects, general anaesthesia during the first year of life or were pre-term or had a low birth weight. Comparison of the prevalence of enamel defects in Klingberg et al. (2002) to the reported prevalence rates in the population is difficult due to wide variations and a lack of standardisation across studies. However, 33% of children aged 3-6 years have been reported to have some kind of enamel defect, 21% with hypoplastic defects and 12% with opacities (Hall 1994). Klingberg et al. (2002) reports figures in the normal population ranging from 5-15%.

Klingberg et al. analysed 38 exfoliated primary teeth from patients in their initial study under polarised light microscopy, microradiography, scanning electron microscopy, X-ray microanalysis and secondary ion mass spectrometry (Klingberg et al. 2005). Whilst the overall morphology of enamel was normal, a number of aberrations were found including hypoplastic defects located along the neonatal line, and hypomineralised pre- and postnatal enamel.

The patients included in the study had multiple medical complications, including low birth weight, hypocalcaemia and/or hypoparathyroidism, congenital heart malformations, intubation during the first year and frequent infections. The authors hypothesised that the hypomineralised areas in both the pre- and postnatal enamel were due to medical complications that caused long periods of impaired metabolism. In
particular, a high frequency of enamel defects were found in those patients with hypocalcaemia and/or hypoparathyroidism (Klingberg et al. 2005). The findings in this study are in accordance with case presentations on the oral findings in DiGeorge syndrome (Borglum Jensen et al. 1983; Fukui et al. 2000).

1.2.6.2 Abnormalities of tooth number

Klingberg et al. (2002) found hypodontia in 13% of the study population, however this figure may be an underestimation as radiographs were not taken as a standard procedure. The teeth most commonly missing were permanent lower incisors (5 patients), lower second premolars (3 patients), and upper permanent lateral incisors (2 patients). Eight patients also presented with peg-shaped or narrow teeth, most commonly upper lateral incisors. Nine patients showed delayed dental development which was > 2 standard deviations compared to normal data (Klingberg et al. 2002). Case reports in the literature have not reported hypodontia as a feature in their subjects (Borglum Jensen et al. 1983; Fukui et al. 2000). The frequency of missing permanent teeth (other than third molars) in the general population is 2.3-9.6% for children under 15 years of age (Hall 1994). The frequency of patients with missing permanent teeth in Klingberg et al. (2002) study population was 0.1%.

Other oral features of note include several case reports of patients with Velocardiofacial syndrome also presenting with solitary median maxillary central incisor syndrome (SMMC) syndrome (Hall et al. 1997; Oberoi et al. 2005; Yang et al. 2005).
1.2.6.3 Caries and oral health

The children with 22q11 deletion syndrome in the study by Klingberg et al. (2002) had a mean value of 2.8 dft/DFT and an average of 1.8 carious teeth. These findings were not compared to national averages. Fifty-three percent of the children in the group were caries free and when these were removed from the data analysis, the remainder had an average of 9.2 carious or filled teeth each. A high number of decayed teeth (dt/DT 5-18) were found in 12 out of 47 children, half of whom also had severe gingivitis (Klingberg et al. 2002). Carious lesions were found in unusual locations such as on cusps and incisal edges.

The reasons stated by the authors for impaired oral health include frequent infections leading to cariogenic dietary habits, impaired oral hygiene when the child is ill, and the infection itself affecting salivary function (Klingberg et al. 2002), however no references are given for these statements.

1.2.6.4 Salivary dysfunction

In their initial study on the oral manifestations in 22q11 deletion syndrome, Klingberg et al. (2002) hypothesised that salivary function may be impaired, however at the time there was no evidence to confirm this.

Hibbert et al. (2004) investigated salivary function in Velocardiofacial syndrome in a pilot study and found stimulated salivary flow rate was low to very low in 83% of
subjects. Buffering capacity was low to very low in 58% of subjects and unstimulated salivary pH was found to be moderately or highly acidic in 77% of subjects.

Klingberg et al. (2007) investigated their hypothesis from their previous study with a study on caries-related salivary factors (Klingberg et al. 2007). Their sample consisted of 21 patients with 22q11 deletion syndrome with an age range of 6-36 years. Control patients were matched for age (±2 months), gender, and caries (dmfs/DMFS ±5).

Stimulated whole saliva was collected over 5 minutes and then tested for buffering capacity, mutans streptococci and lactobacillus counts, total salivary protein, salivary IgA, and electrolytes. Bloods were taken for all patients and analysed for serum IgA, calcium, phosphate and parathyroid hormone.

The mean dmfs/DMFS of the study group was 8.1. When compared to the control group, the average stimulated whole saliva secretion rate was significantly reduced in the study group, as was buffering capacity and output of electrolytes. The levels of mutans streptococci, salivary protein and IgA concentration were all significantly higher in the study group. There was also a positive correlation between the concentration of IgA in serum and saliva.
1.2.7 ORAL AND CRANIOFACIAL FINDINGS FROM GENETIC MODELS

Despite the lack of research on salivary abnormalities in humans with 22q11 deletion syndrome, there are a number of animal studies in the genetics field that have shown that salivary glands may indeed be affected.

$Tbx1^{-/-}$ mice have been reported to have hypoplastic or missing sublingual and submandibular salivary glands, missing or malformed upper incisors, mandibular hypoplasia, absence of the coronoid process and overt cleft palate (Jerome et al. 2001).

In $Tbx1$ heterozygous and homozygous mice, a number of down-regulated genes have been identified, including $Fgf8$ and $Fgf10$ which are involved in submandibular gland development (Vitelli et al. 2002) and $Pax9$ which is involved in tooth development (Jaskoll et al. 2002). It would therefore be reasonable to postulate that loss of $TBX1$ in humans may cause a disturbance in the development of the oral structures.
1.3 SALIVA

Saliva plays a vital role in protecting the oral soft and hard tissues by maintaining homeostasis in the oral cavity. It contains a number of organic and inorganic components which act together to buffer acids, protect against microbial attack, and clear food debris, epithelial cells and microbes.

Saliva protects the oral soft tissues by forming a physical barrier, lubricates the oral mucosa and facilitates swallowing, speech and mastication. It also has a role in relation to taste as well as digestion in the upper gastrointestinal tract (Pedersen et al. 2002). The combined action of all factors in whole saliva provide multi-factorial protection for the oral cavity that is only compromised when salivary flow rate is substantially reduced (Tenovuo 1997). The link between saliva and caries is well known (Atkinson et al. 2005), and as a result many studies have attempted to link individual aspects of salivary output and composition to caries incidence (Dodds et al. 2005; Leone et al. 2001). With the exception of salivary flow rate, the lack of evidence for any specific component of saliva being linked to caries incidence reflects the fact that saliva is greater than the sum of its parts (Dowd 1999).

1.3.1 DEVELOPMENT OF SALIVARY GLANDS

Salivary gland development occurs by the processes of differentiation (development of specific cellular phenotypes), proliferation (cell growth and division), and
morphogenesis (development of shape and form). These processes depend on a number of intrinsic and extrinsic factors, including the programmed pattern of cell-specific gene expression, cellular interactions and growth factors (Avery 1994).

The sequence of events in the development of salivary glands involves epithelial-mesenchymal interactions, where the presence of mesenchyme in close proximity to the epithelium is required for normal epithelial development. This is known as secondary induction, and it results in both the initiation and growth of the glandular tissue, and the cytodifferentiation of cells within the salivary glands.

All salivary glands follow a similar pattern of development. The functional glandular tissue (parenchyma) develops as an epithelial outgrowth (or bud) of the buccal epithelium that invades the underlying mesenchyme. The mesenchyme is composed of cells derived from both the mesoderm and neural crest cells, and is often called ectomesenchyme. The ectomesenchyme is essential for the normal differentiation of the salivary glands. In addition, the extracellular matrix components secreted by the mesenchymal connective tissue cells provide important signals that direct morphogenesis and differentiation of the glandular bud (Ten Cate 1998).

The origin of the buds is believed to be ectoderm in the parotid and minor salivary glands and endodermal in the submandibular and sublingual glands. The breakdown of the buccopharyngeal membrane during the 4th week of development, however, permits the intermingling of stomodeal ectoderm and cranial foregut endoderm, which complicates the identification of specific germ layer origin of the salivary glands. The
parotid glands develop by the 6th week in utero, and the submandibular at the end of
the 6th week. The sublingual glands form at around the 8th week. The minor salivary
glands do not begin to develop until the 12th week in utero (Avery 1994).

Salivary gland development can be divided into six stages: (1) induction of the oral
epithelium by the underlying mesenchyme, (2) formation and growth of the epithelial
cord, (3) initiation of branching in terminal parts of the epithelial cord and continuation
of glandular differentiation, (4) repetitive branching of epithelial cord and lobule
formation, (5) canalisation of the presumptive ducts, and (6) cytodifferentiation of the
functional acini and intercalated ducts (Avery 1994).

Branching is the primary morphogenic process during salivary gland development. It
begins with cleft formation in the bud, followed by co-ordinated cell proliferation. An
intact basal lamina and the presence of mesenchyme are required for normal
branching, however growth and branching remain independent events.

The final structure of the salivary glands consists of branching ducts leading to acini,
which contain about 5-7 secretory acinar cells. There are three types of secretory end-
pieces: serous, mucous and mixed. Surrounding the duct and secretory system is the
connective tissue capsule that extends into the glands as septa dividing the parenchyma
into lobes and lobules. It is essential as a framework for support of the glands and
contains nerves, blood vessels and lymphatics.
1.3.2 PHYSIOLOGY OF SALIVA

The mixed fluid in the mouth is called whole saliva, and it is derived from the three pairs of major salivary glands (parotid, submandibular and sublingual) and the minor salivary glands which are located in the oral mucosa. The major glands form about 90% of the total fluid secretions, and the minor glands account for the remaining 10%. Saliva also contains gingival crevicular fluid, microorganisms from dental plaque and food debris (Edgar 1990).

The parotid glands produce about 50% of the total volume of saliva in the mouth when stimulated, however in the resting state the submandibular glands produce about two-thirds of the volume. The sublingual glands produce only a small fraction of the volume of whole saliva but produce a large fraction of salivary proteins.

Salivary glands differ according to the nature of their secretions, which can be purely serous, mucous, or mixed types. Table 1.5 outlines the characteristics of the salivary glands in relation to their acinar cell type and fluid characteristics.
Table 1.5. Characteristics of the salivary glands*

<table>
<thead>
<tr>
<th>Salivary gland</th>
<th>Acinar cell type</th>
<th>Fluid characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Major salivary glands</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parotid gland</td>
<td>Serous</td>
<td>watery, amylase-rich</td>
</tr>
<tr>
<td>Submandibular gland</td>
<td>Mixed, mainly mucous</td>
<td>viscous, mucin-rich</td>
</tr>
<tr>
<td>Sublingual gland</td>
<td>Mixed, mainly mucous</td>
<td>viscous, mucin-rich</td>
</tr>
<tr>
<td><strong>Minor salivary glands</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palatine</td>
<td>Mucous</td>
<td>mucin-rich</td>
</tr>
<tr>
<td>Buccal</td>
<td>Seromucous</td>
<td>mucin-rich</td>
</tr>
<tr>
<td>Labial</td>
<td>Seromucous</td>
<td>mucin-rich</td>
</tr>
<tr>
<td>Lingual</td>
<td>Serous</td>
<td>watery, lipase-rich</td>
</tr>
<tr>
<td>Retromolar</td>
<td>Mainly mucous</td>
<td>mucin-rich</td>
</tr>
</tbody>
</table>

* Modified from Bardow *et al.* (2004)

Primary saliva is formed by the acinar cells (secretory end-pieces), and moves into the duct system where it is modified continuously until it is secreted into the mouth. The end product varies according gland type, but it is hypotonic compared to serum and generally more than 99% water and less than 1% macromolecules such as proteins and salts (Humphrey *et al.* 2001).

1.3.3 FUNCTIONS OF SALIVA

The important functions of saliva are related to both its fluid characteristics and specific components. Table 1.6 outlines the major functions of saliva in relation to these properties. A number of the most important functions of saliva include lubrication and maintenance of mucosal integrity, antimicrobial functions, maintenance
of mineral content, buffering and oral clearance.

1.3.3.1 Lubrication and mucosal integrity

In addition to water, a number of organic components in saliva are involved in lubricating the oral cavity for mastication, swallowing and speech, as well as hydration and protection of the mucosa against toxins. This function is mainly carried out by glycoproteins called mucins that occur in both high- (MG1) and low- (MG2) molecular weight forms.

Mucins are hydrophilic and maintain hydration of the oral mucosa by binding water. They also form disulfide bridges with other mucin molecules to create a network that protects the oral mucosa from mechanical, thermal and chemical irritation (Humphrey et al. 2001). MG1 contributes to the enamel pellicle and coating of the oral mucosa whereas MG2 does not bind as strongly to enamel, and is more important for aggregation and clearance of bacteria. The hydrophilic and network-forming abilities of mucins give saliva its distinct viscoelastic and lubricating properties (Gerken 1993). Reduced water content of saliva results in a relative increase of mucins, making the saliva more viscous, which presents clinically as ropy or frothy saliva (Walsh 2000).
Table 1.6. Principal functions of saliva*

<table>
<thead>
<tr>
<th>Functions</th>
<th>Salivary characteristics and components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical cleansing and oral clearance</td>
<td>Water</td>
</tr>
<tr>
<td>Lubrication</td>
<td>Water, mucins, proline-rich glycoproteins</td>
</tr>
<tr>
<td>Mucosal integrity and coating</td>
<td>Water, mucins, electrolyte, epidermal growth factor, nerve growth factor</td>
</tr>
<tr>
<td>Maintenance of tooth mineral</td>
<td>Cystatins, histatins, proline-rich proteins, statherins, phosphate, Ca^{2+}</td>
</tr>
<tr>
<td>Buffering</td>
<td>Bicarbonate, phosphate, proteins</td>
</tr>
<tr>
<td>Antimicrobial</td>
<td></td>
</tr>
<tr>
<td><em>Anti-bacterial:</em></td>
<td>Amylases, cystatins, histatins, mucins, lactoperoxidase, lysozyme, lactoferrin, calprotectin, immunoglobulins, chromogranin A</td>
</tr>
<tr>
<td><em>Anti-fungal:</em></td>
<td>Histatins, immunoglobulins, chromogranin A</td>
</tr>
<tr>
<td><em>Anti-viral:</em></td>
<td>Cystatins, mucins, immunoglobulins, secretory leukocyte proteinase inhibitor</td>
</tr>
<tr>
<td>Speech, mastication and swallowing</td>
<td>Water, mucins</td>
</tr>
<tr>
<td>Initial digestion</td>
<td>Water, mucins, amylases, lipases, ribonucleases, proteases</td>
</tr>
<tr>
<td>Taste</td>
<td>Water, gustin, Zn^{2+}</td>
</tr>
</tbody>
</table>

* Modified from Bardow et al. (2004)

1.3.3.2 Salivary proteins with antimicrobial functions

Salivary proteins make up the majority of the organic components in saliva and are secreted into both the saliva and the blood circulation by the acinar and ductal cells. Their concentration depends on both the flow rate and duration of stimulation, however it is less dependent on flow rate than the inorganic components. Individual genetic differences influence the composition and concentration of proteins in saliva, many of which have multifunctional roles (Rudney et al. 1994).

The immunoglobulins IgG, IgM, IgA and secretory IgA (sIgA) are the specific defense mechanisms against the oral microflora. The most abundant immunoglobulin in saliva
is sIgA, which is produced by the salivary glands and plasma cells (Lenander-Lumikari et al. 2000). It is generally accepted that sIgA protects the mucosa through the binding and complexing of antigens. Quantification of immunoglobulin levels in saliva is difficult, and therefore it is uncertain whether they are protective against caries. It has been suggested that salivary IgA may have a role in enzyme inhibition and retarding bacterial colonisation of hard surfaces (Tenovuo 1989b).

Non-specific defense components include lysozyme, histatins, peroxidases and lactoferrin, all of which are affected by salivary flow rate. Lysozyme is secreted from major and minor salivary glands, phagocytic cells and gingival crevicular fluid. It is able to hydrolyse bonds of the bacterial cell wall, resulting in cell lysis and is particularly effective against oral streptococci. Salivary peroxidase is adsorbed onto enamel and bacteria and works to block the metabolic processes of both gram positive and negative species. Lactoferrin is a glycoprotein that binds iron and reduces the availability of this important co-factor for bacterial enzymes, resulting in a bacteriostatic effect. Histatins are small antimicrobial peptides that are particularly lethal to oral fungi such as *Candida albicans* (Amerongen et al. 2002).

### 1.3.3.3 Pellicle formation

Salivary proteins adsorb onto the enamel surface to form a thin organic film called the enamel pellicle, which protects the enamel from dissolution and mediates bacterial adhesion. The formation of the pellicle may decrease the rate of enamel dissolution and
is one of the major ways in which saliva protects the teeth (Lendenmann et al. 2000).

The proteins that form the enamel pellicle also act in liquid phase, and play a role in agglutinating oral bacteria. These aggregates are then flushed away by saliva and swallowed. The ability to either promote adhesion of bacteria to the pellicle, or promote aggregation varies greatly among individuals. It has been suggested that high aggregation and low adhesion activity against S.mutans may explain variations in colonisation susceptibility in individuals (Lenander-Lumikari et al. 2000).

1.3.3.4 Maintenance of mineral content

Saliva plays an important role in maintaining the mineral content of teeth, and controls the equilibrium between mineral gain (remineralisation) and loss (demineralisation). This is a dynamic cycle that is dependent on the pH of the microenvironment and therefore may be modified by a number of intrinsic and extrinsic factors including biofilm quantity and quality, salivary flow rate, buffering capacity and salivary fluoride content.

Under normal physiological conditions, dental hard tissues do not dissolve because saliva is supersaturated with respect to hydroxyapatite Ca_{10}(PO_4)_6(OH)_2, the main mineral component of teeth. Calcium and phosphate are maintained at high concentrations in saliva by calcium-binding proteins including statherin, proline-rich proteins and cystatins (Dodds et al. 2005; Lenander-Lumikari et al. 2000). These
proteins act to prevent the precipitation of calcium-phosphate salts and allow available minerals to enter tooth structure during the remineralisation phase.

The main threat to the mineral content of teeth comes in the form of acids that are produced in the oral cavity through the metabolism of carbohydrates by oral flora, and added to the oral cavity through ingestion of acidic foods and drinks. One of the most important roles of saliva is to maintain a non-harmful pH in the mouth, especially during acid challenges that occur during eating and drinking.

Salivary pH varies between 6.0 and 7.5 in healthy individuals and is highly dependent on the secretion rate (Dowd 1999). A drop in pH to below the critical pH allows demineralisation of dental hard tissues, leading to dental caries or erosion depending on the origin of the acid challenge. The critical pH is on average pH 5.5 (range pH 5.3-5.5) under normal physiological conditions, but its value is not fixed and depends on a number of ion activities that change dynamically as the saliva flow rate varies (Dawes 2003).

The ability of saliva to maintain a non-harmful pH is dependant on its buffering capacity, which is the ability to resist changes in pH when either acids or alkalis are added.

1.3.3.5 Salivary buffer systems and pH regulation

There are three main buffer systems in human saliva; the bicarbonate, phosphate and
protein buffer systems (Humphrey et al. 2001).

a) The bicarbonate buffer system

Bicarbonate is the main buffer in human saliva, and its concentration varies dramatically from about 5 mmol/L in unstimulated flow up to 24 mmol/L in stimulated whole saliva (Bardow et al. 2001). Bicarbonate levels are strongly dependent on salivary flow rates, so that increased flow rates cause a marked rise in bicarbonate levels and therefore increased buffering capacity and salivary pH (Dowd 1999).

The equilibrium of the bicarbonate buffer system in a partly open compartment like the mouth is:

\[
\text{CA} \\
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+
\]

Where CO₂ is the CO₂ in saliva and CA is carbonic anhydrase, which catalyses the hydration of CO₂ to carbonic acid (Tenovuo 1989a).

b) The phosphate buffer system

The total phosphate concentration in saliva is also dependent on the flow rate, however in contrast to the bicarbonate system, phosphate concentration decreases with increasing flow rates. In saliva, phosphate is most effective as a buffer at pH 6.8, and contributes to about 50% of the total buffer capacity in unstimulated flow (Bardow et al. 2004). Due to the flow-dependent decrease upon stimulation, and the
large increase in the bicarbonate concentration, phosphate makes only a small contribution to the stimulated buffer capacity (Humphrey et al. 2001).

c) The protein buffer system

Many of the proteins present in saliva may act as buffers when the pH is above or below their isoelectric point (pI). Below their isoelectric point, proteins can accept protons whereas above their isoelectric point, protons are released. The pI of most salivary proteins is around pH 7.0, and they contribute substantially at pH values less that pH 5. Some salivary proteins also increase the viscosity of saliva at acidic pH values, acting to physically protect the dental tissues against an acid attack (Nordbo et al. 1984).

1.3.3.6 Flow rate and oral clearance

The dilution and removal of acids and sugars in the mouth is crucial in protecting the oral tissues (Lenander-Lumikari et al. 2000; Walsh 2000). Since the salivary flow rate is intrinsically linked to buffering capacity and release of organic compounds, it can be considered its most important caries protective property (Tenovuo 1997).

The term ‘oral clearance’ refers to the time between the introduction of a substance into the oral cavity, and the moment that substance can no longer be detected. The time taken to clear substrates varies widely between individuals and is dependent on a number of factors besides flow rate. These include metabolism by micro-organisms,
adsorption onto oral structures and degradation by salivary enzymes (Luke et al. 1999). It has been reported that patients with oro-motor dysfunction (Gabre et al. 2005), and children with cleft lip and palate have longer oral clearance times (Ahluwalia et al. 2004).

Salivary flow is stimulated in response to taste and results in a maximum volume of about 1.1mL of saliva. A swallow (~0.3mL) is initiated, and results in the substance being contained in about 0.8mL of residual saliva. The remainder of the substance is then progressively diluted by the saliva entering the mouth until the maximum volume is reached and another swallow occurs (Lagerlof et al. 1994). When the substance has reached a sufficiently low level, it will no longer stimulate saliva secretion and the salivary flow returns to its unstimulated rate. Depending on the type of sugars ingested and the food consistency, levels of substrate can still be detected from 15 minutes to well over 1 hour after ingestion (Luke et al. 1999). Therefore, the unstimulated flow of saliva has the greatest overall impact on the oral clearance time, because it is present for the longest period during the clearance time (Dodds et al. 2005).

1.3.4 THE ROLE OF SALIVA IN THE CARIES PROCESS

Caries is a multifactorial disease that is dependent on a number of factors for its development (Fejerskov 2004). Figure 1.1 illustrates the complex interplay between saliva, dietary habits, and the many biological and external factors that influence the
development of caries. The inner circle represents biological determinants of biofilm composition and metabolism. Outside the circle lists various behavioural, genetic and social factors that may influence the likelihood for caries development.

Figure 1.1 An illustration of factors affecting the development of dental caries.*

* Modified from Fejerskov (2004)

The caries attack is a series of events that starts with the ingestion of fermentable carbohydrates which then dissolve in saliva. The sucrose diffuses quickly down its concentration gradient into plaque fluid. In the plaque, micro-organisms (notably the mutans streptococci and lactobacilli) produce acids such as lactic, formic and acetic acids. These acids diffuse into enamel via water-filled pores in the biofilm and dissociate, decreasing the pH of the fluid surrounding the enamel crystals. The enamel liquid becomes unsaturated with respect to the tooth mineral, resulting in calcium and
phosphate ions diffusing out of the tooth into plaque fluid and saliva. The strength and frequency of these caries attacks determines whether net demineralisation and caries progression will occur (Lagerlof et al. 1994).

The importance of saliva in protecting against caries was demonstrated in early studies on de-salivated rats, which showed that a complete lack of saliva resulted in carious lesions on smooth and root surfaces that developed within days (Bowen et al. 1988). A similar effect can be seen in patients with severe salivary hypofunction, however, in patients where the clinical picture is not so obvious it is not clear how much saliva is enough to ensure a caries protective effect.

There are a large number of salivary factors that are considered to be preventive against the caries attack, however salivary flow rate and salivary content of fluoride are considered to be the two most important (Lagerlof et al. 1994; Tenovuo 1997).

1.3.4.1 Salivary flow rate in relation to caries

A reduction of salivary flow rate leads to a corresponding reduction of buffering capacity and components of saliva. As a result, there is slow elimination of microbes, sugars and acids as well as increased plaque accumulation.

The degree of saturation of calcium and phosphate salts with respect to tooth mineral is also reduced with low salivary flow. A reduction of salivary flow and these
protective factors increases the likelihood of demineralisation, and therefore increases
caries susceptibility and tooth surface loss (Cunning et al. 1998; Dowd 1999; Walsh
2000). The resultant low pH that occurs with a reduction in flow rate favours an
aciduric and acidophilic oral microflora including *Streptococcus mutans, Lactobacillus*
and yeasts. High salivary *Lactobacillus* counts are strongly associated with low
secretion rates and the number of lactobacilli in saliva has been positively correlated
with caries activity (Bardow et al. 2001).

The evidence for a link between salivary flow rates and an increased risk of caries is
conflicting, however it is generally accepted that there is no *linear* relationship
between salivary flow rates, caries activity or caries scores (DMFT/DMFS) on a
population level (Lenander-Lumikari et al. 2000; Tenovuo 1997). However, on an
individual level there is clear evidence that patients with severely reduced flow rates
(e.g. head and neck oncology patients) show a dramatic increase in the incidence of
dental caries (Dodds et al. 2005; Papas et al. 1993).

1.3.4.2 Salivary fluoride in relation to caries

Whilst there is no doubt that fluoride is one of the major components of saliva involved
in controlling caries activity, the baseline fluoride concentration in saliva is generally
very low and essentially independent of flow rate (Dawes et al. 1990; Lagerlof et al.
1994). The salivary fluoride concentration is mostly determined by the degree of
fluoride exposure to the mouth by external sources, e.g. drinking water, food, beverages
and fluoride-containing prophylactic agents (Lagerlof et al. 1994).
The ability of fluoride to protect against caries was previously thought to be pre-eruptive incorporation of fluoride into enamel. More recent studies, however, suggest that the maintenance of a certain fluoride level in the oral fluids is the most important factor in prevention and inhibition of caries (Lagerlof et al. 1994).

Fluoride introduced into the oral cavity will diffuse according to its concentration gradient, and therefore be distributed in saliva, plaque fluid and enamel crystal fluid (Lagerlof et al. 1994). Fluoride in the fluid surrounding the enamel crystals has been shown to reduce the demineralisation rate of enamel and reduce bacterial acid production and acidurance (Lagerlof et al. 1994; Shellis et al. 1994).

During remineralisation, the chemical composition of the reformed mineral depends on which ions are available in the surrounding fluid. If fluoride is incorporated, the resulting crystals (fluorapatite) are more resistant to acid dissolution and the critical pH is slightly lowered. Fluoride also stimulates and enhances remineralisation by driving precipitation of mineral back into the tooth surface (Lagerlof et al. 1994; Shellis et al. 1994).
1.4 SALIVARY DYSFUNCTION

Salivary gland dysfunction is defined as any qualitative and/or quantitative change in the output of saliva. Therefore salivary gland dysfunction includes either an increase (hyperfunction) or decrease (hypofunction) in salivary output. Salivary glands and their secretions can be affected by several mechanisms, including central or peripheral neural regulation, salivary gland receptor systems, stimulus secretion coupling, membrane transporters, protein synthesis or protein release mechanisms (Bardow et al. 2004).

The term xerostomia is often used interchangeably with salivary hypofunction, however xerostomia refers to the subjective feeling of dry mouth. The term salivary hypofunction refers to an objective reduction of salivary flow. Xerostomia generally occurs when the unstimulated flow is reduced to approximately 50% of its normal value in any given individual. Xerostomia does not always correlate to objective measures of saliva production and vice versa (Rossie 1993).

1.4.1 SALIVARY GLAND HYPOFUNCTION

Salivary gland hypofunction may occur as a result of many localised or systemic disease processes and disorders of development. It is a common problem in adults, however salivary hypofunction in children is rare (Bardow et al. 2004). A reduction in the quantity or quality of saliva can be responsible for difficulties in eating and
speaking, taste alterations, increased plaque, erosion, halitosis, mucosal irritation, candidal infections and caries (Walsh 2000). Salivary hypofunction does not always cause symptoms, and dental caries may be the only sign of an underlying salivary gland disorder (Hodgson et al. 2001). The amount and pattern of caries is particularly important, as caries caused by salivary hypofunction often occurs in unusual locations such as lower incisors, cusp tips, palatal surfaces of incisors and root surfaces (Gelbier et al. 1995; Li et al. 1993).

Salivary hypofunction can be caused by a number of localised salivary gland diseases as well as systemic diseases and iatrogenic causes. Causes of salivary hypofunction (including both hyposalivation and changes in composition) are listed in Table 1.7.

Whilst it is apparent that a number of diseases affect the salivary glands both directly and indirectly, there is conflicting evidence for a number of these in regards to a significant, objective reduction in salivary flow rate. The diagnosis of salivary hypofunction in a number of systemic conditions is often confounded by either the medications used to treat the disease, or associated lifestyle factors. The conditions for which it is generally accepted that there is a measurable reduction in flow rate include radiation or surgery to the head and neck (Cunning et al. 1998), Sjögren syndrome (Rossie 1993), graft vs. host disease (Nagler et al. 2004), Hepatitis C (Carrozzo 2001), HIV (Fox 1991; Lin et al. 2003) and certain medications.
Table 1.7 Common causes of hyposalivation and/or changes in saliva composition

<table>
<thead>
<tr>
<th>Category</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iatrogenic</td>
<td>Medications, chemotherapy, radiotherapy to the head and neck, surgery</td>
</tr>
<tr>
<td>Immunologic diseases</td>
<td>Sjögren syndrome, graft vs. host disease, sarcoidosis, oral lichen planus, amyloidosis</td>
</tr>
<tr>
<td>Neurological</td>
<td>Cerebral Palsy, Bell’s palsy, Melkersson-Rosenthal syndrome, endogenous depression, Holmes-Adie syndrome</td>
</tr>
<tr>
<td>Endocrine disease</td>
<td>Diabetes mellitus,</td>
</tr>
<tr>
<td>Infections</td>
<td>Hepatitis C, HIV</td>
</tr>
<tr>
<td>Genetic and developmental disease</td>
<td>Cystic fibrosis, agenesis of salivary glands</td>
</tr>
<tr>
<td>Metabolic disorders</td>
<td>Dehydration, malnutrition, bulimia, anorexia nervosa, hyperlipoproteinaemia type V</td>
</tr>
<tr>
<td>Local salivary diseases</td>
<td>Sialolithiasis, sialadenitis, carcinoma</td>
</tr>
<tr>
<td>Other</td>
<td>Sialodenoasis</td>
</tr>
</tbody>
</table>

Table 1.7 sources: (Bardow et al. 2004; Bergdahl et al. 2000; Carrozzo 2001; Cunning et al. 1998; Lin et al. 2003; Nagler et al. 2004; Rossie 1993)

1.4.1.1 Medications

The control of salivation is complex and is regulated by both the parasympathetic and sympathetic nervous system. Decreased salivary flow is likely to be caused by drugs that reduce stimulation of glandular receptors or affect the level of hydration of the oral tissues (Dowd 1999). These include neuroleptics, antihistamines, antidepressants, antihypertensives and diuretics (Dowd 1999; Tenovuo 1989a). There are relatively few controlled studies conducted on the effect of various drugs on salivary flow rate, and only a few groups of drugs have been found to decrease saliva to a degree that it can be detected by a salivary flow rate test (Tenovuo 1989a).

Salivary hypofunction can also be caused by taking a number of medications on a daily
basis (polypharmacy), regardless of whether the individual medications cause hypofunction. The use of four or more medications causes lower unstimulated and stimulated flow rates compared to non-medicated individuals (Narhi et al. 1992). Polypharmacy is a common problem in the older population, however medically compromised younger people may also fit into this category.

1.4.1.2 Radiation and surgery to the head and neck

Radiation therapy to the head and neck induces severe hyposalivation with alteration of pH, buffering capacity, electrolytes and protein content, (Dowd 1999) and rapid progression of caries. Total body irradiation prior to bone marrow transplant has been shown to significantly decrease salivary function in children (Bagesund et al. 2007; Dahllöf et al. 1997).

Patients who undergo radical neck dissection with removal of salivary glands also suffer a significant reduction in flow rates (Cunning et al. 1998; Jacob et al. 1996). Surgery to control drooling in patients with oro-motor function disorders may involve retro-positioning of the submandibular ducts, excision of submandibular glands and/or parotid duct ligation (Stern et al. 2002). This results in changes to both the flow rate and the properties of saliva often causing a subsequent increase in caries (Hallett et al. 1995).

The pattern of decay in patients who have undergone surgery to salivary glands
presents in a similar fashion to cases of congenital absence of salivary glands (Gelbier et al. 1995). In one group of cerebral palsy patients who underwent repositioning of submandibular glands, the caries prevalence in the lower anterior region as a proportion of the total DFMS was more than 100 times higher than in the general population (Arnrup et al. 1990).

1.4.1.3 Sjögren Syndrome

Sjögren syndrome is an autoimmune disorder characterised by chronic lymphocytic infiltration of the salivary and lacrimal glands. Primary Sjögren syndrome affects salivary and lacrimal glands whereas secondary Sjögren syndrome describes the additional involvement of connective tissues such as rheumatoid arthritis, systemic lupus erythematosus, scleroderma or primary biliary cirrhosis. It primarily affects middle-aged women, however it may also present in paediatric patients. It is relatively rare, with only 200 paediatric cases of primary Sjögren syndrome reported (McGuirt et al. 2002). Symptoms may include parotid swelling, xerostomia, xerophthalmia and oral ulceration. Diagnosis is on the basis of parotid or lip biopsy and laboratory investigations (McGuirt et al. 2002).

1.4.1.4 Genetic and developmental disease

Agenesis of one or more of the major salivary glands is a rare but significant cause of hyposalivation in children and young adults (Hodgson et al. 2001). Agenesis may be unilateral or bilateral and may occur in isolation, or be associated with other
developmental defects including first and second branchial arch and ectodermal defects (Gelbier et al. 1995). Other salivary gland defects include salivary duct atresia, diverticula, congenital fistulas, accessory ducts and ectopic salivary glands (Greenberg et al. 2003; Rossie 1993).

There are a number of case reports in the literature describing congenital absence of salivary glands in children (Daniel et al. 2003; Heath et al. 2006; Hodgson et al. 2001; Kwon et al. 2006). Reported associations with other ectodermal defects include the absence of lacrimal glands (Milunsky et al. 1990; O'Malley et al. 1993), Lacrimoauriculo-dento-digital syndrome (LADD) and the ectodermal dysplasias (Wiedemann 1997).

There are several reports of salivary gland agenesis occurring in conjunction with congenital conditions. These include hemifacial microsomia, Treacher Collins syndrome, Melkersson-Rosenthal syndrome (Greenberg et al. 2003), cleft lip and palate (Matsuda et al. 1999), Down syndrome (Ferguson et al. 2005; Yarat et al. 1999), Prader-Willi syndrome (Hart 1998) and Papillon-Lefèvre syndrome (Lundgren et al. 1996).

An audit was carried out on the results of salivary gland scan (scintiscans) at The Children's Hospital at Westmead over a period of 9 years (Eggers et al. 2005). Of the 29 patients diagnosed with major salivary gland hypofunction, 10 had no associated medical conditions, however of the remaining 19, 7 had 22q11 deletion syndrome.
Other medical conditions included Prader-Willi syndrome, ectodermal dysplasia, cleft palate, Brown syndrome, Sturge Weber syndrome, 4A syndrome, cleft palate, osteopenia and Incontinentia pigmentosa.
1.5 DIAGNOSIS OF SALIVARY HYPOFUNCTION

Identifying an individual with salivary gland dysfunction requires careful and systematic evaluation. The clinical evaluation should include a detailed history of the presenting symptoms, medical history, and evaluation of the salivary glands, duct orifices, oral mucosa and the dentition (Navazesh et al. 2003a). Objective evidence of salivary function is essential for diagnosis, prognosis and monitoring the progress of disease and therapy (Booker et al. 2004).

Table 1.8 outlines the signs and symptoms that are often related to salivary hypofunction. It should be noted that some patients with salivary hypofunction report no symptoms (Gelbier et al. 1995), and certainly paediatric patients vary in their ability to identify and report such symptoms.

1.5.1 DIAGNOSTIC TESTS

A range of diagnostic tests are available, however sialometry (saliva function testing) and imaging studies are the most common in the paediatric population. Tests such as microbiology, serology and histology are also available for the diagnosis of medical conditions such as Sjögren syndrome, sarcoidosis and other connective tissue disorders.
Table 1.8 Oral signs and symptoms related to salivary hypofunction*

<table>
<thead>
<tr>
<th>Symptoms</th>
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<tbody>
<tr>
<td>Burning oral sensation</td>
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<tr>
<td>Difficulties in speech</td>
</tr>
<tr>
<td>Difficulty chewing dry food</td>
</tr>
<tr>
<td>Taste impairment</td>
</tr>
<tr>
<td>Sensation of thirst</td>
</tr>
<tr>
<td>Signs</td>
</tr>
<tr>
<td>Dry, glazed and red oral mucosa</td>
</tr>
<tr>
<td>Lobulation or fissuring of the dorsal tongue</td>
</tr>
<tr>
<td>Atrophy of filiform papillae</td>
</tr>
<tr>
<td>Dry, cracked lips</td>
</tr>
<tr>
<td>Increased caries activity</td>
</tr>
<tr>
<td>Increased frequency of oral infections (e.g. recurrent oral candidiasis, angular chelitis)</td>
</tr>
<tr>
<td>Changes in saliva viscosity (e.g. frothy or ropy saliva)</td>
</tr>
</tbody>
</table>

* Modified from Bardow et al. (2004), Walsh (2000).

Subjective visual evidence of salivary hypofunction is important in a diagnostic work-up, and particular attention should be paid to visible alterations in the salivary film. This includes pooling and frothing of saliva, and increased viscosity that results in mucinous strands (Walsh 2000). Because saliva becomes more viscous and mucinous in nature as the resting flow reduces, a simple test for increased viscosity of saliva is useful. This involves lifting pooled saliva from the floor of the mouth or buccal mucosa with a dental mirror. Normal saliva can maintain a strand for a distance of approximately 5cm, whereas the strand distance for more viscous saliva can be as long as 15 cm (Walsh 2000).

1.5.2 SIALOMETRY

If salivary hypofunction is suspected, clinical examination should be followed by measurement of salivary flow rates, buffering capacity and pH. Saliva may be collected under both resting and stimulated conditions. It should be remembered, however, that
obtaining true resting saliva is almost impossible as salivary flow is always influenced by some kind of stimulation. Salivary parameters can also be influenced by factors relating to the test situation and the individual including anxiety, acute illness and masticatory dysfunction, age, sex, and circadian rhythms (Tenovuo 1989a). There is evidence to suggest that salivary flow follows a circadian rhythm, peaking at noon or in the middle of the afternoon, with variations of up to 50% over a 24 hour period (Ghezzi et al. 2000). Therefore, saliva collection should be standardised to a certain time of day to minimise the effects of this phenomenon.

1.5.2.1 Collection of unstimulated whole saliva

When unstimulated saliva is obtained the patient should sit in a forward position with the elbows resting on the knees. The facial muscles and jaw should be moved as little as possible and swallowing should be avoided. A number of collection methods for unstimulated saliva have been described (Tenovuo 1989a):

a) Draining method. After swallowing, the patient allows saliva to drain out of the mouth into a collection vessel. At the end of the collection time, the patient expectorates the remaining saliva into the vessel.

b) Spitting method. This method is similar to the draining method, however the saliva is collected with closed lips and saliva is expectorated at regular intervals into a vessel.
c) **Suction.** A slow-speed saliva ejector tip is connected to a vacuum pump and placed under the tongue. The saliva is led by a plastic tube into a collection vial. At the end of the collection period, the suction tip is moved around the mouth to collect remaining saliva.

d) **Swab.** Pre-weighed cotton rolls or absorbent swabs are placed in the mouth under the tongue and adjacent to the parotid ducts. At the end of the collection time, the swabs are removed and weighed immediately.

A study comparing resting salivary collection methods found that all four methods give the same general information on salivary flow rates (Navazesh *et al.* 1982). The swab technique was found to be less reliable and is therefore not recommended. The spitting and draining methods were found to be simple and reproducible and therefore the method of choice for whole mouth unstimulated saliva collection (Navazesh *et al.* 1982). Collection of saliva using the suction method is common in populations who are very young, or may have physical and cognitive impairment (Flaitz *et al.* 1998; Jones *et al.* 2000; Siqueira *et al.* 2005). The open suction method involves the subject’s mouth being relaxed and open and a saliva ejector swept around the mouth in a standardised manner at 15 second intervals for 2 minutes. Both the 2 and 5 minute open suction methods correlate well with the 5 minute spitting method (r=0.79 and 0.68 respectively) (Jones *et al.* 2000).
The suction technique is reliable and reproducible but produces higher flow rates than the other three methods, probably due to its stimulatory potential. It is also dependent on operator technique as the saliva must be collected uniformly from all areas of the mouth (Navazesh et al. 1982). The main advantage of the suction technique is that it does not require the subject to play an active role in the collection. Conversely, the spitting or draining methods requires the subject to be alert and concentrating for the duration of the test as they must be able to resist swallowing, clear their mouth of saliva and be able to accurately expectorate into the small opening of a collection container (Jones et al. 2000).

1.5.2.2 Collection of stimulated whole saliva

There are two methods commonly described for collecting stimulated saliva (Tenovuo 1989a):

a) Masticatory method. The patient chews on a piece of paraffin wax, initially for 2 minutes to soften the paraffin and remove saliva from the mouth. During the collection period, the patient continues to chew on the paraffin whilst expectorating into a container.

b) Gustatory method. The saliva is stimulated by citric acid which is placed onto the tongue every 30 seconds. Before every new drop of acid, the patient is asked to expectorate.
The efficacy of these methods is dependent on a number of procedural considerations. The choice of acid and its concentration affects the flow rate, as does the size and hardness of the paraffin wax. The number of chewing strokes also affects the flow rate when it is outside the normal range of 40-80 strokes/min (Tenovuo 1989a). Other factors such as chewing force and duration cannot be controlled, and may also have an effect on the flow rate (Tenovuo 1989a). Compared to resting flow rates, stimulated flow rates are significantly more variable both between and within subjects (Tenovuo 1989a).

1.5.2.3 Measurement of salivary flow rate

Salivary flow rate may be calculated by dividing the volume (millilitres) of saliva per minute. It also possible to weigh the amount of saliva and express the secretion rate in grams of saliva per minute (Tenovuo 1989a). Navazesh et al. (1982) tested the correlation between these two methods and found that they were very highly correlated (r=0.99), but that volume measurements are less reliable (Navazesh et al. 1982). Therefore, a number of studies in the literature calculate the salivary secretion rate in g/min, which is then reported as mL/min (Anderson et al. 2001; Bardow et al. 2000; Bardow et al. 2001; Jones et al. 2000; Navazesh et al. 1992; Negoro et al. 2000; Wolff et al. 2002).
1.5.2.4 Measurement of Buffering capacity

Buffering capacity can be measured using a variety of laboratory based techniques or simplified saliva testing kits (Tenovuo 1989a).

a) Titration

Titration of saliva to establish buffering capacity can be carried out under oil, open to air or titrated with carbon dioxide. The error with microtitration is about 6%, and therefore simplified methods such as pre-impregnated acid strips can be used with little or no loss of accuracy (Tenovuo 1989a).

b) Ericsson’s method

This simplified method was developed in 1959 and involves mixing hydrochloric acid into a standard amount of saliva (1.0mL). Carbon dioxide is then eliminated by bubbling air through the mixture for 20 minutes with the final pH being read electrometrically. This final pH is related to the original concentration of bicarbonate and is taken as an expression of the buffering capacity of the sample (Tenovuo 1989a).

c) Buffering capacity strips

The Dentobuff strip was created in order to simplify Ericsson’s method for chairside testing of buffering capacity. It consists of a strip of pH indicator paper which has been impregnated with acid. The final pH is read as a colour change in the paper.
Commercial strips have been shown to correlate with both Ericsson's method (Ericson et al. 1989) and hand-held pH meters (Kitasako et al. 2005).

1.5.2.5 Measurement of pH

Measurement of salivary pH is complicated by the bicarbonate buffer system, which is in equilibrium with the air in the mouth. When saliva is removed from the mouth for testing, a new equilibrium is established with room air and the pH shifts (Walsh 2000). It has been suggested that accurate measurement of pH can be undertaken on samples that have been allowed to equilibrate with room air for 20 minutes using a digital pH meter with temperature compensation rather than pH paper (Walsh 2000).

1.5.3 NORMAL SALIVARY VALUES

There are few studies on salivary parameters in children, so it is difficult to establish normal values for comparison. Therefore, a number of studies with a similar methodology have been compiled in order to show age- and sex-appropriate normal values for children (Andersson 1972; Bretz et al. 2001; Crossner 1984; Heintze et al. 1983; Watanabe et al. 1990). These data are shown in Appendix A. In order to evaluate whether it is appropriate to use adult salivary parameters for diagnosis of salivary hypofunction, paediatric salivary parameters have been compared to normal adult values. These studies are summarised in Figures 1.2-1.5.
1.5.4.1 Normal flow rates

Age and sex related differences in salivary flow rates are well established (Heintze et al. 1983). In children, flow rate increases with age up until 15 years when the salivary glands are fully developed (Crossner 1984). Studies in adults have shown that men have a higher flow rates than women for both stimulated and unstimulated saliva (Tenovuo 1989a). Children and adolescents also show the same trend, with consistently higher flow rates in boys (Andersson 1972; Crossner 1984). A number of studies have also found higher flow rates in children than in adults (Crossner 1984; Klock et al. 1977). A number of these age- and sex-related differences are demonstrated in Figures 1.2-1.5.

1.5.4.1.1 Unstimulated flow rates

Figure 1.2 outlines the findings from a number of studies on unstimulated flow rates in children (Andersson 1972; Bretz et al. 2001; Watanabe et al. 1990) which are compared to mean values in adults aged 15-29 years (Heintze et al. 1983). It can be seen that although there is a large variation in normal values, from the age of 10 years, flow rates are similar to adult values.
1.5.4.1.2 Stimulated flow rates

Figures 1.3 and 1.4 outlines stimulated salivary flow rates in males and females (Heintze et al. 1983) and children of the same sex for comparison (Andersson 1972; Crossner 1984). The stimulated flow rates are expressed in percentile bands and according to age.

Figure 1.3 Stimulated flow rates in boys and adult males
Figure 1.4 Stimulated salivary flow rates in girls and adult females

It can be seen that for both males and females, approximate adult flow rates were reached by the age of 13 years. Figure 1.5 compares stimulated flow rates in boys and girls by age and percentile band (Andersson 1972; Crossner 1984).

Figure 1.5 Stimulated flow rates in boys and girls as percentile bands
1.5.4.2 Normal buffering capacity values

The buffering capacity of saliva appears to show little variation within adults (Tenovuo 1989a). There is, however, some evidence to suggest that adult females have a lower buffering capacity than males (Heintze et al. 1983), and that girls have a lower buffering capacity than boys (Andersson 1972; Klock et al. 1977; Tukia-Kulmala et al. 1993). Buffering capacity has been found to be significantly correlated to the stimulated flow rate in adults (p<0.001)(Heintze et al. 1983).

Normal buffering capacity values were previously judged according to those established by Ericsson and Hardwick in 1959 using the titration method (Ericsson et al. 1978; Tenovuo 1989a). With the advent of buffering capacity strips, buffering capacity is now more commonly evaluated by the various commercial saliva testing kits. In the GC™ Saliva Check® kits, the strip is scored according to colour changes and grouped as either very low (0-5), low (6-9) or normal (10-12).

1.5.4.3 Normal pH values

Klock et al. (1977) found that girls aged 9-12 years had significantly lower salivary pH when compared to boys. Andersson (1972), however, found no age or sex difference in pH values of 7-13 year olds. Values of salivary pH in children appear to be similar to those reported in adults for both unstimulated and stimulated saliva. The salivary pH of stimulated saliva from 655 children (aged 9-12 years) ranged from 5.91-7.80, however 93% of samples fell within the interval of pH 6.8-7.7 (Klock et al. 1977).

Normal values in adults have been reported as pH 6.5-6.9 for unstimulated saliva and
pH 6.8 to 7.5 for stimulated saliva (Tenovuo 1989a). Very low values for salivary pH have been reported as pH <6.3 for unstimulated saliva and pH <6.8 for stimulated saliva (Tenovuo 1989a). Values for unstimulated and stimulated pH in children are shown in Table 1.9 with adult values shaded for comparison.

<p>| Table 1.9 Normal pH values of unstimulated and stimulated saliva |
|-------------------|-------------------|-------------------|-------------------|</p>
<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
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<th>Stimulated pH ± SD</th>
<th>Reference</th>
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<tr>
<td>7</td>
<td>Boys</td>
<td>7.4 ±0.3</td>
<td></td>
<td>Andersson (1972)</td>
</tr>
<tr>
<td>7</td>
<td>Girls</td>
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</tr>
<tr>
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<td>Girls</td>
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<td>Andersson (1972)</td>
</tr>
<tr>
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<td>7.4 ± 0.2</td>
<td>Andersson (1972)</td>
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<tr>
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<td>7.4 ± 0.2</td>
<td>Andersson (1972)</td>
</tr>
<tr>
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<td>6.5 - 6.9</td>
<td>7.0 - 7.5</td>
<td>Ericsson et al. (1978)</td>
</tr>
</tbody>
</table>

Table 1.9 data sources: (Andersson 1972; Ericsson et al. 1978; Klock et al. 1977)

1.5.4 SALIVARY PARAMETERS FOR HYPOFUNCTION IN CHILDREN

A number of studies have attempted to establish normal population values (Heintze et al. 1983), and lower limits for normal salivary parameters in adults (Ericsson et al. 1978; Navazesh et al. 1992). Attempts to formalize a cut-off value distinguishing normal from abnormal function may be confounded by heterogeneous study populations (Ship et al. 1991), and wide variations in the normal range of salivary flow rates both within the population and individuals (Ghezzi et al. 2000; Tukia-Kulmala et
al. 1993). It is also uncertain how much saliva is required to maintain oral health in a particular individual, therefore the use of such cut-off values for salivary hypofunction must always be taken into consideration with the whole clinical picture.

At present, there are no established values for salivary hypofunction in children. Considering the age and sex differences in flow rates, care should be taken when diagnosing hypofunction in children by the use of sialometry. For this reason, in addition to limited co-operation in young children, salivary gland scans are frequently utilised in paediatric populations (Cameron et al. 2003).

Despite the wide variation in normal whole saliva, it is generally accepted that flow rates in hyposalivation are significantly lower than normal. Salivary flow rates of <0.12-0.16 mL/min for unstimulated flow (Navazesh et al. 1992), and <0.7 mL/min for stimulated flow have been suggested as hypofunction in adults (Navazesh et al. 2003b). If individualised baseline rates have been established, then a 50% reduction in flow in an individual may also be considered hypofunction (Ship et al. 1991). Other reported cut-off values in the literature include those used by several studies to determine salivary hypofunction in paediatric oncology patients. These studies have used values of <0.1 mL/min for unstimulated flow rates and <0.5 mL/min for stimulated flow rates as cut-off values for hypofunction (Bagesund et al. 2000; Dahllöf et al. 1997), however no references are given for the use of these values. One of the criteria for diagnosis of Sjögren syndrome is an unstimulated flow rate of ≤0.1
mL/min. For diagnosis of salivary hypofunction in Sjögren syndrome this test has a reported sensitivity of 71.9% and specificity of 74.7% (Vitali et al. 1996).

These values could be considered as a guide in older children, however it would be inappropriate to use these values in age groups where flow rates are greatly lower than in adults. For stimulated flow this would include children ≤13 years of age (see Figures 1.3 and 1.4) (Andersson 1972). Unstimulated flow rates in children are more consistent across age groups and appear to be similar to adult values from the age of 10 years (see Figures 1.2). For younger age groups, it may be more appropriate to consider a diagnosis of hypofunction only when values are well outside the normative range.

1.5.5 SALIVARY IMAGING

Scintigraphy with technetium (Tc) 99m pertechnetate is a dynamic test to assess salivary gland function and to determine abnormalities in gland uptake and excretion. It is the only salivary imaging technique that provides information on the functional capabilities of the glands and correlates well with salivary output (Greenberg et al. 2003). Technetium is taken up by the salivary glands following intravenous injection, transported to the glands and then secreted into the oral cavity. The uptake, concentration and excretion of the pertechnetate anion is imaged with a gamma detector that records both the number and location of the gamma particles released during a given period of time (Greenberg et al. 2003). Most radiologists read Tc 99m scans by using visual interpretation and clinical judgement, however semi-quantitative
time-activity computer analyses are also available (Booker et al. 2004; Greenberg et al. 2003).
1.6 RESEARCH OUTLINE AND OBJECTIVES

1.6.1 RESEARCH AIMS

In order to expand on the research commenced in a pilot study by Dr Sally Hibbert and Associate Professor Richard Widmer in 2003 (Hibbert et al. 2004), it was decided to investigate a larger group of children with 22q11 deletion syndrome. In view of the lack of literature on the oral findings and salivary function in 22q11 deletion syndrome, further investigation and clarification in a number of areas was needed.

The hypothesis of this study is that an increased caries rate in 22q11 deletion syndrome noted in the pilot study is caused by salivary hypofunction.

The aims of this research are:

1. To establish the prevalence of dental caries in a group of children with 22q11 deletion syndrome
2. To carry out testing of salivary function in the same cohort of children
3. To examine paediatric salivary parameters in the literature in order to establish age and sex appropriate normal limits and cut-off values for salivary hypofunction in children.
1.6.2 SIGNIFICANCE OF THE RESEARCH

Children with 22q11 deletion syndrome have a high incidence of congenital cardiac anomalies and are therefore at risk of bacterial endocarditis. This research aims to establish causative factors of caries in these children so that appropriate screening and preventive measures can be implemented at an early age. Preventing the need for dental treatment in 22q11 deletion syndrome is of high importance as the behaviour profile of the group means that many of these patients require management under general anaesthetic. Whilst general anaesthesia is generally safe, it is costly and has an associated level of morbidity and should be avoided wherever possible.

Currently, only two cohorts of patients with 22q11 deletion syndrome have been studied in regards to their oral findings and salivary function (Hibbert et al. 2004; Klingberg et al. 2007). By outlining the oral findings in a different cohort of patients, this research contributes to the body of literature on the spectrum of features found as part of 22q11 deletion syndrome.

At present, there is a lack of information regarding diagnosis of salivary hypofunction in children by sialometry. There are no age and sex appropriate cut-off values similar to those already established for adults. This makes diagnosis of salivary hypofunction in children difficult, and may necessitate the use of more invasive salivary function tests such as salivary imaging. Compiling normative data in the literature in order to establish age- and sex-appropriate cut-off values for salivary hypofunction would
allow the use of sialometry with more certainty for diagnosis of salivary hypofunction in children.
2. MATERIALS AND METHODS

2.1.1 STUDY DESIGN

Information on the oral findings in the study group were obtained via:

a) Medical and dental history
b) Dental examination
c) Salivary function tests
d) A questionnaire designed to collect information on medical and dental history, diet, oral hygiene, fluoride exposure and socioeconomic status

Ethics committee approval for the study was obtained from the Children’s Hospital at Westmead Ethics Committee.

2.1.2 SUBJECT ASCERTAINMENT

The study group consisted of children with a confirmed diagnosis of Velocardiofacial Syndrome (22q11 deletion syndrome) obtained from the Department of Speech Pathology at the Children’s Hospital at Westmead, New South Wales, Australia.

Ninety-three children between 5-16 years of age were sent an invitation letter and an information sheet asking for their participation in the study. The information sheet outlined the nature and requirements of the study as approved by the Ethics
Committee. Twenty-one subjects indicated that they would like to participate and were contacted for an appointment. Participation was voluntary and subjects were permitted to withdraw their permission at any stage.

2.1.3 CONSENT

Informed parental consent was obtained from all participating subjects regarding requirements for the study.

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

a) Medical and dental history
b) Dental examination by the author
c) Dental radiographic records if unavailable or not available for viewing
d) Saliva function testing
e) Completion of a questionnaire

2.1.4 MEDICAL AND DENTAL HISTORY

A full medical and dental history was obtained, including the presence of systemic disease, cleft palate, previous operations, medications and allergies, regularity of dental visits and previous dental work.
2.1.5 DENTAL EXAMINATION

Dental examination was carried out by one examiner (RE) in a dental chair with lighting, mirror and probe. A clinical data sheet was completed for each patient. A dental charting was completed that included decayed, missing and filled surfaces, disturbances of enamel formation and soft tissue pathology. Teeth were recorded as missing only if they had been extracted due to dental pathology. If the tooth had a stainless steel crown it was scored as having 5 filled surfaces. Disturbances of enamel formation were classified according to a discrete opacity, diffuse opacity or hypoplastic defect.

Bitewings x-rays were taken if none had been taken in the last 6 months, or were not available to view. Limited co-operation precluded the taking of x-rays in several patients.

2.1.6 SALIVARY FUNCTION ASSESSMENT

The patients were requested not to eat, drink or brush for 1 hour prior to their appointment. All appointments were conducted between 9am and 11.30 am to reduce the effects of circadian rhythms on the results of the saliva test.

A subjective visual examination of the subject’s saliva viscosity was recorded prior to saliva testing and included descriptive terms such as clinically normal, ropy or frothy. ‘Frothy’ saliva was recorded if the saliva had multiple small bubbles on the floor of the
mouth or buccal mucosa. 'Ropy' saliva was recorded if a web of saliva had a distance of >5cm when lifted from the floor of the mouth with a dental mirror. 'Clinically normal' saliva was recorded if no abnormalities of saliva viscosity were detected.

Unstimulated saliva was collected with a suction device that consisted of a size 14 gauge Y-suction catheter emptying into a 50mL centrifuge tube. The lid of the centrifuge tube was modified to accept both the catheter and a slow-speed saliva ejector. The centrifuge tube was then connected to the slow-speed suction on the dental cart via the saliva ejector. A photograph of the device is included in Appendix B.

Saliva was collected for 5 minutes with the subject seated in the dental chair with their head forward in a resting position. The subject was advised to keep as still as possible and to avoid swallowing. The collection device was weighed before and after saliva collection. The specific gravity of the saliva was assumed to be equal that of water and hence 1g = 1mL. The weight of the saliva in grams was converted to a flow rate measurement in mL/min.

The pH of the unstimulated saliva was tested using the litmus paper included in the GC™ Saliva-Check® kits. Initially a pH probe was used in addition to the pH paper in order to obtain a more accurate reading (WP-80 pH-mV-temperature meter, Daintree Scientific, Australia). Due to lack of saliva in most of the children and technical difficulties, the use of the pH probe was abandoned.
Stimulated saliva was collected using the standard piece of paraffin wax provided in the GC™ kits. Due to limited co-operation from the study group, the subjects were asked to chew 10 times, after which the examiner (RE) removed the wax from the patient’s mouth in order for the subject to spit into the pre-weighed collection cup. This was repeated and continued for 5 minutes after which the collection cup was weighed and the weight converted into a measurement in mL/min.

A stimulated saliva pH was carried out using the litmus paper provided in the GC™ kits and scored against the colour coded card provided.

A test for buffering capacity was carried using the acid impregnated strips from the GC™ kit. Due to insufficient volumes of saliva produced, a titration with hydrochloric acid was unable to be performed.

Salivary hypofunction was diagnosed on the basis of an unstimulated flow rate of ≤0.1 mL/min or a stimulated flow rate that was <5th centile for the subject’s age and sex. Borderline hypofunction was diagnosed as an unstimulated flow rate of ≤0.16 mL/min or a stimulated flow rate between 5th-10th centile for age and sex.

2.1.7 QUESTIONNAIRE

A questionnaire was written specifically for this project with the help of Professor Jennifer Peat (Epidemiologist and Consultant Biostatistician) as advised by the Ethics Committee at the Children’s Hospital at Westmead. A number of questions were
modelled on those present in the NSW Child Health Survey ('New South Wales Child Health Survey 2001'). The questionnaire aimed to control for causative factors of caries including socioeconomic factors, diet, fluoride exposure and oral hygiene practices. Questions regarding causative factors of enamel defects were also included. The questionnaire is included in Appendix C.

2.1.8 STATISTICAL ANALYSIS

Data was cross-checked and randomly sampled for consistency prior to undertaking the statistical analysis. Appropriate statistical methods (chi square test for proportions and independent t-test or ANOVA for continuous outcomes) were used to explore differences for categorical and continuous outcomes. Statistical tests for continuous outcomes were undertaken in both the parametric (independent t) and distribution-free form (Mann-Whitney – U) in order to account for non-normality of variable(s), inequality of variances between different groups, and the small sample size. If a variable was normally distributed than the parametric tests are utilized, otherwise non-parametric tests were used to explore associations. Descriptive statistics (e.g. means and proportions) were computed for all study variables.

The Kolmogorov-Smirnov test was used to assess whether each variable was derived from a population with a normal distribution. Assessment of correlation of normally distributed variables was undertaken using the Pearson correlation co-efficient. Variables that were not normally distributed, or if the sample size was small (less than 20), were analysed using the Spearman’s rank order correlations (rho) to determine the
correlations between variables. The statistical significance level for all analyses was set at 0.05.

Analyses of variance were carried out to test for differences in stimulated flow, unstimulated flow, stimulated ph, unstimulated ph, and buffering capacity between different groups. All analysis was undertaken using SPSS™ Version 14.0. The analysis was undertaken by a statistician from Macquarie University, Sydney, Australia.
3. RESULTS

3.1.1 PATIENT SAMPLE

A total of 21 patients (range 5-16 years) participated in the study, of which 13 were female and 8 were male. The mean age of the group was 9 years 8 months. A number of the patients were unco-operative and were unable to complete some or all of the saliva testing. All 21 patients were able to co-operate for dental examination and questionnaires were completed for all patients.

3.1.2 RESULTS OF THE DENTAL EXAMINATION

Twenty-one patients were co-operative for a dental examination and a summary of the findings are shown in Table 3.1. The mean dmft/DMFT for the group was 4.8 (SD=3.9), and the mean dmfs/DMFS was 12.3. Six children (28.6%) were caries free, and when these subjects were removed from the analysis, the mean dmft/DMFT score increased to 6.73 (SD = 2.8). The decay scores were not significantly correlated to any variable analysed in the data, including both the salivary parameters and the questionnaire.
Table 3.1 Results of the dental examination

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<td>0</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>20</td>
<td>15.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>6</td>
<td>27</td>
</tr>
<tr>
<td>21</td>
<td>16.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>6</td>
<td>9</td>
</tr>
</tbody>
</table>

$d = \text{decayed primary teeth, } m = \text{missing primary teeth, } f = \text{filled primary teeth. Capital letters denote the equivalent in permanent teeth. dmf/DMFT = the total number of decayed, missing and filled teeth, dmfs/DMFS = the total number of decayed, missing and filled surfaces.}$

Eleven subjects (52.3%) had $\text{dmft/DMFT} \geq 6$, three subjects (14.3%) $\text{dmft/DMFT} 3-5$ and seven subjects 33.3% had a $\text{dmft/DMFT}$ score $< 3$. The subjects had a mean of 1.5 decayed teeth ($d+D$), 1.0 missing teeth ($m+M$) and 2.4 filled teeth ($f+F$) each. Untreated decay ($d+D \geq 1$) was present in 52.4% of the study group. Missing teeth due to caries ($m+M \geq 1$) was present in 33% of the study group and filled teeth ($f+F \geq 1$) in 61.9%.

For primary teeth, the mean number of filled teeth (1.9) was greater than the number of
decayed (0.9) or missing teeth (0.7). For permanent teeth the mean number of decayed teeth (0.6) was greater than filled (0.5) or missing teeth (0.3). The mean, ranges and standard deviation for decayed, missing and filled teeth are shown in Table 3.2 and the distribution of dmft/DMFT according to age is shown in Figure 3.1.

Table 3.2 Mean, minimum and maximum values for decayed, missing and filled teeth.

<table>
<thead>
<tr>
<th>Statistic</th>
<th>d</th>
<th>m</th>
<th>f</th>
<th>D</th>
<th>M</th>
<th>F</th>
<th>dmft/DMFT</th>
<th>dmfs/DMFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.9</td>
<td>0.7</td>
<td>1.9</td>
<td>0.6</td>
<td>0.3</td>
<td>0.5</td>
<td>4.8</td>
<td>12.3</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>1.64</td>
<td>1.35</td>
<td>2.54</td>
<td>0.93</td>
<td>1.15</td>
<td>1.12</td>
<td>3.89</td>
<td>11.83</td>
</tr>
<tr>
<td>Minimum</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maximum</td>
<td>5</td>
<td>4</td>
<td>7</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>11</td>
<td>37</td>
</tr>
</tbody>
</table>

$d =$ decayed primary teeth, $m =$ missing primary teeth, $f =$ filled primary teeth. Capital letters denote the equivalent in permanent teeth. dmft/DMFT $=$ the total number of decayed, missing and filled teeth, dmfs/DMFS $=$ the total number of decayed, missing and filled surfaces.

Figure 3.1 Decayed, Missing and Filled Teeth of the study group according to age
Table 3.3 outlines the data from the dental examination of the study group and compares it to data taken from the NSW Child Dental Health Survey (2000) (Armfield et al. 2003). In order to make a comparison to the NSW data (maximum age 15 years) the subject aged 16.8 years was removed from the analysis.

**Table 3.3** A comparison of the study group to data from the Child Dental Health Survey NSW, 2000.

<table>
<thead>
<tr>
<th></th>
<th>NSW 5-8 years</th>
<th>Study group 5-8 years</th>
<th>NSW 9-12 years</th>
<th>Study group 9-12 years</th>
<th>NSW 13-15 years</th>
<th>Study group 13-15 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean dmft</td>
<td>1.05</td>
<td>4.7</td>
<td>0.57</td>
<td>3.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d/dmft</td>
<td>67.0%</td>
<td>38.3%</td>
<td>46.1%</td>
<td>4.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dmft=0</td>
<td>65.3%</td>
<td>30.0%</td>
<td>76.5%</td>
<td>42.9%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean DMFT</td>
<td>0.45</td>
<td>0.29</td>
<td>1.20</td>
<td>5.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D/DMFT</td>
<td>55.7%</td>
<td>0%</td>
<td>51.8%</td>
<td>43.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMFT=0</td>
<td>79.9%</td>
<td>85.7%</td>
<td>61.6%</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean dmft or DMFT for the group ranged from 4.44-6.26 times the NSW average depending on age. However, the proportion of decayed teeth to dmft/DMFT (d/dmft and D/DMFT) was less than the NSW average for all ages in the study group. When compared to NSW data, the proportion of patients who were caries free was lower in the study group except for permanent teeth in 9-12 years olds (85.7% caries free compared to 79.9% caries free in NSW). However, the dmft in 9-12 year olds in the study group was still 6 times higher than the NSW average.
3.1.3 RESULTS OF SALIVA TESTING

Eighteen patients were able to be tested for unstimulated saliva, and sixteen for stimulated saliva. The remainder of the group were un-cooperative for saliva testing. A summary of the results of the saliva test are shown in Table 3.4.

Table 3.4 Summary of results of the saliva test

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Clinical saliva</th>
<th>U.S flow rate (mL/min)</th>
<th>U.S pH</th>
<th>S flow rate (mL/min)</th>
<th>S pH</th>
<th>B.C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.6</td>
<td>F</td>
<td>Frothy</td>
<td>0.39</td>
<td>6.9</td>
<td>0.08</td>
<td>6.9</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>5.7</td>
<td>M</td>
<td>Normal</td>
<td>0.25</td>
<td>7.2</td>
<td>0.47</td>
<td>7.6</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>6.1</td>
<td>F</td>
<td>Ropy</td>
<td>0.07</td>
<td>7.0</td>
<td>0.15</td>
<td>6.9</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>6.3</td>
<td>F</td>
<td>Ropy</td>
<td>0.06</td>
<td>7.2</td>
<td>0.12</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>6.8</td>
<td>F</td>
<td>Frothy</td>
<td>0.00</td>
<td>6.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>7.2</td>
<td>F</td>
<td>Frothy</td>
<td>0.12</td>
<td>6.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7.3</td>
<td>M</td>
<td>Frothy</td>
<td>0.12</td>
<td>6.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>8.2</td>
<td>F</td>
<td>Frothy</td>
<td>0.12</td>
<td>6.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>8.3</td>
<td>F</td>
<td>Normal</td>
<td>0.16</td>
<td>6.8</td>
<td>0.37</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>8.3</td>
<td>M</td>
<td>Frothy</td>
<td>0.16</td>
<td>6.8</td>
<td>0.37</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>9.2</td>
<td>F</td>
<td>Normal</td>
<td>0.53</td>
<td>7.0</td>
<td>0.99</td>
<td>7.6</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>9.8</td>
<td>M</td>
<td>Normal</td>
<td>0.51</td>
<td>7.6</td>
<td>0.40</td>
<td>7.8</td>
<td>4</td>
</tr>
<tr>
<td>13</td>
<td>9.8</td>
<td>M</td>
<td>Normal</td>
<td>0.09</td>
<td>6.7</td>
<td>0.16</td>
<td>6.8</td>
<td>7</td>
</tr>
<tr>
<td>14</td>
<td>10.6</td>
<td>F</td>
<td>Frothy</td>
<td>0.02</td>
<td>7.0</td>
<td>0.08</td>
<td>6.8</td>
<td>3</td>
</tr>
<tr>
<td>15</td>
<td>10.9</td>
<td>F</td>
<td>Ropy</td>
<td>0.02</td>
<td>6.5</td>
<td>0.08</td>
<td>6.7</td>
<td>7</td>
</tr>
<tr>
<td>16</td>
<td>10.9</td>
<td>F</td>
<td>Normal</td>
<td>0.26</td>
<td>7.0</td>
<td>0.44</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>17</td>
<td>12.4</td>
<td>F</td>
<td>Normal</td>
<td>0.64</td>
<td>7.8</td>
<td>0.61</td>
<td>7.8</td>
<td>12</td>
</tr>
<tr>
<td>18</td>
<td>13.3</td>
<td>M</td>
<td>Normal</td>
<td>0.30</td>
<td>6.6</td>
<td>0.81</td>
<td>7.4</td>
<td>10</td>
</tr>
<tr>
<td>19</td>
<td>14.3</td>
<td>F</td>
<td>Frothy</td>
<td>0.05</td>
<td>6.7</td>
<td>0.17</td>
<td>6.9</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>15.2</td>
<td>M</td>
<td>Normal</td>
<td>0.37</td>
<td>7.4</td>
<td>0.96</td>
<td>7.8</td>
<td>7</td>
</tr>
<tr>
<td>21</td>
<td>16.8</td>
<td>M</td>
<td>Frothy</td>
<td>0.00</td>
<td>6.0</td>
<td>0.28</td>
<td>5.8</td>
<td>2</td>
</tr>
</tbody>
</table>

U.S = unstimulated, S = stimulated, B.C = buffering capacity score, grey shading represents patients unable to give a saliva sample.
3.1.3.1 Clinical presentation of saliva

Saliva was clinically normal in 43%, ropy in 14% and frothy in 43% of subjects. Not all of the patients examined clinically were co-operative for saliva testing. Those with ‘frothy’ saliva had a lower unstimulated flow rate (0.11mL/min), stimulated flow rate (0.20 mL/min) and buffering capacity score (2) compared to the group with ‘normal’ saliva, however this was not statistically significant. The patients with ‘ropy’ saliva had even lower flow rates (mean unstimulated flow rate of 0.05 mL/min and mean stimulated flow rate of 0.12 mL/min) and a mean buffering score of 4. The respective values for the ‘normal’ group were 0.37mL/min (unstimulated flow rate), 0.61mL/min (stimulated flow rate) and 7 (buffering capacity score).

3.1.3.2 Unstimulated flow rates

Eighteen patients were able to provide an unstimulated saliva sample. Unstimulated flow rates ranged from 0.00mL/min to 0.64mL/min, with a mean of 0.21mL/min (SD=0.20). The mean for males (n=7) was 0.24 mL/min (SD = 0.17) and for females (n=11) the mean was 0.19 mL/min (SD = 0.22). The difference between the sexes was not statistically significant. Figure 3.2 shows the unstimulated flow rates of the study group according to age and sex.
Figure 3.2 Unstimulated flow rates of the study group according to age and sex.

The unstimulated flow rates in the study group were compared to population means for as outlined previously in Figure 1.2. The results for males in the study group are shown in Figure 3.3, and in Figure 3.4 for females in the study group.
Two out of the seven males in the group had unstimulated flow rates that were $\leq 0.1$ mL/min, and one patient had an unstimulated flow rate of $0.16$ mL/min. The remainder of the male subjects (57%) had flow rates that would be considered normal for their age.

**Figure 3.4** Unstimulated flow rates of the study group (girls) vs. population means

Six out of eleven females (55%) in the group had unstimulated flow rates of $\leq 0.1$ mL/min. One patient had an unstimulated flow rate of $<0.16$ mL/min. The remainder of the females (36%) had flow rates that would be considered within the normal range for their age.
3.1.3.3 Unstimulated pH

Eighteen patients were tested for unstimulated pH, which ranged from 6.0-7.8. The mean of the group was pH 6.9 (SD=0.42), there was no significant difference between the sexes, and all fell within the normal range. The unstimulated pH was significantly correlated to the unstimulated flow rate (r = 0.60, p<0.01).

3.1.3.4 Stimulated flow rates

Sixteen patients were able to provide a stimulated saliva sample. The flow rates ranged from 0.08-0.99mL/min, with a mean of 0.39mL/min (SD=0.31). The mean for males (n= 7) was 0.49 mL/min (SD=0.29) and for females (n=9) was 0.30 m/L/min (SD=0.31). The difference between the sexes was not significant. Stimulated flow rates according to age and sex are shown in Figure 3.5. The stimulated flow rates were significantly correlated to the unstimulated flow rates (r = 0.63, p<0.01).

Figure 3.5 Stimulated flow rates of the study group according to age and sex.
The stimulated flow rates in both sexes were compared to population values in children expressed as percentile bands as outlined previously in Figure 1.5. This comparison is shown in Figures 3.6 and 3.7.

**Figure 3.6** Stimulated flow rates of the study group (boys) vs. population flow rates expressed as percentile bands.

Five out of the seven males in the group (71%) had stimulated flow rates $\leq 5^{th}$ centile for their age, one patient was between the $5^{th}$-$10^{th}$ centile and one patient between the $10^{th}$-$25^{th}$ centile.
Figure 3.7 Stimulated flow rates of the study group (girls) vs. population flow rates expressed as percentile bands

Six out of the nine females in the group (67%) had stimulated flow rates <5th centile for their age, one subject had a flow rate that fell between the 5th-10th centile, one subject between the 10-25th centile and one between the 50th-75th centile.

3.1.3.5 Stimulated pH

Stimulated pH was testing in 16 patients. The samples ranged from pH 5.8-7.8, with a mean of pH 7.1. One patient had a pH value that would be considered outside the normal range. The difference between the sexes was not significant. The stimulated pH was significantly correlated to the stimulated flow rate (r = 0.76, p<0.01).
3.1.3.6 Buffering capacity

Sixteen patients were tested for buffering capacity (7 males and 9 females). The range was from 1-12. The mean buffering capacity score for females was 4 and for males the mean was 6, however the difference between the sexes was not significant. According to the categories provided by the GC™ Saliva Check® kits, 9 patients had very low buffering capacity, 5 patients had low buffering capacity and 2 patients had normal buffering capacity. Figure 3.8 shows the distribution of buffering capacity as percentages. Buffering capacity was significantly correlated to the stimulated salivary pH (r = 0.56, p<0.05), however it was not significantly correlated to the stimulated flow rate.

Figure 3.8 Buffering capacity according to the GC™ Saliva Check® kits.
3.1.4 RESULTS OF QUESTIONNAIRE

3.1.4.1 Medical History

A parent or guardian of all 21 patients in the study group completed the questionnaire. One patient was adopted and therefore some information was not available for this patient. Information regarding the medical history of the study group is presented in Table 3.5.

<table>
<thead>
<tr>
<th>Medical Condition</th>
<th>Number of patients</th>
<th>Medical condition</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cardiovascular</strong></td>
<td></td>
<td><strong>Respiratory</strong></td>
<td></td>
</tr>
<tr>
<td>Cardiac condition</td>
<td>14</td>
<td>Pneumonia</td>
<td>3</td>
</tr>
<tr>
<td><strong>Immunological</strong></td>
<td></td>
<td>Whooping cough</td>
<td>1</td>
</tr>
<tr>
<td>Hypoparathyroidism</td>
<td>1</td>
<td>Asthma</td>
<td>2</td>
</tr>
<tr>
<td>Seizures</td>
<td>2</td>
<td>Bronchiolitis</td>
<td>1</td>
</tr>
<tr>
<td>IgA deficiency</td>
<td>1</td>
<td><strong>Otolaryngological</strong></td>
<td></td>
</tr>
<tr>
<td>Thymus aplasia</td>
<td>4</td>
<td>Otitis media</td>
<td>11</td>
</tr>
<tr>
<td>Calcium deficiency</td>
<td>1</td>
<td>Hearing loss</td>
<td>5</td>
</tr>
<tr>
<td><strong>Gastrointestinal</strong></td>
<td></td>
<td>Ear reconstruction</td>
<td>2</td>
</tr>
<tr>
<td>Malrotation of bowel</td>
<td>1</td>
<td>Laryngeal webs</td>
<td>1</td>
</tr>
<tr>
<td>Pyloric stenosis</td>
<td>1</td>
<td>Paralysed vocal cord</td>
<td>1</td>
</tr>
<tr>
<td>Gastrointestinal infections</td>
<td>1</td>
<td><strong>Genitourinary</strong></td>
<td></td>
</tr>
<tr>
<td>Reflux</td>
<td>2</td>
<td>Inguinal hernia</td>
<td>1</td>
</tr>
<tr>
<td>Constipation</td>
<td>2</td>
<td><strong>Velopharyngeal</strong></td>
<td></td>
</tr>
<tr>
<td>Umbilical hernia</td>
<td>2</td>
<td>Cleft soft palate</td>
<td>4</td>
</tr>
<tr>
<td><strong>Orthopaedic</strong></td>
<td></td>
<td>Submucous cleft</td>
<td>3</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>1</td>
<td>Velopharyngeal</td>
<td>9</td>
</tr>
<tr>
<td>Scoliosis</td>
<td>1</td>
<td>insufficiency</td>
<td></td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General anaesthetic under 12 months</td>
<td>14</td>
<td>Low birth weight</td>
<td>2</td>
</tr>
</tbody>
</table>

Five children (23.8%) were taking medication(s) for various medical conditions. These included methylphenidate hydrochloride (Ritalin), omeprazole magnesium (Acimax,
Losec), Respiradone, benztropine mesylate (Cogentin), salbutamol sulphate (Ventolin), fluticasone propionate (Serotide) and citalopram hydrobromide (Cipramil). The patients taking medications did not have significantly lower unstimulated flow rates, stimulated flow rates or buffering capacity. Fourteen patients had a cardiac condition, however there was no correlation between the presence of a cardiac condition and dmft/DMFT scores or an increase in untreated decay (d+D) in the same subjects.

3.1.4.2 Dental History

Seven patients had their first dental visit from the age of 1-2 years, 11 patients attended from 3-5 years and 3 patients over the age of 8 years. For 16 patients, their last visit was less that 6 months ago, 2 patients attended a dentist 6-12 months ago, 1 patient 1-2 years ago, 1 patient 2-4 years ago and 1 patient had never previously seen a dentist. Forty-seven percent of patients received their previous dental care at a private dental practice, whereas 38% had attended a public clinic. The type of dental treatment received by the study group in the last 12 months is presented in Table 3.5.

Table 3.6 Dental treatment received in the previous 12 months

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fillings</td>
<td>4</td>
<td>19%</td>
</tr>
<tr>
<td>Extraction</td>
<td>2</td>
<td>10%</td>
</tr>
<tr>
<td>Check-up</td>
<td>19</td>
<td>91%</td>
</tr>
<tr>
<td>Fluoride treatment</td>
<td>5</td>
<td>24%</td>
</tr>
<tr>
<td>Scale and clean</td>
<td>7</td>
<td>33%</td>
</tr>
</tbody>
</table>
3.1.4.3 Oral hygiene and preventive history

A summary of results from the oral hygiene section of the questionnaire are included in Table 3.7. Brushing was commenced between the ages of 1-2 years for 48% of the group, and 62% brushed their teeth twice per day.

<table>
<thead>
<tr>
<th>Oral hygiene variable</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age child started brushing</strong></td>
<td></td>
</tr>
<tr>
<td>6-12 months</td>
<td>6</td>
</tr>
<tr>
<td>1-2 years</td>
<td>10</td>
</tr>
<tr>
<td>2-3 years</td>
<td>3</td>
</tr>
<tr>
<td>3-4 years</td>
<td>1</td>
</tr>
<tr>
<td>4-5 years</td>
<td>1</td>
</tr>
<tr>
<td><strong>Frequency of brushing</strong></td>
<td></td>
</tr>
<tr>
<td>1 times per day</td>
<td>8</td>
</tr>
<tr>
<td>2 times per day</td>
<td>13</td>
</tr>
<tr>
<td><strong>Use of fluoride supplements</strong></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>17</td>
</tr>
<tr>
<td>Yes</td>
<td>4</td>
</tr>
</tbody>
</table>

There was no correlation between either the frequency of brushing per day or the age the patient started brushing and dmft/DMFT scores.

3.1.4.4 Diet history

Analysis of early childhood feeding practices revealed that 62% of the group had been breastfed. Fifty four percent of the group stopped breastfeeding at 0-6 months, 23% at 6-14 months and 23% at 15-23 months. Seventy-six percent of patients were fed infant formula regularly; 6% stopped formula feeding at 0-6 months, 69% at 6-14 months, 12.5% from 15-23 months and 12.5% later than 36 months.
Eighty-one percent of patients were given drinks from a bottle other than breastmilk and formula, the results being summarised in Table 3.8.

**Table 3.8 Drinks given from a bottle other than breastmilk and formula**

<table>
<thead>
<tr>
<th>Drink</th>
<th>Number of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow's milk</td>
<td>11</td>
<td>52.4</td>
</tr>
<tr>
<td>Water</td>
<td>15</td>
<td>71.4</td>
</tr>
<tr>
<td>Fruit juice</td>
<td>13</td>
<td>61.9</td>
</tr>
<tr>
<td>Cordial</td>
<td>3</td>
<td>14.3</td>
</tr>
<tr>
<td>Soy milk</td>
<td>1</td>
<td>4.8</td>
</tr>
<tr>
<td>Sugar-free drinks</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Soft drink</td>
<td>1</td>
<td>4.8</td>
</tr>
</tbody>
</table>

The age at which all bottle feeding was stopped is summarised in Table 3.9.

**Table 3.9 The age at which bottle feeding was stopped**

<table>
<thead>
<tr>
<th>Months</th>
<th>Number of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-14 months</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td>15-23 months</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td>24-36 months</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>above 36 months</td>
<td>3</td>
<td>14</td>
</tr>
</tbody>
</table>

A dietary history, designed to assess the consumption of cariogenic and erosive foods and drinks was undertaken. Sweets were consumed by 38% of the group 1-2 times per day, juice 2-3 times per day by 62% and other sugar-containing beverages 1-2 times per day by 24%. The results the dietary history are summarised in Table 3.10. The consumption of juice or any other sugar-containing beverage on a daily basis was not correlated with the dmft/DMFT scores.
Table 3.10 Consumption of cariogenic foods and drinks

<table>
<thead>
<tr>
<th>Dietary variable</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweets 1-2 times/day</td>
<td>8</td>
</tr>
<tr>
<td>Sweets 1-3 times/week</td>
<td>7</td>
</tr>
<tr>
<td>Sweets 4-5 times/week</td>
<td>5</td>
</tr>
<tr>
<td>Juice 1 times/day</td>
<td>2</td>
</tr>
<tr>
<td>Juice 2-3 times/day</td>
<td>13</td>
</tr>
<tr>
<td>Juice 1-2 times/week</td>
<td>1</td>
</tr>
<tr>
<td>No juice</td>
<td>4</td>
</tr>
<tr>
<td>Other sugar containing drink 1-2 times/day</td>
<td>5</td>
</tr>
<tr>
<td>Other sugar containing drink 1-2 times/week</td>
<td>5</td>
</tr>
<tr>
<td>Other sugar containing drink 5-6 times/week</td>
<td>4</td>
</tr>
<tr>
<td>No other sugary drinks</td>
<td>6</td>
</tr>
</tbody>
</table>

3.1.4.5 Social History

All 21 patients were born in Australia, and all parents were born in Australia with the exception of 3 mothers and 3 fathers. English was spoken as the primary language in all but one household (Arabic). The education level of the parents of the study group is outlined in Table 3.11.

Table 3.11 Education level of parents

<table>
<thead>
<tr>
<th>Education level</th>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
<tbody>
<tr>
<td>Some high school</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Completed School certificate, year 10</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Completed HSC, year 12</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>TAFE certificate or diploma, including trade certificate</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>University or tertiary institute degree or higher</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>
4. DISCUSSION

4.1.1 PATIENT SAMPLE

Of the 93 subjects with 22q11 deletion syndrome invited, 21 indicated that they would like an appointment to participate. The participation rate of 23% could be considered low, however the invited group were geographically diverse with a number of the subjects living in country NSW and outside the Sydney West Area Health Service, making it difficult for them to attend. Another possible reason for the low participation rate may be that this group of patients are required to attend numerous medical appointments and therefore an additional appointment at the dental department did not fit into their schedule. Therefore the study group may be biased towards parents or carers who have good dental awareness, or to subjects that were experiencing dental problems. Whilst this may have an impact on a number of the study variables, it would be expected that the measurement of salivary function is independent of this bias. The small number of subjects in the group also impacted on the statistical analysis, and a larger cohort would have made analysis more reliable. However, considering the rarity of the syndrome, a study group size of 21 could be considered reasonable and is similar to the cohort size in Klingberg et al. (2007).

An age- and sex-matched control group of healthy children was planned as part of the initial protocol. The Ethics Committee at the Children’s Hospital at Westmead specified that the control group was to come from the same population as the study group (The Children’s Hospital at Westmead) in order to avoid population bias. The
author attempted for over one year to obtain suitable patients, however it was not possible due to the lack of healthy patients at The Children's Hospital at Westmead willing to participate.

4.1.2 DENTAL EXAMINATION

The findings of the dental examination indicate that the majority of the group (53%) had high decay scores (dmft/DMFT >6) (see Table 3.1). The mean dmft or DMFT for the group ranged from 4.44-6.26 times the NSW average depending on age (see Table 3.3).

Missing teeth due to caries (m+M ≥ 1) was present in 33.0% of the study group (ages 5-15), compared to 2.7% reported in NSW. Filled teeth (f+F ≥ 1) were present in 61.9% of the study group compared to 19.5% reported in NSW (Armfield et al. 2003). Untreated decay (d+D ≥ 1) was present in 52.4% of the study group, which is higher than the maximum reported for NSW in any age group (31.8% for 7 years olds) (Armfield et al. 2003). However, the proportion of decayed teeth to the total decay score (d/dmft or D/DMFT) was less than the NSW average for all ages in the study group (see Table 3.3). This indicates that the study group has a high level of untreated decay, but an even greater proportion of treated (missing and filled teeth) decay. These findings may reflect both a high caries activity and large amounts of previous treatment. Seventy-six percent of the study group had seen a dentist in the six months prior to the examination, however decay had been left untreated in 56% of these patients. This may reflect the difficult behaviour displayed by many of the study group
during the dental examination and salivary function testing. It would be expected that completing dental work in the chair in this group of children is difficult and the majority of the study group would require a general anaesthetic to complete dental treatment. As many of the group are medically compromised, general anaesthesia may need to be carried out with paediatric support and appropriate hospital facilities. These patients are therefore limited to services with extensive waiting lists.

Whilst average decay experience scores for a population provides good summary statistics, they can hide the existence of people within that population who have considerable decay experience (Armfield et al. 2006). The Significant Caries Index (SiC) was designed to bring attention to those individuals with the highest scores in a population. The SiC is the average dmft/DMFT of the 30% of the population with the highest decay scores. A modified index, the SiC$^{10}$, is the average dmft/DMFT of the 10% of children with the highest decay scores (Armfield et al. 2006). Use of this data allows comparison of the study group to the subjects in the population who carry a high burden of disease. For 5-8 year olds in Australia (2001) the average dmft in the 30% of the population with the highest decay scores was 5.4 (Armfield et al. 2006). The average dmft for 5-8 year olds in the study group was 4.7. The SiC (dmft) for 9-10 year olds was 4.6, compared to an average dmft of 4.2 in the study group. The SiC (DMFT) for 12-15 year olds was 4.1, compared to the average DMFT for 12-15 year olds in this study which was 4.4. Therefore the caries prevalence in the study group is similar to that which occurs in the 30% of the population with the highest decay scores.
When compared to NSW data, the proportion of patients who were caries free was lower in the study group, except for permanent teeth in 9-12 years olds. In this age group, 85.7% of the study group were caries free compared to 79.9% in the NSW population. However, the dmft scores for this age group were over 6 times higher than the NSW average. These results may be due to delayed dental development leading to more deciduous and less permanent teeth being present in this age group. Delayed eruption has been reported in Klingberg et al. (2002), however dental development was not investigated in this study.

Comparison of caries data in the study group to national and NSW population data is useful as a guide. However, in order to confirm the increased prevalence of dental caries found in this study, an age- and sex-matched control group from a similar population would be required. There are a number of confounding factors that need to be controlled for, including the presence of cardiac disease, which has been reported to result in increased decay rates (Stecksen-Blicks et al. 2004) and an increased proportion of untreated decay (Franco et al. 1996). In this study, there was no statistically significant increase in the decay scores, or the amount of untreated decay (d+D) in the children with cardiac disease. This may reflect the small sample size, however it may also indicate that cardiac disease is not a major contributing factor to the development of caries in this group. Comparison of children with 22q11 deletion syndrome to a control group with cardiac disease in addition to a healthy control group would allow investigation of this hypothesis.

The presence of enamel defects, even at a subclinical level, has also been implicated in
the development of early childhood caries (Bell 2005; Pascoe et al. 1994). Whilst enamel defects were not analysed in this study, Klingberg et al. (2002) found a high prevalence of subjects with hypoplastic and hypomineralised enamel in their study group. Most likely this was due to a high incidence of medical complications during the peri- and post-natal period. Controlling for this confounding factor in the development of caries would be beneficial in this study group, especially for the younger children in the study.

The results of this study cannot be compared to many of the results in the study by Klingberg et al. (2002) due to several differences in methodology. Adults were included in their analysis, subjects up to the age of 19 years were regarded as children and missing teeth were not recorded. Therefore, the prevalence of decay cannot be compared across studies. However, Klingberg et al. (2002) found the children in their study group had an average of 1.8 carious teeth each, which is similar to the findings of this study (average of 1.5 carious teeth each). A larger proportion of their study group was caries free (53%), compared to this study (29%). When the caries free children in their study group were removed from the analysis, the remainder had an average of 9.2 carious or filled teeth (dft+DFT), compared to 5.1 for this study. The results of this study also revealed that a number of carious lesions occurred in unusual locations such as lower incisors, buccal surfaces of molars and cusp tips which is similar to the findings in Klingberg et al. (2002).
The pilot study conducted by Hibbert et al. (2004) examined 22 children for decay scores. The study group had an age range of 5-16 years (mean 10.6 years), which is similar to the age range of subjects investigated in this study. Their subjects had a mean dmft of 7.7, with a range of 0-20. The mean DMFT was 3.9, with a range of 0-13. The results of this study found the mean dmft was 4.2, with a range of 0-11 and the mean DMFT was 1.4 with a range of 0-7. Therefore the decay scores found in the pilot study were higher than those found in this study.

4.1.3 SALIVARY FUNCTION TESTING

Salivary function testing was complicated by the limited co-operation in the group. Whilst attempts were made to standardise procedures at all stages, the behaviour profile of this group of children has undoubtedly impacted on the collection of data. In particular, some children had difficulty not swallowing during the collection of unstimulated saliva. It was also difficult to standardise the number of chewing strokes per minute during the stimulated saliva testing, as some children had difficulty chewing the paraffin wax and co-ordinating spitting into the collection cup. The majority of the patients were young (mean age 9.7), and most were extremely nervous during the examination and saliva testing, however the impact of this is difficult to quantify.
4.1.3.1 Clinical presentation of saliva

Whilst there was no statistically significant correlation between the clinical appearance of saliva and flow rates, the subjects with ‘frothy’ and ‘ropy’ saliva had lower average unstimulated flow rates, stimulated flow rates and buffering capacity compared to the subjects with ‘normal’ saliva. This may indicate that the clinical appearance of saliva may a useful indicator of salivary hypofunction, however a larger sample size would be required to confirm this by statistical analysis.

4.1.3.2 Unstimulated flow rates

The traditional 5 minute drooling test for unstimulated saliva was modified to a suction device in order to minimise the need for patient co-operation. This technique results in increased flow rates, probably due to the stimulation by the suction device (Navazesh et al. 1982). Unstimulated flow rates have not previously been reported in the literature for subjects with 22q11 deletion syndrome and therefore comparisons between studies are not possible. The unstimulated flow rate should be considered essential in the diagnosis of salivary hypofunction, and indeed the use of sialometry as one of the diagnostic criteria for Sjögren syndrome includes only the use of unstimulated flow rates (Vitali et al. 1996).

Eighteen of the 21 patients were co-operative enough to complete the unstimulated saliva test. Eight patients (44%) had very low unstimulated flow rates (≤0.1 mL/min) and could be considered the have salivary hypofunction. Two patients (1 male, 1 female) had unstimulated flow rates of ≤0.16 mL/min which could be considered
borderline hypofunction. The remainder of the patients could not be diagnosed with salivary hypofunction even though some were below average for their age.

Considering the relative consistency of unstimulated flow rates for patients over the age of 10 years, the use of adult values for hypofunction was considered reasonable. However, for patients less than 10 years of age who were diagnosed with hypofunction on the basis of unstimulated flow rates (3 patients), these results should be interpreted with caution. More normative data on unstimulated flow rates is required for patients aged 5-10 years to allow application of this cut-off value with more certainty. The use of a cut-off value of \( \leq 0.1 \text{ mL/min} \) is lower than some cut-off values reported for use in adults (Navazesh et al. 1992), however the use of this more conservative estimate is in accordance with values suggested as a diagnostic criteria for Sjögren syndrome.

The mean unstimulated flow rate for the group (0.21±0.20 mL/min) is similar to that reported in a group of paediatric bone marrow transplant patients (0.24±0.17 mL/min) (Bagesund et al. 2000). Twenty-six percent of this group had flow rates of \( <0.1 \text{ mL/min} \). Therefore the prevalence of salivary hypofunction diagnosed on the basis of unstimulated flow rates in the study group (44%) is higher than that reported in bone marrow transplant patients who have undergone total body irradiation.

4.1.3.3 Stimulated flow rates

The stimulated flow rates in the study group were significantly correlated to the unstimulated flow rates \( (r = 0.63, \ p<0.01) \), which is similar to findings in adult subjects (Heintze et al. 1983).
The adult value for hypofunction is a stimulated flow rate of <0.7 mL/min (Navazesh et al. 1992), however this could only be considered appropriate for patients ≥ 13 years due to age and sex related variations. A cut-off value for a stimulated flow rate of <0.5 mL/min has been used previously in groups of paediatric oncology patients (Bagesund et al. 2000; Dahllöf et al. 1997). Considering that values of <0.5 mL/min are within normal limits for 5,6 and 7 year olds, and 8 and 10 year old girls (Andersson 1972; Crossner 1984), it was considered inappropriate to use this value. The use of this value may lead to incorrect diagnosis some of the younger children with salivary hypofunction. Therefore the use of data from the general population (see Figure 1.5) was used in order to compare the study group to age- and sex-appropriate percentile bands for stimulated flow rates. It is uncertain whether this data (Andersson 1972; Crossner 1984) is derived from a normal population, however the number of subjects in these studies was large, and these studies were considered the best available for comparison. The use of the 5th centile band as the cut-off value for hypofunction is an arbitrary decision, however it was considered that the flow rate values below this centile band were well below the mean ± SD for each age and sex. The values between the 5th-10th centile were chosen for the diagnosis of borderline hypofunction and these values were also outside the mean ± SD for all age groups. The use of values ± 2 SD from the mean as a cut-off for hypofunction was not possible due to the large variation in the population.

Eleven subjects (69%) (5 males and 6 females) had stimulated flow rates ≤5th centile for their age group. Two subjects (1 male, 1 female) fell in the 5th-10th centile which could be considered borderline hypofunction. The mean of the group was 0.39 ± 0.31
mL/min which is lower than the mean described by Klingberg et al. (2007) of 0.71 ± 0.46 mL/min, however their sample included adults.

With the exception of one patient, all patients diagnosed with salivary hypofunction on the basis of unstimulated flow rates were also diagnosed on the basis of stimulated flow rates. This one subject was unable to co-operate for stimulated saliva sampling. Four patients (3 males, 1 female) were diagnosed with salivary hypofunction on the basis of stimulated flow alone.

Due to the large variation of salivary parameters in the population, it is uncertain whether age and sex-matched controls would have contributed to being able to make a diagnosis of salivary hypofunction in the study group.

4.1.3.4 Buffering capacity

For the estimation of buffering capacity, the acid-impregnated strips in the GC™ kits were used. Initially the protocol for the study stated that titration would be used, however the lack of saliva volume in most patients meant that this had to abandoned. In addition, it was felt that titration would not have any benefits over the buffering capacity strips. This is mainly due to the inaccuracy of determining buffering capacity outside of a closed system which is required to prevent loss of carbon dioxide (Bardow et al. 2004). Buffering capacity was significantly correlated to the stimulated pH (r = 0.56, p<0.05), however it was not significantly correlated to either the stimulated flow rate or the decay scores.
A high proportion of the group had very low buffering capacity (56%) or low buffering capacity (31%). It is generally accepted that buffering capacity is correlated to the stimulated flow rate (Heintze et al. 1983), however in this group there was no correlation. Very low buffering capacity was present in a number of children who had some of the high flow rates, in fact the two patients with the highest flow rates had low buffering capacity. Whilst the sample size was too small for reliable statistical analysis, this may suggest that there may be another variable besides flow rate contributing to the low buffering capacity in this group of children. Direct comparison to Klingberg et al. (2007) is difficult due to the use of a different brand of buffering capacity strip. However, similar results were found, with 54% presenting with low buffering capacity, 32% intermediate and 14% high buffering capacity.

4.1.3.5 Unstimulated and stimulated pH values

Only one patient in the study group had very low pH values, and this was for stimulated saliva. Interestingly, the unstimulated pH was significantly correlated to the unstimulated flow rate, and the stimulated pH was significantly correlated to the stimulated flow rate. These results need to be interpreted with caution, as measurement of salivary pH is difficult due to loss of CO₂ as discussed in 1.5.2.4. Since salivary pH is dependent on the buffering capacity and therefore the flow rate, its measurement should be considered secondary to these parameters in making a diagnosis of salivary hypofunction. There was no statistically significant difference between the sexes, which is in agreement with the findings in Andersson (1972).
4.1.3.6 Comparison to the literature on 22q11 deletion syndrome

There are only two studies in the literature on salivary function in 22q11 deletion syndrome (Hibbert et al. 2004; Klingberg et al. 2007). Klingberg et al. (2007) focussed on detecting differences in the composition of saliva (proteins and electrolytes) between the study group and controls. Unfortunately, from a diagnostic point of view, the concentration of electrolytes in saliva is highly variable both between and within subjects. In addition, it is rare for either oral or systemic conditions to affect the concentration of any particular ion. Only sodium and chloride have been linked to glandular pathology (Tenovuo 1989a).

Stimulated flow rates and buffering capacity were also examined in Klingberg et al. (2007), where the average flow rate for their study group was compared to the average flow rate in a control group (Klingberg et al. 2007). The stimulated flow rate was found to be significantly lower in the study group. However, it could be considered inappropriate to average the flow rates of such a wide age range of patients (6-36 years) due to the age- and sex- related variations discussed previously. In addition, the patients who could not produce enough saliva volume to carry out the laboratory based saliva analysis were excluded from their study, resulting in selection bias. It was not mentioned if any of the study group were taking medications, and whether this was controlled for. Considering the high prevalence of mental illness in patients with 22q11 deletion syndrome (Goldmuntz 2005), it is possible that a number of adults in the group may have been taking medications that have an impact on salivary flow rates (Bardow et al. 2004).
Hibbert et al. (2004) carried out sialometry using the GC™ Saliva Check® kits on 12 of the 24 children in the pilot study. It was found that stimulated salivary flow rate was low to very low in 83% of subjects. Buffering capacity was low to very low in 58% of subjects and unstimulated salivary pH was found to be moderately or highly acidic in 77% of subjects. The results regarding the salivary pH is in contrast to the findings of this study and highlights the variability in this parameter, and therefore its reduced value in the diagnosis of salivary hypofunction.

Six children in the pilot study had previously had scintiscans performed as part of their normal clinical care, which showed markedly reduced salivary function. Four of these children had markedly reduced function in all major salivary glands, and two had reduced function in parotid glands only.

The proportion of children diagnosed with salivary hypofunction on the basis of stimulated flow rates in the pilot study is higher than in this study. In the pilot study, the values that were used for diagnosis of hypofunction were those suggested by the Saliva Check® kits. These values are based on adult flow rates and this is likely to have resulted in a number of the younger children being incorrectly diagnosed with salivary hypofunction.

4.1.4 QUESTIONNAIRE

The prevalence of cardiac anomalies in the study group (67%) is less than reported in the literature (75-85%) (Goldmuntz 2005). As discussed above, there was no
correlation between the presence of a cardiac condition and decay scores. Five children (23.8%) were taking various medications for medical conditions. A number of these have been linked to decreased salivary function, including Respiradone (an antipsychotic) and Cipramil (a selective serotonin re-uptake inhibitor). A number of patients were also taking Ritalin, which has been linked anecdotally to decreased salivary function, however there is no evidence for this in the literature. The patients taking medications did not have significantly lower unstimulated flow rates, stimulated flow rates or buffering capacity. Whilst no statistical significance could be established, this may be due to the small sample size. It should be kept in mind that the use of such medications may be a contributing factor to salivary hypofunction in a small number of patients in the study group.

An IgA deficiency was present in only 1 patient (5%) which is less than the reported prevalence of 13% in the literature (Smith et al. 1998). Thymus aplasia was present in four subjects and hypoparathyroidism in one subject, which may have implications for calcium metabolism and therefore enamel defects as discussed by Klingberg et al. (2005). As reported in the literature, otitis media was a common finding, and was present in 53% of the study group. Velopharyngeal abnormalities were present in 78% of the study group, which is similar to the figures reported by Goldmuntz (2005) and Shprintzen (2000a) (75-81%). A submucous cleft was present in 14% which is much lower than reported by Shprintzen (2000) of 44%.

The subjects that participated in this research project may be skewed in regards to their
dental awareness and/or known dental problems in the child. Without a control group it is not possible to establish the dental awareness of the group, however it appears that most patients attended the dentist regularly, with the majority having had a check-up in the last 6 months. There were, however, a number of subjects in the group who were irregular attenders, and one had never seen a dentist prior to the examination for this research. Brushing was commenced prior to the age of 2 years for 76% of the study group, however only 62% brushed their teeth twice per day. Considering the high decay scores present in the group, these findings may mean that there is further scope for education and an emphasis on preventive dentistry.

Analysis of early childhood feeding practices revealed that 62% of the group had been breastfed, compared to 90% reported in the general NSW population ('New South Wales Child Health Survey 2001'). This may reflect feeding difficulties reported in children with 22q11 deletion syndrome (Robin et al. 2005). The figures for duration of breastfeeding were similar to those reported in the NSW population ('New South Wales Child Health Survey 2001'). A number of subjects were given drinks from a bottle which included fruit juice, cordial and soft drinks. In addition, 14% of patients could be considered to have had an increased duration of bottle feeding (>36 months). These factors have been linked to an increased prevalence and severity of early childhood caries (Hallett et al. 2003), however this variable was not analysed due to difficulty established the prevalence of early childhood caries in the older subjects in the group. The consumption of juice or any other sugar-containing beverage on a daily basis was not correlated with the dmft/DMFT scores.
Whilst analysis of the social demographics was not possible due to a lack of a control group, all patients and the majority of parents were born in Australia. Sixty-seven percent of parent had completed some form of tertiary education.

4.1.5 SALIVARY GLAND HYPOFUNCTION IN 22q11 DELETION SYNDROME.

It has been hypothesised that a deletion at 22q11 disrupts either the neural crest cells, or the cells with which they interact, at a critical phase of organogenesis (Scambler 2000). The affected structures are those derived from the branchial arch and pharyngeal pouch system (Maynard et al. 2002; Scambler 2000). Murine studies have established that the pharyngeal pouches are absent, which is postulated to result in disordered migration of neural crest cells into the pharyngeal arches (Baldini 2002).

The normal development of salivary glands depends on secondary induction, which requires the presence of mesenchyme for the initiation, growth and branching morphogenesis of glandular tissue. Since the mesenchyme surrounding the developing salivary glands is derived from neural crest cells, it could be hypothesised that a disturbance in the development of the salivary glands is due to the disordered neural crest cell migration. Considering the wide phenotype found in patients with 22q11 deletion syndrome, it would be expected that the degree of developmental disturbance of the salivary glands would be variable. This is in keeping with the results of this study, where the majority of the subjects showed some form of salivary hypofunction,
however there were a number who appeared to have normal glandular function. To analyse the structure and function of the salivary glands in patients with 22q11 deletion syndrome, further testing would be required, and may include scintigraphy, sialography and biopsy of glandular tissue.
5. CONCLUSION

5.1.1 THE PREVALENCE OF DENTAL CARIES

The mean dmft/DMFT for the group was 4.8 (SD=3.9), which was 4.44–6.26 times the NSW average depending on age. High levels of decay (dmft/DMFT ≥ 6) were present in 53% of the group, and 29% were caries free. Untreated decay was present in 52.4% of the study group, missing teeth due to caries in 33.0%, and filled teeth in 61.9% of the study group. These levels were all higher than NSW averages, indicating both a high level of untreated and treated decay in the study group. The prevalence of caries in this cohort is similar to the decay scores reported in the most severely affected 30% of the Australian population. A control group is required to confirm these findings.

5.1.2 SALIVARY FUNCTION IN 22q11 DELETION SYNDROME

Twelve patients (66.7%) out of those who were able to produce a saliva sample were diagnosed with salivary hypofunction. Seven (58.3%) of these were diagnosed on the basis of both low unstimulated and stimulated flow rates, four subjects (33.3%) by stimulated flow rate only, and one subject (8.3%) by unstimulated flow rate only. Three subjects (16.7%) had flow rates that could be considered borderline for a diagnosis of salivary hypofunction.
5.1.3 PARAMETERS FOR THE DIAGNOSIS OF SALIVARY HYPOFUNCTION IN CHILDREN

Unstimulated flow rates of $\leq 0.1$ mL/min as the basis for diagnosing salivary hypofunction can be considered reasonable for subjects over the age of 10 years. Whilst unstimulated flow rates are similar to adults for patients less than 10 years, the application of this cut-off value should be carried out with caution. More data is required on normal unstimulated flow rates in this younger age group of children. Therefore in children under 10 years of age, the whole clinical picture is of increasing importance in making a diagnosis of salivary hypofunction.

Diagnosis of salivary hypofunction on the basis of stimulated flow rates in children using adult values could be considered inappropriate for patients $\leq 13$ years. The normative data from the literature was assessed by the statistician, and it was decided that due to the large variance in the normative data it was not feasible to provide an arbitrary cut-off point such as $\pm 2$ SD from the mean. Therefore, it was not possible to establish a limit for normal function in children based on the stimulated flow rate data in the literature. In this study, a comparison was made to age- and sex- appropriate percentile bands for stimulated flow rates. A value of $\leq 5^{th}$ centile for the subject’s age and sex was considered salivary hypofunction. A value of between $5^{th}$-$10^{th}$ centile was considered borderline salivary hypofunction. Both of these percentile bands were well outside the mean $\pm 1$ SD.
5.1.4 FUTURE DIRECTIONS

Further investigations into the oral health and salivary function in 22q11 deletion syndrome should be undertaken in a larger cohort of children with a control group for comparison. Salivary gland scans could be carried out to further quantify salivary gland hypofunction in the group. The type of glands affected could also be evaluated by this method. Whilst this is a more invasive test, it is not subject to the many variables associated with sialometry in a group of children with a difficult behaviour profile. A control group would allow comparisons of decay scores, dental awareness, oral hygiene practices, social demographics and diet.

In order to diagnose children with salivary hypofunction through the use of sialometry, age- and sex-appropriate limits for normal function need to be established. Ideally, this would involve correlation of salivary flow rates with the results of salivary gland scans and establishing normal flow rates in children.
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## APPENDIX A

### Unstimulated Flow Rates:

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number</th>
<th>Sex</th>
<th>Flow rate (mL/min)</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>60</td>
<td>Mixed</td>
<td>0.22 ± 0.14</td>
<td>5 min draining</td>
<td>Watanabe et al. (1990)</td>
</tr>
<tr>
<td>5.3 ± 0.8</td>
<td>31</td>
<td>Mixed</td>
<td>0.25 ± 0.27</td>
<td>3 min draining</td>
<td>Bretz et al. (2001)</td>
</tr>
<tr>
<td>5.3 ± 0.7</td>
<td>111</td>
<td>Mixed</td>
<td>0.37 ± 0.28</td>
<td>3 min draining</td>
<td>Bretz et al. (2001)</td>
</tr>
<tr>
<td>5.4 ± 0.8</td>
<td>100</td>
<td>Mixed</td>
<td>0.48 ± 0.37</td>
<td>3 min draining</td>
<td>Bretz et al. (2001)</td>
</tr>
<tr>
<td>5.5 ± 0.9</td>
<td>90</td>
<td>Mixed</td>
<td>0.34 ± 0.23</td>
<td>3 min draining</td>
<td>Bretz et al. (2001)</td>
</tr>
<tr>
<td>6.1±1.2</td>
<td>46</td>
<td>Mixed</td>
<td>0.19 ± 0.15</td>
<td>3 min draining</td>
<td>Bretz et al. (2001)</td>
</tr>
<tr>
<td>10</td>
<td>53</td>
<td>Boys</td>
<td>0.39 ± 0.19</td>
<td>5 min spitting</td>
<td>Anderson (1972)</td>
</tr>
<tr>
<td>10</td>
<td>47</td>
<td>Girls</td>
<td>0.38 ± 0.22</td>
<td>5 min spitting</td>
<td>Anderson (1972)</td>
</tr>
<tr>
<td>13</td>
<td>49</td>
<td>Boys</td>
<td>0.46 ± 0.24</td>
<td>5 min spitting</td>
<td>Anderson (1972)</td>
</tr>
<tr>
<td>13</td>
<td>47</td>
<td>Girls</td>
<td>0.33 ± 0.18</td>
<td>5 min spitting</td>
<td>Anderson (1972)</td>
</tr>
<tr>
<td>15-29</td>
<td>84</td>
<td>Male</td>
<td>0.34</td>
<td>5 min draining</td>
<td>Heintze et al. (1983)</td>
</tr>
<tr>
<td>15-29</td>
<td>95</td>
<td>Female</td>
<td>0.25</td>
<td>5 min draining</td>
<td>Heintze et al. (1983)</td>
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### Stimulated Flow Rates:

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Mean ± SD</th>
<th>Min</th>
<th>Percentiles</th>
<th>Max</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5 10 25 50 75 90 95</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Boys</td>
<td>0.67±0.38</td>
<td>&gt;0.1</td>
<td>0.1 0.2 0.4 0.6 0.9 1.3 1.3</td>
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</tr>
<tr>
<td>5</td>
<td>Girls</td>
<td>0.52±0.34</td>
<td>&gt;0.1</td>
<td>0.1 0.1 0.6 0.4 0.7 1.0 1.2</td>
<td>1.4</td>
</tr>
<tr>
<td>6</td>
<td>Boys</td>
<td>0.91±0.44</td>
<td>&gt;0.1</td>
<td>0.3 0.4 0.6 0.9 1.2 1.5 1.7</td>
<td>2.5</td>
</tr>
<tr>
<td>6</td>
<td>Girls</td>
<td>0.77±0.41</td>
<td>0.1</td>
<td>0.3 0.3 0.4 0.7 1.0 1.3 1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>7</td>
<td>Boys</td>
<td>1.09±0.65</td>
<td>0.2</td>
<td>0.3 0.4 0.6 1.0 1.5 1.8 2.3</td>
<td>3.5</td>
</tr>
<tr>
<td>7</td>
<td>Girls</td>
<td>0.80±0.42</td>
<td>0.2</td>
<td>0.3 0.3 0.5 0.7 1.1 1.4 1.4</td>
<td>2.1</td>
</tr>
<tr>
<td>8</td>
<td>Boys</td>
<td>1.17±0.53</td>
<td>0.2</td>
<td>0.3 0.5 0.7 1.2 1.5 1.9 2.1</td>
<td>3.3</td>
</tr>
<tr>
<td>8</td>
<td>Girls</td>
<td>0.87±0.50</td>
<td>0.1</td>
<td>0.2 0.3 0.5 0.8 1.2 1.6 1.8</td>
<td>2.2</td>
</tr>
<tr>
<td>10</td>
<td>Boys</td>
<td>1.23±0.49</td>
<td>0.3</td>
<td>0.5 0.6 0.9 1.2 1.5 2.1 2.2</td>
<td>2.4</td>
</tr>
<tr>
<td>10</td>
<td>Girls</td>
<td>1.01±0.54</td>
<td>0.2</td>
<td>0.3 0.3 0.6 0.9 1.2 1.6 2.0</td>
<td>3.1</td>
</tr>
<tr>
<td>13</td>
<td>Boys</td>
<td>1.90±0.48</td>
<td>0.7</td>
<td>0.9 1.1 1.4 1.8 2.4 3.0 3.2</td>
<td>3.4</td>
</tr>
<tr>
<td>13</td>
<td>Girls</td>
<td>1.59±0.67</td>
<td>0.3</td>
<td>0.6 0.7 1.0 1.6 2.1 2.5 2.6</td>
<td>3.1</td>
</tr>
<tr>
<td>14</td>
<td>Boys</td>
<td>2.25±0.90</td>
<td>0.3</td>
<td>0.9 1.1 1.5 2.2 2.8 3.5 3.7</td>
<td>4.5</td>
</tr>
<tr>
<td>14</td>
<td>Girls</td>
<td>1.83±0.76</td>
<td>0.3</td>
<td>0.7 0.8 1.3 1.8 2.2 2.5 3.0</td>
<td>4.5</td>
</tr>
<tr>
<td>15</td>
<td>Boys</td>
<td>2.24±0.96</td>
<td>0.5</td>
<td>1.0 1.2 1.5 2.0 2.6 3.2 4.2</td>
<td>5.4</td>
</tr>
<tr>
<td>15</td>
<td>Girls</td>
<td>1.92±0.70</td>
<td>0.7</td>
<td>0.9 1.1 1.4 1.9 2.1 2.8 3.3</td>
<td>3.8</td>
</tr>
</tbody>
</table>

*Modified from Crossner (1984)
APPENDIX C

Questionnaire

Please complete this short questionnaire on behalf of your child. Please answer the following questions by ticking the most appropriate answer unless directed otherwise.

(i) Today's date__/__/____
(ii) Date of birth__/__/____
(iii) Sex: Female ☐ / Male ☐
(iv) Age: ________years________months

1. What is your relation to the child?
   (a) Mother________________________☐
   (b) Father________________________☐
   (c) Stepmother____________________☐
   (d) Stepfather____________________☐
   (e) Grandmother___________________☐
   (f) Grandfather___________________☐
   (g) Legal guardian________________☐
   (h) Other (please specify)____________

2. What was the length of the pregnancy?
   (a)________weeks

3. What was your child's birthweight?
   (a)______pounds______ounces OR
   (b)________grams

4. Did your child have a general anaesthetic, or intubation (help breathing with a tube) before the age of 12 months?
   (a) General anaesthetic___________☐
   (b) Intubation____________________☐

5. Did your child have a prolonged or serious illness or infection under the age of 3 years?
   (a) Yes (Go to qu. 6)_______________☐
   (b) No (Go to qu. 7)________________☐

6. Please tick all of the illnesses that apply for under the age of 3 years.
   (a) Vitamin D deficiency___________☐
   (b) Hypoparathyroidism____________☐
   (c) Kidney disease________________☐
   (d) Calcium deficiency____________☐
   (e) Rickets_______________________☐
   (f) Liver disease__________________☐
   (g) Cardiac condition______________☐
   (h) Fevers________________________☐
   (i) Cancer or leukaemia____________☐
   (h) Rubella_______________________☐
   (i) Measles_______________________☐
   (k) Chicken Pox___________________☐
   (l) Pneumonia____________________☐
   (m) Gastrointestinal infections_______☐
   (n) Other________________________☐

7. Has your child ever been breastfed?
   (a) Yes (Go to Qu 8)_______________☐
   (b) No (Go to Qu 9)________________☐
   (c) Don't know___________________☐

8. Age at which breast feeding was stopped?
   (a) 0-6 months____________________☐
   (b) 6-14 months___________________☐
   (c) 15-23 months__________________☐
   (d) 24-36 months__________________☐
   (e) Above 36 months_______________☐
9. Was your child given infant formula regularly (at least once per day)?
   (a) Yes (Go to Qu 10)__________________ □
   (b) No (Go to Qu 11)__________________ □
   (c) Don't know________________________ □

10. Age at which formula feeding was stopped?
    (a) 0-6 months________________________ □
    (b) 6-14 months________________________ □
    (c) 15-23 months________________________ □
    (d) 24-36 months________________________ □
    (e) Above 36 months____________________ □

11. Was your child given any other type of drink from a bottle regularly?
    (a) Yes (Go to Qu 12)__________________ □
    (b) No (Go to Qu 14)__________________ □
    (c) Don't know________________________ □

12. What type of drink was used in the bottle? (please tick all that apply)
    YES NO
    (a) Cow's milk________________________ □ □
    (b) Water_____________________________ □ □
    (c) Fruit juice_________________________ □ □
    (d) Cordial___________________________ □ □
    (e) Soy milk_________________________ □ □
    (f) Sugar free juice or cordial_______ □ □
    (g) Soft drink________________________ □ □
    (h) Other___________________________ □ □

13. At which age was bottle feeding stopped?
    (a) 0-6 months________________________ □
    (b) 6-14 months________________________ □
    (c) 15-23 months________________________ □
    (d) 24-36 months________________________ □
    (e) Above 36 months____________________ □

14. How often does your child eat sweets (including chocolates)?
    (a) ______ times per day □
    (b) ______ times per week □
    (c) Not at all________________________ □

15. How many cups of fruit juice does your child usually drink in a day? (1 cup = 250ml, a household tea cup or a popper)
    (a) ______ cups per day □
    (b) ______ cups per week □
    (c) Doesn't drink fruit juice__________________ □
    (d) Don't know________________________ □

16. How many cups of soft drink, cordial or sports drink does your child usually drink in a day?
    (a) ______ cups per day □
    (b) ______ cups per week □
    (c) Doesn't drink fruit juice__________________ □
    (d) Don't know________________________ □

17. How often did your child eat citrus fruit per day (eg. oranges, lemons, grapefruit)? 1 serve = 1-2 pieces of fruit
    (a) ______ serves per day □
    (b) ______ serves per week □
    (c) Doesn't eat citrus fruits__________________ □

18. At what age did your child first attend the dentist?
    (a) ______ months □
    (b) ______ years □
    (c) Never attended________________________ □

19. How long ago did your child see a dental professional about his/her teeth or gums?
    YES NO
    (a) Less than 6 months ago________ □ □
    (b) Less than 12 months ago________ □ □
    (c) One to two years ago___________ □ □
    (d) Two to four years ago___________ □ □
    (e) Never attended____________________ □ □

20. Where was your child's last dental visit made at?
    (a) Private dental practice__________ □
    (b) School dental service___________ □
    (c) Other government or public dental clinic__ □
    (d) Health fund dental clinic________ □
    (e) Any other place (please specify)________ □
    (f) Never attended dentist__________ □
21. What type of dental treatment did your child have in the last 12 months?

(a) Fillings
(b) Tooth removed
(c) Check-up
(d) Fluoride treatment
(e) Cleaning
(f) Braces
(g) Treatment of an injury
(h) None
(i) Other treatment (please specify)

22. At what age did your child start brushing his/her teeth?

(a) 6-12 months
(b) 1-2 years
(c) 2-3 years
(d) 3-4 years
(e) 4-5 years
(f) Over 5 years
(g) Doesn’t brush yet

23. How often does your child brush their teeth at present?

(a) ________ times per day
(b) ________ times per week
(c) ________ times per month
(d) Not at all

24. How often do you help your child to brush his/her teeth?

(a) ________ times per day
(b) ________ times per week
(c) ________ times per month
(d) Not at all

25. Does your child use toothpaste when brushing?

(a) Every time
(b) Nearly always
(c) Hardly ever
(d) Not at all

26. What type of toothpaste does your child usually use?

(a) Junior toothpaste
(b) Adult toothpaste
(c) Fluoride free toothpaste
(d) If uncertain please indicate brand name (eg Colgate Milk Teeth)

27. Have you ever given your child fluoride supplements eg tablets, mouthrinses?

(a) Yes
(b) No

28. How often does your child usually floss their teeth?

(a) More than once a day
(b) Once a day
(c) Several times a week
(d) Once a week
(e) Less than once a week
(f) Never

29. Do you live in an area with fluoridated water?

(a) Yes
(b) No
(c) If uncertain, please indicate area

30. What country was your child born in?

(a) Australia
(b) Other country (please specify)

31. What country was the child’s mother/stepmother born in?

(a) Australia
(b) Other country (please specify)

32. What country was the child’s father/stepfather born in?
33. What language do you usually speak at home?
(a) Language (specify) ____________________

34. What is the highest level of education the child’s mother or stepmother has completed?
(a) Never attended school ____________ □
(b) Completed primary school ____________ □
(c) Some primary school (not completed) ____________ □
(d) Some high school ____________ □
(e) Completed School Certificate - Intermediate - year 10/4th form ____________ □
(f) Completed HSC - Year 12 - Leaving - 6th form ____________ □
(g) TAFE certificate or Diploma, including trade certificate ____________ □
(h) University, CAE or some other tertiary institute degree or higher ____________ □
(i) Other (please specify) ____________________ □

35. What is the highest level of education the child’s father or stepfather has completed?
(a) Never attended school ____________ □
(b) Completed primary school ____________ □
(c) Some primary school (not completed) ____________ □
(d) Some high school ____________ □
(e) Completed School Certificate - Intermediate - year 10/4th form ____________ □
(f) Completed HSC - Year 12 - Leaving - 6th form ____________ □
(g) TAFE certificate or Diploma, including trade certificate ____________ □
(h) University, CAE or some other tertiary institute degree or higher ____________ □
(i) Other (please specify) ____________________ □

36. How many hours does your child’s mother or stepmother usually work or study, away from home, each week?
(a) ____________ hours per week
(b) Don’t know ____________ □

37. How would you describe your child’s father or stepfather’s current employment status?
(a) Employed full-time (including self-employed) □
(b) Employed part-time (including self-employed) □
(c) Unemployed ____________ □
(d) Home duties ____________ □
(e) Student and working ____________ □
(f) Student and not working ____________ □
(g) Retired ____________ □
(h) Unable to work due to health problems ____________ □
(i) Other (specify) ____________________ □
(j) Don't know ____________ □

38. Do you or your child receive any of the following benefits?
(a) Disability support ____________ □
(b) Unemployment benefits or Job Search Allowance ____________ □
(c) Sickness benefits or Allowance ____________ □
(d) Parenting payment ____________ □
(e) Family allowance ____________ □
(f) Age pension ____________ □
(g) Repatriation Pension or Service Pension ____________ □
(h) Other type of benefit (please specify) ____________________ □
(i) No benefits ____________ □

39. What suburb do you live in?
(a) ____________ (suburb) □
(b) Don’t know ________ □

40. Is your child of Aboriginal or Torres Strait Islander origin?
(a) Aboriginal but not Torres Strait Islander ____________ □
(b) Torres Strait Islander but not Aboriginal origin ____________ □
(c) Aboriginal and Torres Strait Islander origin ____________ □
(d) Not aboriginal or Torres Strait Islander origin ____________ □
(e) Don’t know ____________ □

Thank you for filling out this questionnaire. Your time is appreciated.