Infraocclusion of primary molars and associated dental anomalies in twins and singletons: what is the underlying aetiology?

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List of abbreviations

AIC	Akaike's Information Criterion
CA	Chronological age
C	Primary canine
CorGE	Genotype-environment correlation
D	Primary first molar
DAP	Dental anomaly pattern
DDA	Demirjian dental age
DZ	Dizygotic (twin pairs)
E	Primary second molar
F	Female
GxE	Genotype by environmental interaction
h^2	Heritability estimate
L	Left
M	Male
Man	Mandibular arch
Max	Maxillary arch
MD	Mesiodistal tooth width
MEF	Mechanical eruption failure
MI	Mild
MLD	Mandibular left first molar
MLE	Mandibular left second molar
MO	Moderate
MZ	Monozygotic (twin pairs)
NI	Non-infraoccluded
OPG	Orthopantomograph
PEF	Primary eruption failure
R	Right
SD	Standard deviation
Se	Dahlberg statistic
SE	Severe
SEM	Structural equation modelling
SE	Standard error
V_A	Additive genetic variance
V_D	Dominance variance (effects between alleles at the same locus)
V_E	Total environmental variance
V_{EC}	Common environmental variance (affecting both twins)
V_{EW}	Individual environmental variance (affecting one twin)
V_G	Total genetic variance
V_I	Epistatic variance (interactions between alleles at different loci)
V_P	Phenotypic variance
WDA	Willems dental age
x diff	Mean difference
6	Permanent first molar

Abstract

The process of tooth eruption involves complex interactions between genetic, epigenetic and environmental factors. 'Infraocclusion' refers to a tooth that is positioned below the normal plane of occlusion. This study aims to determine the frequency of occurrence of infraocclusion in the primary molars and to find out whether there are associations between infraocclusion and several variables. Further, it is planned to clarify the roles of genetic, epigenetic and environmental factors in contributing to observed variation in infraocclusion, and to estimate the frequency of occurrence of some selected dental anomalies in association with infraocclusion.

Orthopantomographs of 1,454 healthy singleton Finnish boys and girls aged between 9-10 years, and study models of 320 Australian twin pairs aged between 8-10 years were examined. Adobe Photoshop CS5 computer software was used to construct reference lines (from the mesial marginal ridge of the mandibular first permanent molar to the cusp tip of the primary canine or the mesioincisal edge of the permanent lateral incisor). The distances between reference points were measured (in mm) for both samples and categorised into non-infraoccluded, mild, moderate, and severe. Genetic modelling was also used to quantify the contribution of genetic and environmental factors to observed variation. The orthopantomographs were examined for the presence of associated dental anomalies. Dental age and tooth size assessment were carried out in individuals showing infraocclusion.

Descriptive statistics, including mean values, standard deviations and percentage frequencies, were used to summarise data within groups and comparisons between groups were made using t-tests and chi-square analyses.

The overall prevalence of infraocclusion was 22% in singletons, and 27 % in twins. The primary mandibular first molar was the most commonly affected tooth (21% in singletons and 28% in twins compared with 6% and 18% for the mandibular second molar in singletons and twins respectively). Genetic modelling indicated a strong genetic contribution (~94%) to

observed variation in the primary mandibular first molar, while common and unique environmental factors contributed to infraocclusion of the primary mandibular second molar. Investigation of MZ twin pairs revealed differences in the expression of infraocclusion within some twin pairs, for example, mirror imaging. These findings reflect epigenetic events and/or environmental disturbances that have occurred during the developmental process. Analysis of dental anomalies in singletons revealed a significant association of ectopic canines and the lateral incisor complex with infraocclusion. Individuals showing infraocclusion displayed delayed dental development and evidence of reduced primary tooth size.

The findings showed that genetic factors play a major role in contributing to infraocclusion of the primary mandibular first molar, whereas environmental factors contribute more to variation in infraocclusion of the second molar. These environmental factors could occur in the prenatal or early postnatal stages of life and may disrupt the network of epithelial rests of Malassez, leading to localised areas of ankylosis.

A possible pleiotropic effect was reflected by the presence of associated dental anomalies with infraocclusion.

These findings are significant in improving understanding of the basic biological mechanisms and associated features of infraocclusion, and should assist clinicians in providing proper counselling, early diagnoses, prevention and treatment planning for affected individuals.

Thesis declaration

Name: **Ruba Mohammed Odeh** Program: **PhD in Dentistry**

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Format of the thesis

This thesis is presented as eleven main chapters. The first two chapters provide an overall introduction and literature review, focusing on setting the scene of this research and identifying gaps in our knowledge, while Chapter 3 presents the aims. The fourth chapter summarises the methods used in this project, while the fifth chapter focuses on reporting the systematic and random errors of the methods. Chapters 6, 7 and 8 present results and are set up to facilitate future publications, so there is some repetition from the literature review and materials and methods presented in previous chapters. For certain topics, a more detailed explanation is included than one might expect in a published paper, for example the section about genetic modelling in Chapter 7. When these findings are submitted for publication, some of these sections will be reduced in length or removed.

Chapter 6 presents descriptive statistics on infraocclusion obtained from the singleton and twin samples. Chapter 7 reports on genetic analysis of infraocclusion in the twin sample. Chapter 8 explores associations between infraocclusion and other dental anomalies in both samples. Chapter 9 presents a series of interesting cases selected from the twin sample, as well as some of their family members. Chapter 10 presents a general discussion of this research, with key findings and suggestions for further research, while Chapter 11 provides general conclusions. A list of references is provided at the end of this thesis, together with some appendices.

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1. Introduction

A substantial amount of research has been conducted to clarify the molecular events occurring during dental development and the altered mechanisms that result in dental anomalies (Vastardis, 2000; Sharpe, 2001). However, a complete understanding of these processes has not yet been reached and further investigations are warranted at both molecular and phenotypic levels. Infraocclusion has been investigated for many decades (Steigman et al., 1973a; Kurol, 1981; Koyoumdjisky-Kaye and Steigman, 1982a; Douglass and Tinanoff, 1991; Shalish et al., 2010), and most of previous reports have suggested that there is some genetic influence on infraocclusion. This proposition is based on the familial tendency of infraocclusion and its strong association with other dental anomalies. However, the genetic basis of infraocclusion has not been investigated in depth.

Agenesis, ectopic canines and small maxillary lateral incisors are three of the most commonly identified dental anomalies to be associated with infraocclusion (Baccetti, 1998; Shalish et al., 2010). Moreover, a relationship has been found between agenesis, reduced tooth size and delayed dental development (Uslenghi et al., 2006; Brook et al., 2009b; Tunc et al., 2011). Likewise, a relationship has been established between ectopic canines and delayed dental development (Zilberman et al., 1990; Becker and Chaushu, 2000; Naser et al., 2011). However, the notion that infraocclusion may be related to delayed dental development and reduced tooth size has not been proposed or investigated in previous studies.

Studying twins and their families allows researchers to determine the contributions of genetic and environmental factors to variation of dental phenotypes (Townsend and Martin, 1992; Hughes et al., 2007; Townsend et al., 2009; Hughes et al., 2013; Hughes and Townsend, 2013). Moreover, discrepancies in some dental phenotypes between monozygotic co-twins have been explained by epigenetic factors which generally refer to changes in phenotypic expression without changes in the DNA sequence of genes (Townsend et al., 2005). However, the studies that have reported previously on infraocclusion in twins (Stewart and Hansen, 1974; Kurol, 1981; Helpin and Duncan, 1986; Dewhurst et al., 1997; Cozza et al., 2004; Zengin et al., 2008), have not enabled any firm conclusions to be drawn about genetic, epigenetic or environmental contributions to observed variations, or about the presence of associated dental anomalies in one or more members of their family.

In this study, the term ‘infraocclusion’ is used as a descriptive term to refer to a tooth that is below the level of the occlusal plane. In doing so, it is acknowledged that the study samples available do not enable a definite judgement to be made of whether a tooth emerged and then subsequently stopped erupting before reaching the occlusal plane, or whether a tooth had erupted to its full occlusal position then stopped while the adjacent teeth continued to erupt, resulting in what appeared to be infraoccluded tooth. Also, the study material available does not enable the diagnosis of the primary eruption failure which, by definition, refers to a tooth that fails to emerge.

This study has implications in further understanding the normal biological processes that are involved in tooth eruption, as well as in helping clinicians in dental practice to manage patients presenting with infraoccluded teeth. By exploring the relationships between infraocclusion, tooth size, dental development and other dental anomalies, it is aimed to provide a foundation for appropriate counselling to patients and care-givers, and for effective treatment planning.

2. Literature review

2.1. Introduction

Understanding of the tooth eruption process and identification of disturbances during this continuing process is important when studying dental anomalies and associated factors. In this chapter the eruption process will be reviewed and previous findings about infraocclusion and other associated dental anomalies by different authors will be discussed. This literature review aims to highlight the current state of knowledge of infraoccluded primary molars and what areas require further investigation.

2.2. The process of tooth eruption and associated disturbances

Stages of tooth eruption

Tooth eruption is a multifactorial process involving the developing tooth and its surrounding tissues, including the alveolar bone. Tooth movement results from a balance between tissue destruction (bone, connective tissue and epithelium) and tissue formation (bone, periodontal membrane and root). The movement can be divided into three stages: pre-eruptive, eruptive and post-eruptive tooth movement. Pre-eruptive tooth movement occurs within the jaw as tooth germs begin to grow and differentiate; eruptive tooth movement occurs as the tooth moves into its functional position in occlusion and this phase can be divided into intraosseous and extraosseous components; post-eruptive tooth movement maintains the tooth in its occlusal position while the jaws continue to grow and this process compensates for occlusal and proximal tooth wear (Nanci and Ten Cate, 2008). All these movements occur as a complex series of continuous events that position the tooth in three-dimensional space.

Timing of tooth emergence

The term ‘tooth emergence’ is used to refer to the point of time at which the erupting tooth becomes apparent within the oral cavity. The complete process of emergence of primary teeth occurs over a period of approximately 2 years, starting at around 8 months of age and ending at around 30 months of age. However, there is some evidence that the period of duration of emergence has apparently became shorter over the years (Woodroffe et al., 2010). On average, there is no significant difference in the sequence of emergence or the timing of emergence (2-3 months) between males and females (Koch et al., 2009a). Likewise, the sequence of emergence of permanent teeth is similar in both sexes. However, the timing of emergence of all permanent teeth is significantly earlier in females than males (over 6 months) (Koch et al., 2009a). The emergence of the permanent dentition starts with the mandibular central incisor at around 6 years of age and ends with the emergence of the maxillary second molars at around 12 years of age, excluding the third molars (Koch et al., 2009a). The timing of tooth emergence is important to determine the dental developmental age, especially in cases where chronological age is unknown. Moreover, investigating the stage of dental development is important for clinicians in orthodontic treatment planning and, in paediatric endocrinopathies, the decision making of diagnosis and prognosis of treatment may be improved in cases where dental age is assessed in accordance with other signs of maturity (Demirjian et al., 1973; Bredy et al., 1991).

Mechanisms of tooth eruption

The eruption path is determined by genetic, epigenetic and environmental factors, which influence the time and direction of tooth eruption. More than 300 genes have been identified in the process of dental development (Thesleff, 2006; Brook, 2009). A series of intercellular and extracellular interactions occur between the ectoderm and mesenchyme by identified genes (Cobourne and Sharpe, 2013). These series of interactions regulate initiation, morphogenesis and differentiation of the developing teeth. Gene mutation affecting the regulatory genes may result in defects in the dental development process (Brook, 2009). Interactions between genes and the environment, known as ‘epigenetic factors’, may play a role in the dental developmental process. Thus, a slight epigenetic variation can result in distinct differences in the clinical expression of teeth, e.g. tooth number and size (Townsend et al., 2005; Hughes and Townsend, 2013).

The mechanism of tooth eruption is not well understood, although there are several theories that have been proposed to explain this mechanism (Wise et al., 2002; Wise and King, 2008; Wang, 2013). One theory states that tooth eruption begins when the root starts developing apically, generating a force in the opposite direction resulting in movement of the tooth occlusally. However, reviews of studies involving humans, monkeys, dogs, and rodents have shown that rootless teeth do erupt into the mouth (Carl and Wood, 1980; Marks and Schroeder, 1996; Wang, 2013). Moreover, rootless tooth crowns were noted to erupt into the mouths of patients with dentin dysplasia Type I and in children treated with irradiation (Carl and Wood, 1980; Ozer et al., 2004). Another theory is that the periodontal ligament promotes eruption through the shrinking and cross-linking of its collagen fibres and the contraction of fibroblasts. However, it has been reported that, in dog premolars and rat molars, there are no organized periodontal ligaments attached to the adjacent alveolar bone until the tooth penetrates the gingiva (Marks et al., 1983; Wise and King, 2008). Furthermore, the ability of rootless teeth to erupt on time, indicates that the periodontal ligament is not essential for the eruption of these teeth. The most accepted theory is that continuous bone formation forces the tooth to the occlusal surface, by bone deposition on the apical side and bone resorption on the coronal side of the tooth (Marks and Schroeder, 1996; Wise and King, 2008). It has been shown that this process of bone remodelling occurring around an erupting tooth is regulated by genes in the adjacent parts of the dental follicle and that both processes are required for tooth eruption (Wise and King, 2008).

In fact, tooth eruption is a multifactorial process that results from the interaction of closely related processes, including alveolar bone growth, root development and periodontal ligament re-organisation (Marks and Schroeder, 1996; Craddock and Youngson, 2004; Wang, 2013). It has been proposed that alveolar bone growth occurs during tooth development and that alveolar bone deficiencies may lead to primary or permanent teeth failing to erupt (Kurol and Koch, 1985). The two major factors in the eruption process are proposed to be the force which is created by cellular proliferation at the root apex, and eruption path clearance by clast cells through active resorption of bone, gingival tissue and primary teeth (Proffit and Frazier-Bowers, 2009). Further investigation of these theories should help to explain to some extent the factors and /or processes which lead to disturbances during the eruption process and result in conditions such as premature tooth eruption, delayed tooth eruption, early exfoliation of primary teeth and retained primary teeth.

Disturbances in the eruption process

Disturbances in the process of tooth eruption and emergence may result from local and/or systemic abnormalities that can lead to functional disturbances or disorders within the erupting dentition (Table 2.1). Examples of systemic factors leading to delayed tooth eruption include endocrine deficiencies and nutritional deficiencies, while examples of local factors include early loss of, or trauma to, a deciduous tooth (Nanci and Ten Cate, 2008).

Table 2.1. Abnormalities related to disturbances in the process of tooth eruption.

Systemic factors	**Local factors**
Cleidocranial dysplasia	Impaction
Ectodermal dysplasia	Ankylosis
Endrocrinopathies:	
Hypothyroidism	
Hypopituitarism	
Hypoparathyroidism	
Vitamin D–resistant rickets	Primary retention
Gardner syndrome	Early loss of primary teeth
Down syndrome	Retained primary teeth
Apert syndrome	Cysts
Mucopolysaccharidosis	Neoplasms
De Lange syndrome	Trauma
Prematurity/low birth weight	Agenesis
Icthyosis	Supernumerary teeth
Hereditary gingival fibromatosis syndromes	

Modified from O'Connell and Torske (1999)

Genetic factors also play a crucial role, for example, genetic mutations can lead to disturbances in metabolic activity or altered blood flow which may hinder the eruption process (Wise et al., 2002).

In syndromes associated with eruptive failure, e.g. cleidocranial dysplasia, hypothyroidism and hypopituitarism, failure to erupt is related to mechanical obstruction such as fibrous gingival tissues, supernumerary teeth or retained primary teeth (Proffit and Frazier-Bowers, 2009).

Eruption failure is represented by a range of phenotypes varying in severity of presentation and occurring at various developmental time points. The dental developmental process of tooth eruption is considered to be a multilayered complex (Figure 2.1). This presentation of the dental developmental process is important as it highlights the critical period during which some teeth will continue or finish the developmental process while, for other teeth, the process just starts (Brook, 2009). It can be said that dental anomalies can occur if this process is interrupted. The timing of the disturbance will determine the type of anomaly and which dentition is affected.

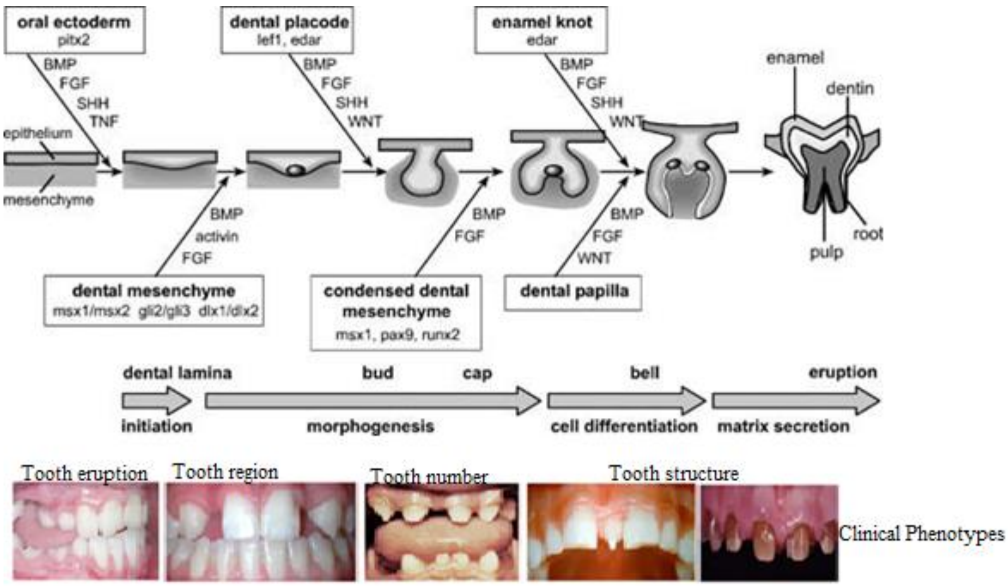


Figure 2.1. A multilayered illustration of the dental developmental process, showing that genetic and environmental disturbances during dental development may result in distinct clinical phenotypes. Derived from Thesleff (2006) and Brook (2009).

Identification of the genes associated with eruption failure should provide a better understanding of the tooth eruption process which may lead to diagnostic tools that can positively distinguish the various subtypes of eruption failure, and offer the potential for more effective treatment and prevention options (Proffit and Frazier-Bowers, 2009).

2.3. Infraocclusion

An example of a disturbance in the eruption process is the so-called ‘infraoccluded’ tooth, which refers to a tooth that is positioned below the normal plane of occlusion (Figure 2.2). Infraocclusion can vary from mild levels of infraocclusion to severe cases where the tooth is completely embedded under the bone (Kurol, 1981).

Many terms have been used to describe the condition of infraocclusion, including arrested eruption, impaction, incomplete eruption, intrusion, buried tooth, depression, disinclusion, reimpaction, reinclusion, secondary retention, shortened tooth, and suppressed eruption, but the most commonly used terms are ankylosis, submergence and infraocclusion (Kurol, 1981). Some of these terms are misleading (e.g. submerged) and some refer to aetiology rather than appearance (e.g. ankylosis), while others can be correctly used in place of the term ‘infraocclusion’. The term ‘submerged’ describes a tooth that has erupted previously but becomes embedded subsequently in the oral tissues (Antoniades et al., 2002). The term ‘ankylosis’ may occur before eruption of the tooth into the oral cavity and refers to the fusion of the tooth with the surrounding bone due to developmental disturbances in the periodontium caused by trauma or other pathology. Clinically, when a tooth is ankylosed, it appears to be below the occlusal plane in relation to the adjacent teeth that continue to erupt (Messer and Cline, 1980; Wise et al., 2002). However, ankylosis is not always present clinically or histologically in association with incomplete eruption of a tooth (Kurol and Magnusson, 1984). The term primary eruption failure (PEF) has been used to describe a non-ankylosed tooth which fails to erupt to the normal functional level due to a disturbance in the eruption process. PEF is characterised by failure of a tooth to erupt through the already cleared pathway of eruption, thus leading to infraocclusion with no associated ankylosis. PEF is distinguished from mechanical eruption failure (MEF) which is associated with ankylosis in all cases (Wise et al., 2002).

In this research, the terms ‘infraocclusion’ and ‘infraoccluded’ will be used because they describe the inferior position of an affected tooth relative to adjacent teeth, regardless of the underlying cause or degree of severity.

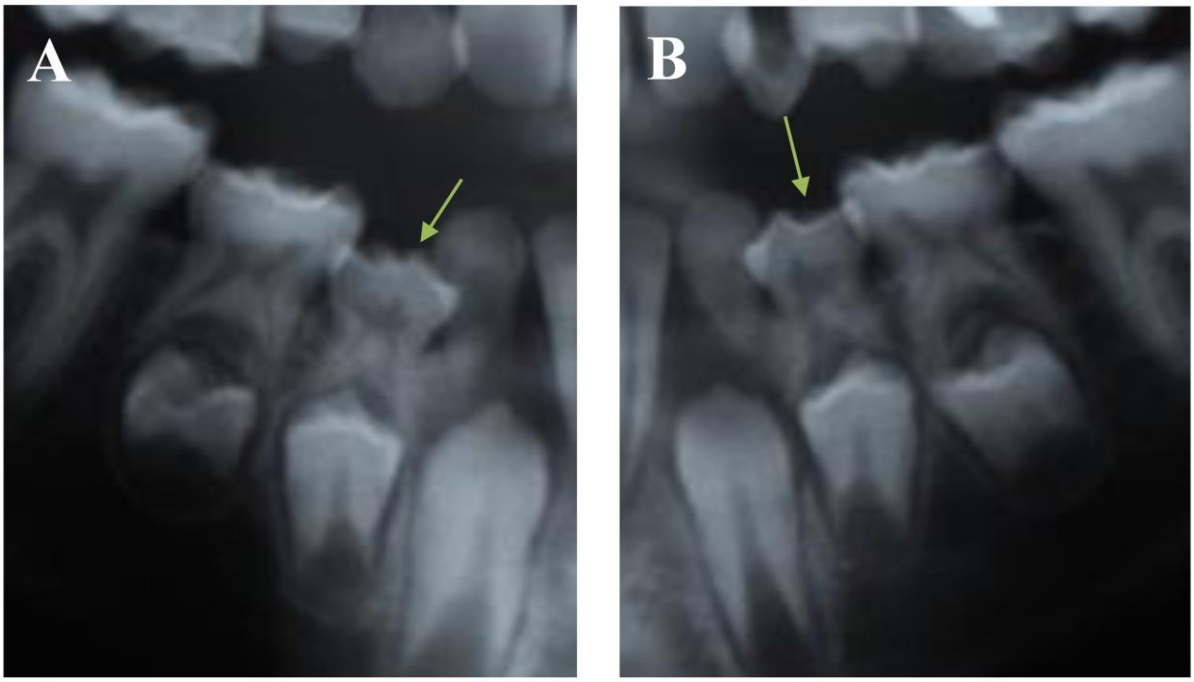


Figure 2.2. Radiographs showing a pair of infraoccluded primary mandibular first molars: A. mandibular right, B. mandibular left.

The prevalence of infraoccluded teeth

Estimates of the prevalence of infraocclusion in the primary dentition have ranged between 1.3% and 38.5% (Via, 1964; Kurol, 1981; Koyoumdjisky-Kaye and Steigman, 1982b; Shalish et al., 2010). This wide degree of variation is thought to be related to differences in terms of study sample sizes, age groups, diagnostic methods and inclusion/exclusion criteria (Table 2.2) (Kurol, 1981). The prevalence of infraocclusion in the permanent dentition is less common, 8.1% in a sample of 221 cases (Spieker, 2000).

Table 2.2. The prevalence of infraocclusion in the primary dentition.

Ethnic group (author)	Year	Sample size	% of infraocclusion
Scandinavian (Brearley and McKibben)	1973	1,000	6.90%
Swiss (Kurol)	1981	1,059	8.90%
USA (Mueller et al.)	1983	1,767	9.20%
Israeli (Koyoumdjisky-Kaye and Steigman)	1982	1,530	24.80%
Italian (Baccetti)	1998	1,000	5.80%

Based on clinical visual examination of 1,059 boys and girls aged from 3 to12 years, Kurol (1981) recorded the presence of infraocclusion if a tooth surface was 1mm or more below the level of the occlusal plane of adjacent teeth. The prevalence of infraocclusion in relation to age and sex, the most affected tooth, and also the side affected were reported. It was found that girls showed more infraocclusion than boys in the age group 3 to 6 years, whereas more boys than girls were affected in the 7 to 12 year age group. At age 3 years, the prevalence of infraocclusion was 3.6% and this gradually increased with age to reach a maximum of 14.3% at 8 to 9 years of age, but declined at age 12 to 1.9%, related to normal exfoliation of teeth at this stage. No differences were reported between right and left sides, but primary mandibular molars showed a higher prevalence of infraocclusion than maxillary molars. Infraocclusion of primary mandibular first molars was twice as common as for the primary second molar in children less than 9 years of age, while the primary second molar was more often infraoccluded in children 9 years of age and above. This finding was correlated with the time of development and eruption of the permanent successors (Kurol, 1981). Despite the fact that Kurol's study comprised a large sample, examination was conducted visually, which could be thought to influence the reproducibility and accuracy of the measurements obtained.

In a study comparing the prevalence of infraoccluded teeth between different ethnic groups using plaster study models, infraocclusion was found to be more common in females than males in some ethnic groups, including Cochins, Kurds, East Europeans and Circassians.

In contrast, the prevalence was higher in males in Druse, Yemenites and North Africans (Koyoumdjisky-Kaye and Steigman, 1982a; 1982b).

The same study showed that the ages at which infraoccluded teeth occur also differed between ethnic groups. The percentage of infraoccluded primary molars varied from 14% to 35%, the highest percentages being noted in Kurds, followed by East Europeans, Druse, Circcassians and North Africans. The groups exhibiting the lowest percentage of infraocclusion were Cochins and Yemenites (Koyoumdjisky-Kaye and Steigman, 1982a; 1982b). The prevalence of infraocclusion reported in this study may be considered an overestimation due to the criteria used to define an infraoccluded tooth, which is if a tooth was 0.5 mm below the occlusal plane, compared with other studies were the definition of infraocclusion was 1.0 mm below the occlusal plane.

A higher prevalence of infraocclusion of primary molars has been reported in the siblings of affected children (Kurol, 1981; Helpin and Duncan, 1986). The prevalence of infraocclusion was reported to be 18.1% in 138 siblings (having one or both parents in common) of children having infraocclusion. Out of the total sibling group, five twin pairs were identified, of whom three pairs displayed infraocclusion. However, the zygosities of these twins were not determined, and whether the co-twins had similar or different patterns of expression of infraoccluded teeth was not mentioned (Kurol, 1981). The findings suggest the possibility of a familial tendency for infraocclusion. Nevertheless, a firm conclusion cannot be drawn from this suggestion because of the small sample size.

Factors associated with infraocclusion

According to several reports, ankylosis has been categorised as the most common factor associated with infraocclusion (Kurol, 1984; Kurol and Magnusson, 1984; Kurol, 2002; Karacay et al., 2007; Kjaer et al., 2008). Ankylosis, as mentioned earlier, is the fusion of the tooth to the surrounding alveolar bone due to disturbances to the periodontium. A study reporting on histological comparisons between infraoccluded primary molars and non-infraoccluded primary molars, in a sample of children aged 6 to 15 years, found signs of ankylosis in 38 out of 48 infraoccluded primary molars with a permanent successor (Kurol and Magnusson, 1984). In the same study, ankylosis was also noted in 12 out of 14 infraoccluded primary molars lacking a permanent successor, in children aged 8 to 17 years (Kurol and Magnusson, 1984). Another study reported 98% of infraoccluded teeth as being ankylosed (Darling and Levers, 1973).

When ankylosis is associated with infraoccluded primary molars it seems to occur during the normal remodelling process following root resorption, as a result of unequal patterns of resorption and repair (Mancini et al., 1995). The epithelial cell rests of Malassez in the periodontium show some differences in distribution in ankylosed primary molars and so may be associated with the aetiology of root resorption and ankylosis (Fujiyama et al., 2004). It has been suggested that ankylosis is a secondary pathology occurring subsequent to other problems, such as deficient eruptive force, infection, deficient vertical alveolar bone growth, or traumatic masticatory force producing local periodontal injuries (Proffit and Vig, 1981; Kurol and Magnusson, 1984). On the other hand, a genetic contribution to ankylosis has been proposed as there appears to be a familial pattern (Karacay et al., 2007).

Infraocclusion can be associated with other factors including crowding, loss of space, trauma to teeth, agenesis of permanent teeth (e.g. missing second premolars or missing maxillary lateral incisors) (Kurol and Thilander, 1984a). Infraocclusion can also result from the primary retention of permanent teeth which is due to localised failure of eruption. This occurs as an isolated condition in the absence of other local and systemic factors (Proffit and Frazier-Bowers, 2009).

Infraocclusion can also be present in some human syndromes affecting the oro-facial region, such as in cleidocranial dysplasia, osteopetrosis, ectodermal dysplasia and Down syndrome (Suri et al., 2004; Ahmad et al., 2006). These syndromes are categorised under systemic causes of infraocclusion in Table 2.1. The causative genes and the modes of inheritance have been identified for most of these syndromes (Proffit and Frazier-Bowers, 2009) but the specific factors affecting infraocclusion are not well known. Other related problems that are associated with these genetic syndromes include dental developmental delay, supernumerary teeth, abnormalities in periodontal tissue development and increased bone density (Suri et al., 2004).

As highlighted previously in relation to prevalence, several studies have indicated that there is a familial predisposition to infraoccluded teeth (Kurol, 1981; Winter et al., 1997; Karacay et al., 2007). However, only a few cases of infraocclusion have been reported in twins. Similarities in the distribution, pattern and severity of infraoccluded teeth have been recorded in monozygotic (MZ) twins, suggesting that genetic factors are important (Stewart and Hansen, 1974; Kurol, 1981; Helpin and Duncan, 1986; Dewhurst et al., 1997; Cozza et al., 2004; Zengin et al., 2008). This is addressed more fully on page 37 and presented in Table 2.5, page 38. A genetic predisposition to infraocclusion has also been postulated because one or more other dental anomalies have been identified to be associated with the condition. Baccetti (2000) found a higher prevalence of associated dental anomalies in subjects displaying one or more anomalies compared to the general population (subjects displaying no anomalies), thus, proposing a common genetic component may link a range of different dental anomalies. The most commonly associated dental anomaly with infraocclusion has been identified as agenesis of the permanent successor of the infraoccluded tooth. This was reported in 17% of cases (Steiner-Oliveira et al., 2007). Infraocclusion has also been shown to display a higher prevalence than many other dental anomalies, including palatal displacement of maxillary canines, rotation of maxillary lateral incisors, agenesis of second premolars and smaller-sized maxillary lateral incisors (Baccetti, 2000). This supports the notion that infraocclusion is not an isolated condition, but it most likely occurs in association with other dental anomalies. Infraocclusion may be the first sign observed by the clinician when other anomalies (e.g. agenesis, ectopic canines and small lateral incisors) are also present (Shalish et al., 2010).

While there is some evidence for genetic, epigenetic and environmental contributions to variation in infraoccluded teeth, the contributions of these influences are not well understood. There is, therefore, a need to carry out further studies in related individuals to clarify the roles of genetic, epigenetic and environmental influences on infraoccluded teeth.

Classification and measurement methods of infraocclusion

Infraocclusion can be identified by direct visual examination (Kurol, 1981); radiographically (using panoramic and cephalometric radiographs) (Becker and Karnei-R'em, 1992b; Aktan et al., 2011) (Figure 2.3); on study models (Koyoumdjisky-Kaye and Steigman, 1982b; Sidhu and Ali, 2001) (Figure 2.4); or through the assessment of intraoral photographs (Frazier-Bowers et al., 2007) (Figure 2.5).

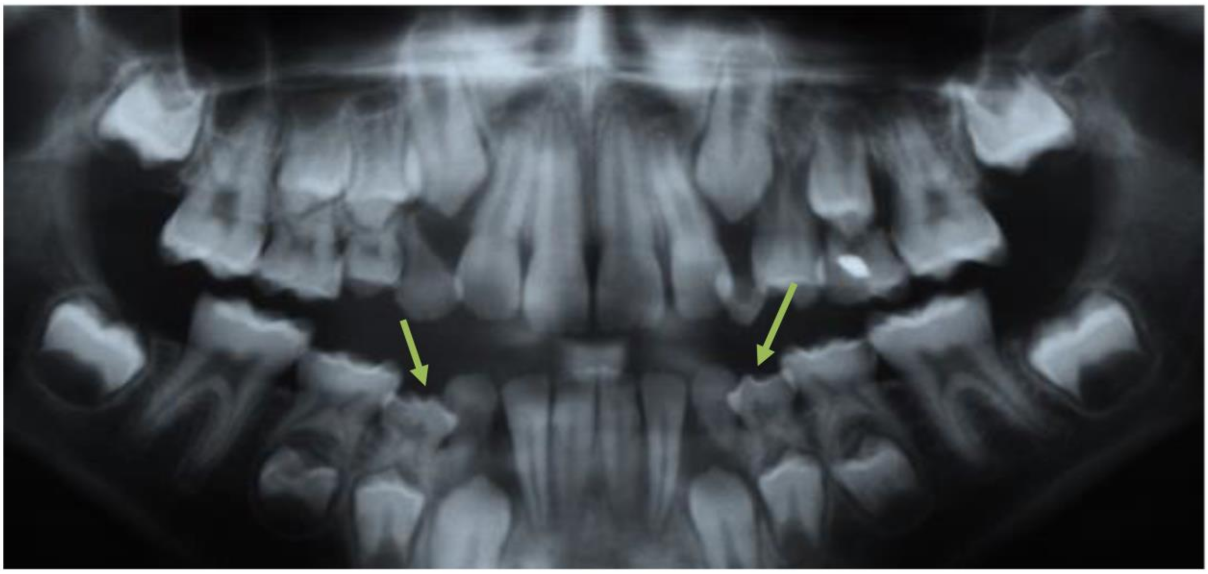


Figure 2.3. An orthopantomograph showing infraoccluded primary mandibular left and right first molars.

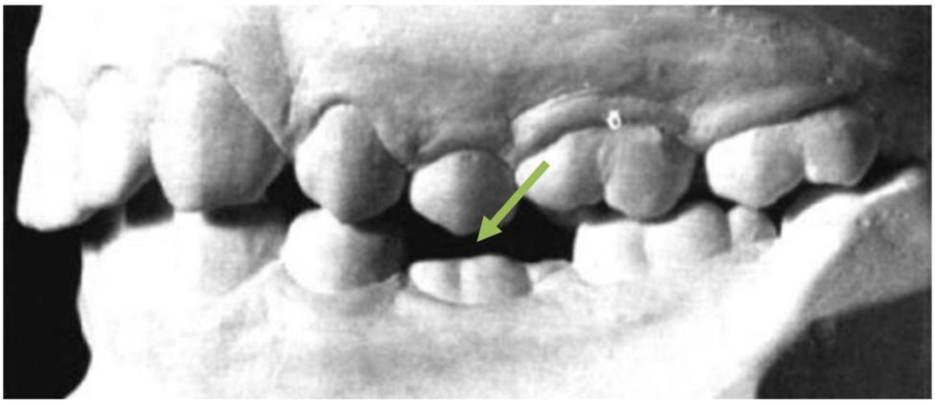


Figure 2.4. A study model showing an infraoccluded primary mandibular left first molar.

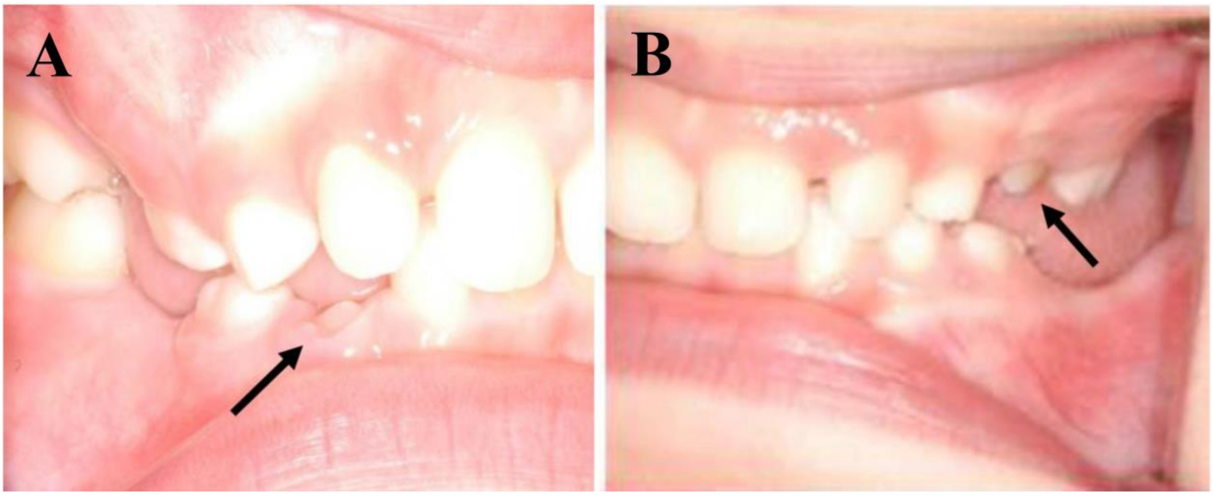


Figure 2.5. Intra-oral photographs showing a infraoccluded primary mandibular right first molar (A) and a primary maxillary left first molar (B).

Previous studies have used various criteria in the diagnosis, measurement and classification of infraocclusion. Some authors have relied on direct visual examination (Steigman et al., 1973b; Kurol, 1981), however, the accuracy and reliability of this technique is questionable, due to subjectivity factors. Other authors have obtained direct measurements from study models, for example, by placing a steel ruler from the mesio-buccal cusp of the permanent first molar to the incisal edge of the central incisor within the same quadrant. From this horizontal plane, using a set of callipers, the vertical distance to the occlusal surface of the infraoccluded tooth can be measured (Kurol and Thilander, 1984a; Shalish et al., 2010) (Figure 2.6). This technique can provide a higher level of accuracy and reliability, especially where reference points can be located in subsequent models, for the same individual over time.

However, there are limitations, e.g. measurements obtained using a hand-held ruler may not provide a standardised approach that can be followed consistently across all models. Another disadvantage of making direct measurements of infraocclusion using study models is that it may lead to accidental chipping of cusp tips or incisal edges of teeth.

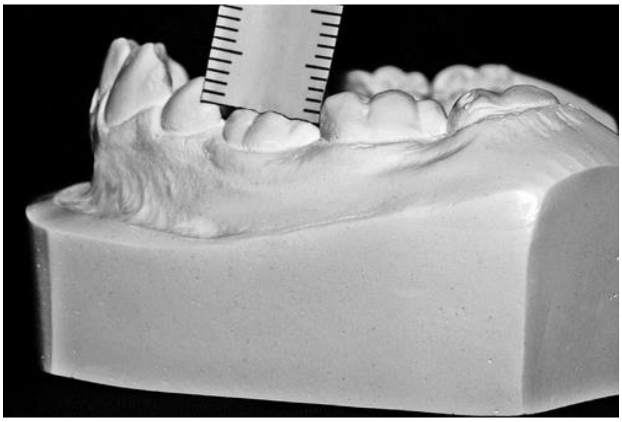


Figure 2.6. Study models showing the direct measurement of infraocclusion using a calibrated ruler (derived from Shalish et al., 2010).

Another example is the method introduced by Darling and Levers (1973). They design measured infraocclusion directly from study models with the aid of a spring-loaded height gauge. The occlusal plane was represented by a small piece of thin sheet-brass 0.16mm thick and the difference between heights represented the measurement of infraocclusion (Figure 2.7). Darling and Levers (1973) also used intra-oral radiographs to measure infraocclusion. The occlusal plane was constructed by joining a mark made at the midpoint between the buccal and lingual cusps of the unaffected teeth adjacent to the affected tooth. Another line, parallel to the first, was drawn through the midpoint between the buccal and lingual cusps of the affected tooth. The vertical distance between the two lines was then measured. These can be considered the most accurate and reliable methods used to measure infraocclusion. However, intraoral radiographs were not available in all cases. Moreover, measurement of the study models using the apparatus described risked damaging the models.

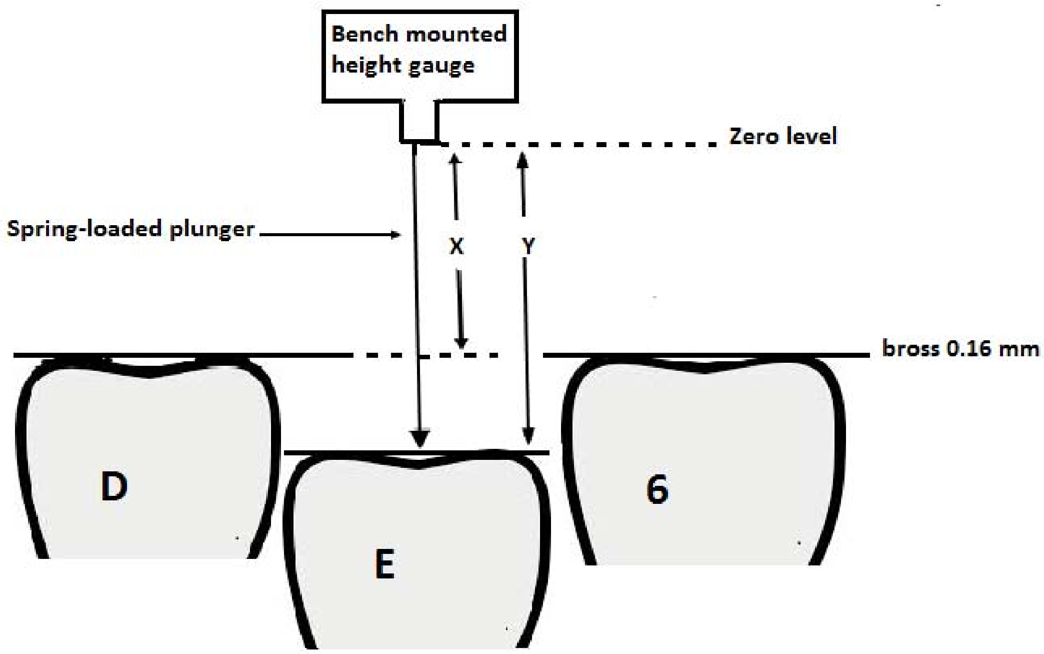


Figure 2.7. Method used to measure infraocclusion from study models by Darling and Levers (1973).

Methods used to describe infraocclusion have been either purely descriptive or have included both descriptive and numerical components.

Examples are as follows:

- Classification based on the degree of infraocclusion according to Darling and Levers (1973):
  - 1 = Slight
  - 2 = Marked
  - 3 = Occlusal surface of tooth only just visible
  - 4 = Very deep
  - 5 = Very deep and "floating" free in an abscess cavity
- Classification based on the degree of infraocclusion in respect to the surrounding hard and soft tissues (Figure 2.8), proposed by Brearley and McKibben (1973). This system has become more commonly used and includes the following categories:
  - Slight – occlusal surface located approximately 1 mm below the expected occlusal plane for the tooth.

- Moderate – occlusal surface approximately level with the contact point of one or both adjacent tooth surfaces.
- Severe – occlusal surfaces level with or below the interproximal gingival tissue of one or both adjacent tooth surfaces (Messer and Cline, 1980; Ekim and Hatibovic-Kofman, 2001; Sidhu and Ali, 2001; Hudson et al., 2007).

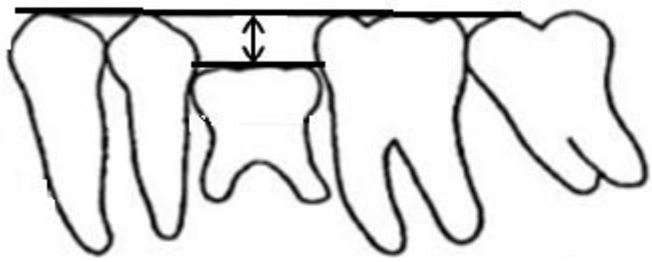


Figure 2.8. Infraocclusion determined from the occlusal plane of the affected tooth to the occlusal plane of the adjacent teeth.

- A similar but modified descriptive classification system to Brearley and McKibben (1973), but using four groupings related to the severity of the condition when compared to the adjacent teeth, proposed by Kjaer et al. (2008):
  - Group I mild

The level of occlusion of the primary molar is equal to or less than half the crown height of the actual primary molar of one or two fully erupted neighbouring teeth

- Group II moderate

The level of occlusion of the primary molar is half to full crown height below the level of the occlusal surface of one or two fully erupted neighbouring teeth.

- Group III severe

The level of occlusion of the second primary molar is equal to or more than full crown height below the level of the occlusal surface of one or two fully erupted neighbouring teeth

- Group IV extreme

  The second primary molar is found deeply, subgingivally retained to such an extent that the occlusal surfaces of the fully erupted neighbouring teeth are located at a distance equal to or more than one and a half crown height of the primary molar compared with the level of the neighbouring teeth.

- A classification system, involving the measurement of infraocclusion, in millimetres, with categories defined according to severity, put forward by Shalish et al. (2010):

  - Mild infraocclusion (1–2 mm)
  - Moderate infraocclusion (>2 mm and <5 mm)
  - Severe cases (>5 mm)

While this classification provides a descriptive and numerical expression of the level of infraocclusion, the intervals chosen were not equally distributed for all categories.

- Others have described infraocclusion as being present when the occlusal level of the tooth is 1mm or more below the occlusal plane of the adjacent tooth, but with no further categorisation describing the level or severity of infraocclusion (Kurol, 1981; Bjerklin et al., 1992).

Consequences of infraoccluded teeth

The condition known as infraocclusion not only places the affected tooth in a malfunctioning position, but also inhibits the harmonious relationship that one would expect to develop through normal eruption and exfoliation.

The main consequences of infraocclusion have been reported in terms of:

- Tilting of adjacent teeth and local space loss
- The type of movement of the adjacent teeth and their vertical development
- Change to dental arch length and the midline position

Figure 2.9. The effect of infraocclusion on the orientation of the trans-septal fibres and tilting of the adjacent teeth.(Becker and Karnei-R'em, 1992b)

In one study using panoramic radiographs of 17 cases, infraocclusion was viewed, traced and measured (Becker and Karnei-R'em, 1992b). The relationship between adjacent teeth was described to be a continuous chain in which teeth are connected to each other by trans-septal fibres. These fibres are thought to be parallel to the occlusal surface and maintain the interproximal contact between teeth (Becker and Karnei-R'em, 1992b). In the case of an infraoccluded tooth, changes were formed to occur in the orientation of these fibres (Figure 2.9). Normal physiological development will cause the adjacent teeth to continue to erupt, resulting in a forced action on the transeptal fibres to reorientate in a diagonal direction downward as the tooth becomes infraoccluded. This action is thought to lead to tilting of the adjacent teeth, under-eruption of the adjacent teeth, and severe displacement of the apices of the adjacent teeth in an anteroposterior direction which correlates with the presence of a centre of rotation in the coronal half of the teeth (Becker and Karnei-R'em, 1992a). It has also been found that infraocclusion tends to result in a midline shift towards the affected side, causing an increase in the arch length of the not affected side when measured to the midline, and more distal positioning of the first permanent molar adjacent to the affected tooth (Becker et al., 1992).

Overall, infraoccluded primary molars may cause several problems in the dental arch, such as alignment problems, tipping of adjacent teeth, supra-eruption of antagonists and dislocation of permanent teeth lying under the primary tooth (Karacay et al., 2007). In a comparison between infraoccluded teeth and normal teeth within the same arch for a group of individuals, minimal differences were noted in their shedding and exfoliation times and no disturbance was found in the development of their permanent successors (Kurol and Thilander, 1984a). In the absence of a permanent successor, it was reported that a progression occurred in the degree of infraocclusion. Also, the infraoccluded teeth did not exfoliate naturally, as was expected, when compared with infraoccluded teeth with permanent successors (Kurol and Thilander, 1984b).

Severe cases of infraocclusion may cause incomplete alveolar process development, lack of normal mesial drift, non-response to orthodontic forces, retained primary teeth and impaction of their successors if present, tipping of adjacent teeth, lateral open bite and higher frequencies of cross bite (Ekim and Hatibovic-Kofman, 2001; Altay and Cengiz, 2002).

A high rate of association (38.4%) has been reported between arrested molar eruption and infraocclusion of deciduous molars when compared to the normal population (4.9%) (Baccetti, 2000). This suggests a relationship exists between the two conditions, with disturbances occurring either in the alveolar bone or in the metabolism process of the periodontal ligament. It is well-known that biological interactions occurring between the alveolar bone and the periodontium are fundamental to tooth eruption and that these interactions are frequently altered in infraoccluded primary molars, leading to tooth ankylosis.

2.4. Associated dental anomalies

As previously mentioned, infraocclusion is considered to be an early indicator for other dental anomalies (Shalish et al., 2010). An increased prevalence of dental anomalies found to be associated with infraocclusion was first described by Peck (2009) as the dental anomaly pattern (DAP). DAP refers to a higher prevalence of a combination of two or more different anomalies in the same patient.

Based on previous studies, Peck summarised the following measurable or visually discrete conditions that can be included as components of biologically-related DAP:

1. Tooth agenesis
2. Microform teeth (e.g. peg-shaped lateral incisors)
3. Tooth-size reduction (generalised or localised)
4. Delay in tooth formation and eruption (generalised or localised)
5. Infraocclusion (most often of deciduous teeth)
6. Palatal displacement of canines
7. Maxillary canine-first premolar transposition
8. Mandibular lateral incisor-canine transposition
9. Distal angulation of unerupted mandibular second premolars

Orthopantomographs and study models of 99 patients with at least one infraoccluded tooth were included in a study investigating the relationship of infraocclusion with other dental anomalies (Shalish et al., 2010). The dental anomalies included:

- Tooth agenesis
- Microdontia of maxillary lateral incisors
- Palatal displaced canines
- Mandibular second premolar distal angulation anomaly

The sample was selected from pre-treatment records of orthodontic patients, including 43 orthodontic patients from Boston, United States, and 56 patients from Jerusalem, Israel. The age range of the selected samples was 8 to 15 years.

The results (Table 2.3) confirmed the presence of a strong association between infraocclusion and other dental anomalies. The fact that these features are not related by anatomical position supports a common genetic origin.

Table 2.3. Prevalence of dental anomalies in association with infraocclusion compared with prevalence in the normal population.

Dental anomaly examined	% of infraocclusion	Reference sample
Tooth agenesis	21.2% (21/99)	5.0% (53/1,064) (Grahnén, 1956)
Agenesis of 3rd molar	38.7% (24/62)	20.7% (427/2,061)(Bredy et al., 1991)
Agenesis of 2nd premolar	20.2% (20/99)	5.8% (58/1,000) (Baccetti, 1998)
Small maxillary lateral incisor	24.2% (15/62)	4.7% (47/1,000) (Baccetti, 1998)
Palatal displaced canine	12.1% (12/99)	1.7% (25/1,450) (Dachi and Howell, 1961)

Derived from Shalish et al. (2010)

In another study by Garib et al. (2009), a sample of 203 Brazilian patients aged 8 to 22 years with agenesis of at least one second premolar were selected from patient files at the orthodontic department of a university dental school and eight private dental offices. Findings revealed a higher prevalence of infraocclusion (24.6%) when compared to the prevalence of infraocclusion in a normal reference population (8.9 %) as reported by Kurol (1981).

The patterns of association between seven types of dental anomalies including infraocclusion were studied (Baccetti, 1998) (Table 2.4). The total sample involved in this study included 4,980 individuals aged 7 to 14 years. The records of this sample were derived from the Department of Orthodontics, University of Florence. No treatment was conducted on these patients.

Infraocclusion was found to be significantly associated with agenesis of second premolars, ectopic eruption of first molars, smaller sized maxillary lateral incisors, enamel hypoplasia, and palatal displacement of maxillary canines when compared to the control group (Table 2.4).

Table 2.4. Prevalence of associated dental anomalies among seven groups of patients.

Dental anomaly examined	% of infraocclusion	% of anomalies in infraoccluded sample	% of anomalies in control group(n=1000)
Agenesis of 2nd premolar	15%	14%	5.8%
Smaller sized maxillary lateral incisors	22%	13%	4.7%
Enamel hypoplasia	20%	12%	4.2%
Ectopic eruption of 1stmolar	20%	18%	4.6%
Supernumerary teeth	4%	4%	3.9%
Ectopic canines	14%	13%	5.2%

Derived from Baccetti (1998)

Results should be interpreted with caution, since the study samples included individuals selected from orthodontic clinics and hospitals, and may not be representative of the normal population. This could be the reason for the high percentage of dental anomalies among the sample.

Bjerklin et al. (1992) studied the association between four groups of eruption disturbances. 1. Ectopic maxillary first permanent molars (n=93), 2. Infraocclusion (n= 9 3), 3. Ectopic maxillary canines (n= 91) and 4. Agenesis of premolars (n= 97). Results revealed that infraocclusion was the most common dental anomaly to occur in association with other dental anomalies. The findings can be summarised as follows:

- Infraocclusion and ectopic maxillary permanent first molars noted in 19 individuals (20.7%)
- Infraocclusion and ectopic maxillary canines noted in 9 individuals (9.9%)
- Infraocclusion and agenesis of premolars noted in 18 individuals (19.4%)

The findings of this study further support the proposition that the occurrence of dental anomalies is often in association with other anomalies rather than occurring as an isolated condition. Moreover, these findings support the idea that infraocclusion is a common dental anomaly which can be used as an early indicator for other anomalies.

An interesting case, reported by Steiner-Oliveira et al. (2007), revealed four infraoccluded primary second molars, agenesis of all second premolars and a peg-shaped maxillary right lateral incisor in the dentition of a healthy 11-year-old girl. As described earlier, tooth development is a complex process, comprising strong genetic control which mediates the signalling pathways controlling the dental developmental process and determining the position, number and shape of several teeth (Brook, 2009). The case of this girl further confirms that dental anomalies may be associated with mutations of one or more genes that play a role in the dental developmental process. However, this does not exclude the possibility that epigenetic and environmental factors also have a role to play in the development of dental anomalies. Since familial tendency has been proposed in the literature (Kurol, 1981; Helpin and Duncan, 1986), it would be interesting to investigate whether any dental anomalies were reported in one or more of the siblings of the affected girl, occurring in the same pattern or occurring with a different pattern of expression.

2.5. Association of dental age with infraocclusion

In a study of the association between agenesis of teeth and dental age it was found that dental development in children with mild-to-moderate agenesis was significantly delayed compared with a control group (Tunc et al., 2011). Indeed, the delay can reach up to 1.5 years, with the severity of the agenesis affecting the magnitude of the delay, and with teeth adjacent to the area being more affected (Uslenghi et al., 2006). Dental age determination has been found to be more accurate when dental development and mineralisation stages are used to determine the age rather than tooth emergence times (Hunt and Gleiser, 1955; Moorrees et al., 1963; Demirjian et al., 1973). This is because the emergence of a tooth is only one point in time, as part of a continuous process involving many stages, until a tooth reaches the occlusal plane.

Tooth emergence timing, as mentioned earlier, is susceptible to local factors such as infection, premature loss of primary teeth, crowding and impaction (Nanci and Ten Cate, 2008). Moreover, tooth emergence can only be used for dental age assessment from six months of age and until the emergence of all the primary teeth at approximately three years of age, and then after six years of age when the permanent teeth start to emerge until 12 years of age. On the other hand, dental development and mineralisation are processes in which all teeth go through the same sequence and they do not appear to be affected greatly by local factors. Also, they can be used over a wider age range (Demirjian et al., 1973).

Methods of dental age assessment

Techniques that can be used to estimate dental age in children include:

1- **Atlas method**: This method consists of using radiographs to compare the stages of dental development and mineralisation between reference material and subjects being examined. An example of this approach is provided by Schour and Massler (1941). The method consists of dividing dental development into 20 stages from early postnatal stages, up to adulthood, with a corresponding chronological age for each stage (Willems, 2001). The atlas method has been further developed to provide dental age estimates for humans, using both tooth development and alveolar eruption between 28 weeks in utero and 23 years (AlQahtani et al., 2010). This approach, developed to aid forensic odontology and mass disaster identification, provides single-year intervals in the stages from early childhood (24 months) until adulthood (23½ years) which are not age specific in terms of months and days.

A system introduced by Moorrees et al. (1963) provided dental maturation tabulated values, derived from radiographs of males and females separately. This was done by dividing dental development into 14 stages, starting from calcification of the tip of the cusp/incisal edge to apical closure. The stages of root development were illustrated by diagrams, with a score calculated (through statistical analysis) for each tooth, at each stage (Moorrees et al., 1963). This method was developed further by Anderson et al. (1976) to include the mean age of attainment of mineralisation of maxillary and mandibular teeth, including all stages of the 3rd molar. Other systems which determine dental age by root development, as well as crown development are considered difficult to apply as they require scoring the root length, where a portion of the root is either missing or not fully developed (Fanning, 1961; Maber et al., 2006). This can be misleading or impossible to do (e.g. age estimation in the living).

2- **Scoring system:** The best known scoring approach was introduced by Demirjian et al. (1973). In this method, dental development is divided into eight stages, A-H, with only teeth in the mandibular left quadrant being scored (from A to H), with individual scores summed to produce a total score. A statistical analysis was done to produce a maturity score for both boys and girls (Demirjian et al., 1973).

This approach has been widely used since it was first introduced. Although it involves several steps, it produces reasonably accurate estimates of dental age because it depends on the stages of development of several teeth. Many authors have chosen this system as their method of choice, as the radiographs, diagrams and clear written instructions provided are very useful in assessing the process of dental age determination (Chaillet et al., 2004; Maber et al., 2006).

When the Demirjian system has been assessed for accuracy (in terms of how closely the dental age and chronological age match) it has been shown to produce a consistent over-estimation of dental age in several populations. For example, an overestimation of 0.5 years for boys and 0.6 years for girls was found in one report, for Belgian boys and girls (Willems et al., 2001); an over-estimation of 1.6 years was found in a sample of 615 South Australian children (McKenna et al., 2002); and in another report using a sample from Sydney, the use of Demirjian system resulted in consistent over-estimation of dental age up to 0.99 years in children under the age of 14 (Blenkin and Evans, 2010). The Demirjian scores were originally derived from a French-Canadian sample. Thus, more accurate results of dental age estimation are obtained when the scoring system is adapted to the specific ethnic group being studied. This can be related to the fact that some histological evidence shows that there is a small variation in the duration of crown formation between different ethnic groups (Maber et al., 2006).

In the literature, associations have been reported between dental age and the occurrence of dental anomalies (Figure 2.11). Specifically, cases that display dental anomalies have been shown to have delayed dental development (Becker and Chaushu, 2000). Patients with maxillary canine displacement have often presented with delayed dental development, prompting the investigation of dental age in association with maxillary canine displacement (Zilberman et al., 1990; Becker and Chaushu, 2000; Naser et al., 2011; Rozylo-Kalinowska et al., 2011). Delayed dental development was found in cases showing canine displacement (Becker and Chaushu, 2000; Rozylo-Kalinowska et al., 2011), while other studies have reported delayed dental age is more commonly associated with palatally displaced canines compared with labially displaced canines (Naser et al., 2011). The latter finding suggests there may be a different aetiology for each condition. Given these studies used different dental age scoring systems, and their samples' age groups and sample sizes varied, making comparisons between the studies is questionable.

2.6. Association of tooth size with infraocclusion

Microdontia (reduction in tooth size), like many of the other dental anomalies, displays a multifactorial aetiology and in most occasions occurs in association with other dental anomalies (Brook et al., 2009b).

Reduction in tooth size and form is apparent in patients with agenesis. Indeed, a direct relationship has been found, that is, the greater the number of missing teeth, the greater are the chances of finding associated microdontia upon clinical examination of the same individual (Figure 2.10) (Garn and Lewis, 1970; McKeown et al., 2002; Brook et al., 2009a). It has been reported that microdont maxillary lateral incisors on one side of the arch may be associated with agenesis of maxillary lateral incisors on the other side of the arch (Rantanen, 1956). Moreover, in cases of agenesis of the third molar, a reduction in the size of the permanent first molar has been reported (Lavelle et al., 1970). In another study, reduced mesiodistal width was reported in association with agenesis (Baum and Cohen, 1971). The association of agenesis with microdontia suggests a common etiological factor or factors (Brook et al., 2009b).

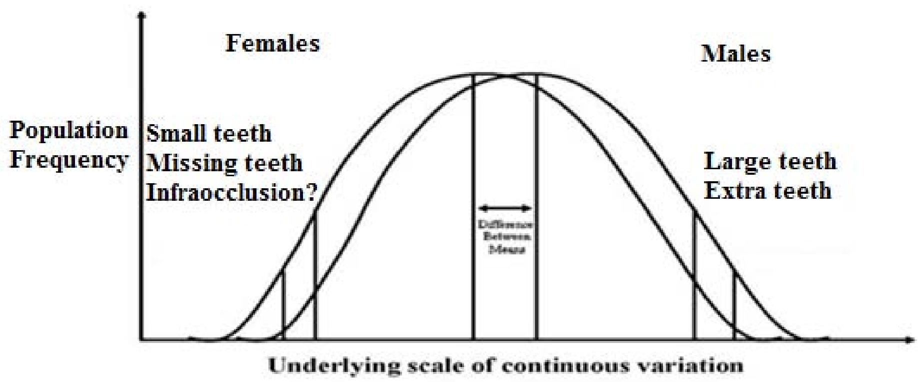


Figure 2.10. Model of normal distribution illustrating the relationships between anomalies of tooth number and size and possible association of infraocclusion, modified from Brook (2009).

Likewise an association exists between infraocclusion of primary molars and agenesis of premolars (Bjerklin et al., 1992; Baccetti, 1998). Agenesis of the second premolars was found to be associated with infraocclusion of the first primary molars in 18% to 22% of subjects (Baccetti, 1998).

Since agenesis has been linked to microdontia and infraocclusion in previous studies, it is possible that there is a relationship between microdontia and infraocclusion (Figure 2.11).

However, to the best of the author's knowledge, there have been no studies investigating the association between microdontia and infraocclusion. Thus, further studies in this area would be beneficial.

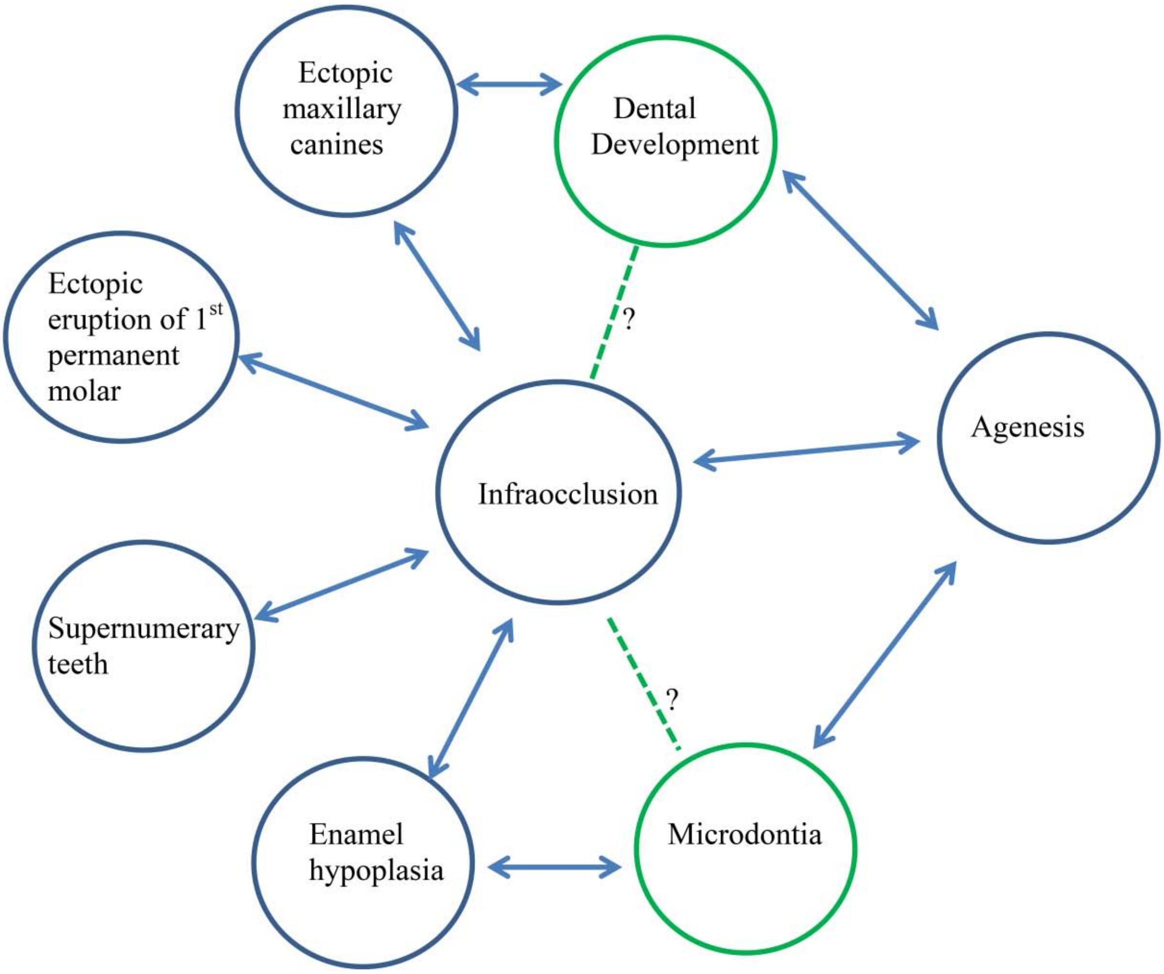


Figure 2.11. Diagram showing the relationships of infraocclusion with other dental anomalies. The double arrow represents a significant relationship, while the dashed line represents no known relationship, modified from Baccetti (1998).

Methods of tooth size measurement

Tooth measurement can be obtained using digital hand-held callipers by direct intraoral measurement or from study models, radiographs, computed tomography, 2D images and 3D scans (Brook et al., 1999; Lahdesmaki and Alvesalo, 2004; Olejniczak and Grine, 2006; Yitschaky et al., 2011). Using hand held callipers, whether by direct intraoral measurement or on study models, is associated with difficulties e.g. difficult to access posterior teeth using direct vision or damage to the study models. Measurements from radiographs are limited due to possible distortion. Using orthopantomographs is better for recording vertical measurements rather than recording horizontal measurements due to the direction of the cone beam. The use of 2D image analysis is a standardised system in which a digital camera is used to obtain high resolution images of study models. The 3D scan is a laser imaging system that produces a 3D image of the study models. This allows more detailed analysis of various dimensions including curvature, angles and volume. These imaging systems allow easy identification of landmarks making measurement more precise and accurate (Ribeiro et al., 2013).

2.7. Application of different twin models to study genetic influences on infraocclusion

Twin studies enable researchers to quantify the relative contributions of genetic and environmental factors to variation in a variety of traits. Two types of twins have been identified, monozygotic (MZ), or so-called identical twins, where the co-twins share 100% of their genes, and dizygotic (DZ), or so-called fraternal twins, who share 50% of their genes on average (Townsend et al., 2009).

When studying twins, several different research methods can be used.
These methods include:

1- The classical twin method involves comparing similarities in MZ twin pairs with those in DZ twin pairs. Assuming similar environmental influences on both twin groups and that they have been sampled from the same gene pool, it is possible to estimate the contribution of genetic and environmental influences to observed variation in traits. The extent of genetic contribution to the variation of phenotypes can be studied by calculating heritability estimates (Townsend et al., 2009). Heritability estimates, denoted by the symbol h^2, can vary from 0 to 1, and they refer to the contribution of genetic factors to observed phenotypic variation (Townsend et al., 2009)

2- The monozygotic and dizygotic reared-apart twin method, involves studying twins who were separated shortly after birth (MZA and DZA). This is a simple but powerful approach as it minimises the confounding effects of common environmental factors and reveals the role of genetic factors on phenotypes. This method has been used mainly to compare the behavior of twins, including their abilities, personalities, interests and social attitudes (Bouchard et al., 1990).

3- The half-sib method, involves studying genetic and environmental contributions to variation in MZ twins, their spouses and their offspring. Its advantage lies in the fact that the children of MZ co-twins who are born to different mothers are themselves genetically half-siblings. This method also offers the opportunity to detect maternal effects and to assess the importance of assortative mating, as multiple associations between MZ twins and their spouses can be explored (Townsend et al., 2009).

4- Opposite-sexed DZ model: This approach focuses on male-female twin pairs and tests whether there are differences in mean values and variances for selected features between these twins compared with other twin types and singletons that might reflect hormonal influences between male and female twins in utero (Townsend et al., 2009).

5- MZ co-twin design: Monozygotic twin pairs are matched perfectly for age and sex and, as they share 100% of their genes, observed differences can be attributed to differences in environmental factors or epigenetic events (alterations in gene expression without changes in nucleotide sequencing) (Townsend et al., 2003). This model provides a powerful approach to clarify the roles of genetic, epigenetic and environmental influences on normal features, or susceptibility to diseases. In this model, MZ twin pairs who show different phenotypic expressions for a particular trait or disease are investigated. Alternatively, researchers can manipulate the environment so that each member of a twin pair is exposed to different environmental conditions; for example, MZ co-twins may be treated with different orthodontic appliances (Pangrazio-Kulbersh and Berger, 1993) and the outcomes compared, or the severity of periodontal disease might be compared between MZ co-twins where one is a smoker and the other is not (Townsend et al., 2006).

A dental example of the MZ co-twin design is that of variation in the expression of missing, peg-shaped, diminutive and normally-formed maxillary lateral incisors within pairs of MZ twins. These associated features conform to a multi-factorial threshold model linking tooth size with presence and absence of teeth (Brook et al., 2009b). Several examples have been reported in Australian MZ twin pairs, where one member of the pair displays agenesis of one or more maxillary lateral incisors, whereas the other shows diminutive or peg-shaped teeth. Discordance in dental expression has also been reported, where one member shows agenesis of a tooth on the left side and the other member shows agenesis of the same tooth on right side, an example of so-called mirror imaging (Townsend et al., 2006). Another example of different phenotypic expression of a dental feature within MZ twin pairs is varying expression in the number of supernumerary teeth (Townsend et al., 2009). Given that considerable insights can be gained by applying this model to only one set of twins, the MZ co-twin method is a feasible and potentially valuable approach to apply within dental practices (Townsend et al., 2003).

Differences in the expression of phenotypes (V_P) may be explained as the result of both genetic and environmental influences. The total genetic influence on a phenotype (V_G) can be categorised into: additive effects of alleles (V_A), dominance effects between alleles at the same locus (V_D), and epistatic interactions between loci (V_I). Likewise, the total environment influence on a phenotype (V_E) can be categorised into: common environmental affecting both twins (V_{EC}) and individual environment affecting one twin (V_{EW}) (Hopper, 1993).

Therefore, there are several components that can contribute to the total phenotypic variation of a multifactorial trait (Dempsey et al., 1999). This can be represented as follows:

$$V_P = V_G + V_E$$

$$V_P = V_A + V_D + V_I + V_{EC} + V_{EW}$$

It is unlikely that the relationship between these different contributions to phenotypic variation is a simple additive one, and other interactions also need to be considered. For example, genotype-environment correlation (CorGE) occurs when the phenotypes expressed by an individual are a result of selected environmental factors correlated with an individual's genotype. An example of this effect would be parents who raise their children in a home environment that is influenced by their own heritable characteristics rather than a randomly selected environment. Another factor is genotype by environmental interaction (GxE) which describes how different genotypes maybe expressed differently according to the environment (Hopper, 1993). An example of this would be found among individuals who are carriers of xeroderma pigmentosum (XP) mutation. The effect of the XP mutation on an individual is a significantly increased risk of developing skin cancer when exposed to ultraviolet light, compared to individuals who are not carriers of the XP mutation. In theory, if individuals with XP mutations completely avoid ultraviolet light their risk of skin cancer becomes similar to the risk factor in the general population (Hunter, 2005).

As mentioned earlier, MZ co-twins share 100% of their genes while DZ twins share, on average, 50% of their genes. Because twins develop prenatally in the same uterus it is expected they would share the same common environmental factors, however, this is not always the case. An example is Twin to Twin Transfusion Syndrome (TTTS) where an unequal distribution of blood occurs between MZ twins sharing the same placenta. Post-natally, some of the common environment experienced by twins may be imposed by their parents or other family members, while other common environmental factors may be elected by the twins such as similar interests and behaviours due to the fact that they spend most of the time together (Morris-Yates et al., 1990; Neale and Cardon, 1992). An underlying assumption of the traditional twin model is that common environmental factors acting on MZ twins are similar to those acting on DZ twins. Thus, the component of phenotype variation due to common environmental factors is assumed to be similar. It can be postulated that the expected phenotypic covariance of MZ twins is equal to $V_A + V_D + V_C$, and the expected phenotypic covariance of DZ twins is equal to $0.5\ V_A + 0.25\ V_D + V_C$. Therefore, the maximum expected correlation (r) for MZ twins is 1.0 and for DZ twins is 0.5 and the concordance of the MZ twins is 100% and for DZ twins is 50%. This information forms the baseline for genetic structural equation modelling (GSEM), a process in which information from twin data provides maximum likelihood estimates of variance components. For example, if the correlation between DZ twin pairs is reduced, leading to the value of correlation between MZ twin pairs being more than twice as large as the value of correlation between DZ twin pairs, then some dominant genetic variance (V_P) is expected. On the other hand, if the correlation between MZ twin pairs is less than twice as large as the correlation between DZ twin pairs, there is evidence of common environmental variance (V_C) (van Dongen et al., 2012).

Heritability estimates (h^2) indicate how much of the expressed phenotype is due to genetic variation. Broad-sense heritability is the percentage of phenotypic variation due to all genetic sources of variance, and is estimated as V_G/V_P. Narrow-sense heritability is the percentage of phenotypic variation due to additive genetic variance, and is estimated as V_A/V_P (Hopper, 1993; Dempsey et al., 1999). Heritability is a population concept, referring to the proportion of genetic variation within a given population at a particular time and should not be applied to a single individual but, rather, to a group of individuals (Townsend et al., 2006).

In this study, the classical twin method will be used to clarify whether there is a genetic contribution to variation in expression of infraocclusion. Initially, comparisons of similarities in infraocclusion among MZ twin pairs will be made with similarities in infraocclusion among DZ twin pairs, assuming similar environmental factors, to determine whether there is any evidence of a genetic influence on variation of infraocclusion. Another method that will be used is the MZ co-twin model which will help to reveal evidence of environmental and/ or epigenetic factors contributing to different expressions of the condition in members of the same twin pair. More sophisticated genetic modelling will also be performed.

As mentioned earlier, there are only a few previous reports of infraocclusion in twins (Table 2.5). One case report of a pair of 8-year-old male MZ twins described how both co-twins showed exactly the same pattern of infraoccluded teeth, the teeth affected being the primary maxillary and mandibular first molars, and the primary mandibular second molars (Stewart and Hansen, 1974). In an epidemiological and familial study, comprising five twin pairs, three of the pairs were found to have infraocclusion in both co-twins, but the zygosity and teeth involved were not reported (Kurol, 1981). In another report, the example of a pair of 10-year-old male MZ twins was described, with both members of the twin pairs showing a similar pattern of infraocclusion, and the rest of the teeth between co-twins being similar in appearance (Helpin and Duncan, 1986). A further report on two MZ twin pairs described, one pair of 9-year-old males in whom both co-twins had infraocclusion of the primary second mandibular molars and another pair of 12-year-old females who expressed a different pattern of infraoccluded teeth. For the MZ twin females, all primary maxillary and mandibular first molars were infraoccluded but, in addition, the primary mandibular second molars were also infraoccluded in one of the co-twins (Dewhurst, Harris and Bedi, 1997).

Another study reported 19-year-old MZ twin pair males with similar tooth agenesis and impacted primary teeth (Zengin et al., 2008). In one member of the twin pair the primary maxillary first molars on both sides were retained with agenesis of the permanent successor and the primary mandibular second molars on both sides were retained with agenesis of the permanent successor. In the other member of the pair the primary maxillary first molars on both sides and the primary maxillary right second molar were retained with agenesis of the permanent successor and the primary mandibular left second molar was retained with agenesis of the permanent successor.

Strong familial tendency of infraocclusion was reported in the case of a 10 year-old girl with retained primary maxillary right and left second molars and primary mandibular right first and second molars associated with severe infraocclusion. On radiographic examination it was revealed that the permanent successors were present and the mandibular right lateral incisor was missing. On examination of the girl's siblings, 8.5-year-old male twins, it was shown that both members of the twin pair had infraocclusion of the primary maxillary second molar, one twin had infraocclusion on the right side while the other member had infraocclusion on the left side. The zygosity of this twin pair was not reported (Cozza et al., 2004).

Table 2.5. Summary of the published findings on infraocclusion in twins.

	No. of twin pairs	Zygosity	Sex	Age	Infraocclusion Twin 1	Infraocclusion Twin 2	Teeth	
Stewart and Hansen (1974)	1	MZ	M	8	✓	✓	D E D	D D E
Kurol (1981)	1	?	?	?	✓	✓	?	
	2	?	?	?	✓	✓	?	
	3	?	?	?	✓	✓	?	
Helpin and Duncan (1986)	1	MZ	M	10	✓	✓	ED 	 DE
Dewhurst, Harris and Bedi (1997)	1	MZ	M	9	✓	✓	 E	 E
	2	MZ	F	12	✓	✓	D D	D DE*
Cozza (2004)	1	?	M	8.5	✓	✓	E(twin 1) 	E(twin 2)
Zengin (2008)	1	MZ	M	19	✓	✓	ED* E*	E E

✓ Infraocclusion present
? Unknown
* These teeth are affected in one co-twin only

2.8. Management of infraoccluded teeth: prevention and treatment

Treatment of infraoccluded primary teeth is influenced by the degree of the abnormality, presence of a succeeding permanent tooth, and the time of onset (Karacay et al., 2007). However, it has been suggested that infraoccluded teeth should not be extracted as a treatment of choice to overcome the risk of space loss (Kurol and Thilander, 1984b). Rather, it has been proposed it is better to wait for normal exfoliation to occur, except in specific circumstances, such as severe tipping of adjacent teeth with space loss, or irregular primary root resorption related to severe infraocclusion (Kurol and Thilander, 1984a). In other cases associated with tipping of adjacent teeth and supra-eruption of the antagonist, treatment may include orthodontic intervention followed by extraction (Ekim and Hatibovic-Kofman, 2001).

In situations where an infraoccluded tooth has good remaining root and crown structure with only aesthetic improvement required, treatment can be provided using the following assessment criteria:

- General assessment: patient's health, motivation, expectations and oral health
- Local assessment: clinical examination of the coronal shape, colour and structural integrity of the primary teeth. The gingival level of the infraoccluded teeth and their relationship to the occlusal plane should be noted as it is often coronal to that of the permanent teeth. Interocclusal space may be reduced if primary teeth have worn allowing over-eruption of opposing teeth.
- Radiographic assessment: assessment of the length and form of the remaining root structure, apical status and periodontal support. The rate of root resorption can be noted by comparing a series of radiographs, if available.

By completing a detailed assessment, it should be possible to predict the prognosis of the retained primary tooth, leading to better decision making in regards to treatment planning. Several studies have shown mandibular and maxillary primary canines and second molars have a much better prognosis than mandibular and maxillary primary incisors and first molars (Stanley et al., 1996; Haselden et al., 2001). A retrospective radiographic study (mean examination time span 12.4 years) comprising 20 individuals with retained primary teeth and a total of 24 teeth, showed minimal root length changes associated with the retained teeth. This finding is encouraging in terms of retaining primary teeth and avoiding extraction as a treatment of choice (Sletten et al., 2003). Another important factor that can influence management options is the degree of bone loss. Bone loss was studied in 68 individuals with 119 infra-occluded primary molars, with only two cases of significant bone loss being noted (Kurol and Olson, 1991).

Treatment options

It is generally agreed that treatment of infraocclusion should aim to provide the most effective yet conservative and minimally invasive treatment. Treatment should also satisfy patients in terms of aesthetics and function, as well as cost effectiveness.

Treatments options include:

- Retention of the primary tooth and reshaping with a direct composite or indirect restoration, e.g. composite, porcelain or gold onlays. This approach has the advantage of reduced intervention and preservation of the underlying bone and soft tissue.
- Extraction of the primary infraoccluded tooth in the case of crowding, followed by orthodontic treatment for space closure.
- Extraction of the primary infraoccluded tooth, in the case of periodontal or periapical disease and replacement with a prosthesis where there are poor aesthetics, e.g. fixed replacement, conventional bridge, resin-bonded bridge, dental implant supported crown or bridgework (Robinson and Chan, 2009; Aktan et al., 2011).

As part of the management process, general screening of the erupting dentition and the permanent succedaneous teeth should be conducted by obtaining the required radiographic images e.g. orthopantomographs periodically. This is justified by the fact that infraocclusion may occur in association with other dental anomalies and can be the first sign noted clinically. An early radiograph with proper follow up as required at the time of initial diagnosis allows the clinician to provide early diagnosis and treatment planning. Importantly it provides the clinician with the required information for counselling and reassuring the patient and care-givers.

2.9. Summary

The term ‘infraocclusion’ refers to a tooth that is positioned below the normal level of occlusion. This condition has been identified in association with local and systemic disturbances. A genetic influence on infraocclusion has been proposed following reports that have shown that the condition tends to occur in siblings. Also, a higher prevalence (12%-39%) of infraocclusion has been found in cases displaying one or more other dental anomalies, a situation described as Dental Anomaly Pattern (DAP) (Peck, 2009; Shalish et al., 2010).

Previous estimates of the prevalence of infraocclusion involving at least one primary tooth in an individual vary between 1.3% and 38.5%, with no significant differences reported between males and females. The primary first mandibular molar has been reported as being the most commonly affected tooth. It has often been anticipated in the past that infraocclusion is associated with agenesis of the succedaneous tooth. However, this is the case in only 18 % to 24.6 % of individuals (Kurol and Thilander, 1984b; Baccetti, 1998). In the remaining cases, the permanent tooth is present.

Ankylosis has been suggested as an important factor in the development of infraocclusion. However, not all infraoccluded teeth are ankylosed. Indeed, infraocclusion can be associated with many other factors, including crowding, loss of space, trauma to teeth, agenesis of second premolars, and also agenesis of maxillary lateral incisors. In several previous reports, agenesis of permanent teeth has been associated with a significant delay in dental development (Kan et al., 2010; Tunc et al., 2011) and also reduction in tooth size (McKeown et al., 2002; Brook et al., 2009a). Since infraocclusion is associated with agenesis, further investigation is needed to clarify whether there are associations between infraocclusion, delayed dental development and reduced tooth size. This could involve comparing the group of cases where infraocclusion occurs together with the presence of permanent succedaneous teeth, to those cases where infraocclusion occurs with no permanent successors present.

Infraocclusion can be identified using orthopantomographs, study models and intraoral photographs. These recording methods allow measurement of the condition and also categorisation of the level of infraocclusion. However, there is no one standardised technique of measurement or a single categorisation system, making it difficult to compare between different studies. Thus, a deficiency in available measurement techniques required the adoption of new measurement technique in this study involving both numerical and categorical criteria.

The consequences associated with infraocclusion can result in complex problems in the dental arch, such as: alignment issues, tipping of adjacent teeth, supra-eruption of antagonists and dislocation of permanent teeth lying under the primary tooth. Management options are determined by the severity of the situation and the associated consequences. Extraction is not considered the treatment of choice, with retention of the affected tooth until normal exfoliation a more favoured option.

The main factors associated with infraocclusion have not yet been clearly identified and, although a genetic correlation with other dental anomalies has been suggested, the nature of any genetic influences has not been elucidated. Moreover, the epigenetic and environmental factors that contribute to this condition have not been investigated in any detail. Thus, further understanding of these factors will enhance the knowledge base of clinicians in order to provide proper counselling, early diagnosis, prevention and treatment planning for affected individuals. If a strong genetic association is shown to exist, further genetic analyses may be undertaken in order to identify the genes involved. This is particularly critical where infraocclusion is found to occur in association with other dental and systemic anomalies and/or syndromes.

3. Aims, hypotheses and significance

The broad aim of this study is to investigate the occurrence of infraocclusion in samples comprising singletons and twins to advance understanding of the presentation and aetiology of the condition. This will involve determining whether there are associations between infraocclusion and several variables, including sex, age, arch type (maxillary/mandibular), arch side (right/left) and tooth type. Further, it is planned to clarify the roles of genetic, epigenetic and environmental factors on infraocclusion by applying the traditional MZ/DZ twin model, as well as the MZ co-twin model, to data collected from a sample of twins.

In addition, it is aimed to determine the frequency of occurrence of some selected dental anomalies in association with infraocclusion in the singleton sample, including the following:

- Ectopic maxillary canines
- Lateral incisor complex including: agenesis, diminutive or peg-shaped lateral incisors
- Agenesis of the premolars

Another aspect of this research will be to investigate dental development, which will be assessed by comparing the dental age of the singletons with infraocclusion to their chronological age, in order to clarify whether the presence of infraocclusion is associated with delayed dental development.

Furthermore, to investigate if an association between infraocclusion and tooth size exists, the maximum mesiodistal crown width of selected teeth from the twins' study models displaying infraocclusion will be measured and compared to a similar sample with no infraocclusion.

Major hypotheses to be tested are:

1- The primary mandibular first molar is the most commonly infraoccluded tooth in singletons and twins

2- The frequency of occurrence of infraocclusion is similar in samples of singletons and twins

3- MZ twin pairs display greater similarity in expression of infraoccluded teeth compared to DZ twin pairs

4- Some MZ twin pairs show differences in the expression of infraocclusion, as well as presenting examples of mirror-imaging

5- Infraocclusion is not an isolated condition but can occur in association with other dental anomalies

6- Dental age is delayed in cases of infraocclusion in singletons and twins

7- Dental crown size is reduced in cases of infraocclusion in singletons and twins

8- Family members of twin pairs displaying infraocclusion are more likely to show infraocclusion and/ or other dental anomalies

9- Study models examined at three different stages of dental development (primary, mixed, permanent) display signs of infraocclusion in the first stage and display infraocclusion, associated anomalies and/ or consequences of infraocclusion in the third stage.

Significance

This study will be significant in improving our understanding of the basic biological mechanisms and associated features of infraocclusion. It is also anticipated that the findings will assist clinicians in providing proper counselling, early diagnoses, prevention and treatment planning for affected individuals. If a strong genetic association is shown to exist, further genetic analyses may be undertaken in order to identify the genes involved.

4. Methodology

4.1. Study samples

This study analysed data collected from two existing samples.

1. Singleton sample (n=1,454 orthopantomographs)
2. Twin sample (n=320 individual study models)

The first sample comprised healthy singleton children, Finnish boys and girls (all of European ancestry), aged between 9-10 years. Orthopantomographs (OPGs) (n=1,454) obtained during the period 1980-1997 by the Health Centre of Lapinlahti Council, Finland, were made available for this study by a colleague, Dr. Raija Lahdesmaki (University of Oulu, Finland). Originally, the purpose of obtaining the radiographs was to evaluate dental development, including missing or supernumerary permanent teeth, as well as abnormal eruption patterns. However, during the early stages of this research planning, Dr Lahdesmaki was in Adelaide and it was agreed that the OPGs would provide a valuable resource to assess infraocclusion. The radiographs were obtained using Cranex dc 2 (Serial number 852315) and Planmega proline xc (serial number xc426206) machines. The OPG files were then scanned and de-identified during 2006/07 at the Health Centre of Lapinlahti Council, Finland, following permission being obtained from the Head of the Dental Care Department, Mr Risto Mannberg.

The second sample comprised MZ and DZ twins, aged between 8-11 years, who form part of a larger study conducted over the past 30 years by the Craniofacial Biology Research Group, School of Dentistry, University of Adelaide, led by Professor Grant Townsend. The entire study includes records from over 1,200 pairs of twins. These are divided into three main cohorts with approximately 300 twin pairs in each of cohorts 1 and 2, and around 600 twin pairs in cohort 3 (Hughes et al., 2013). The zygosities of over 600 MZ and same-sex DZ twins have been confirmed. For the twins in cohort 1, zygosity was determined by comparisons of genetic markers in the blood, including serum enzyme polymorphisms and protein polymorphisms. For twins in cohort 2, zygosity was confirmed using DNA from buccal cells to analyse six highly variable genetic loci on six different chromosomes (Hughes et al., 2013). The probability of dizygosity, given concordance for all systems, was less than 1 per cent (Townsend et al., 2005). Information available from cohorts 1 (n=300 pairs) and 2 (n=300 pairs), which will be used in this study, includes records of teeth present, dental caries, other dental problems (e.g. anomalies in the pattern of eruption), study models and intraoral photographs (Townsend et al., 2006; Hughes et al., 2013).

4.2. Measurement methods and criteria for selection

Pilot study

To establish the criteria for assessment of infraocclusion, 20 OPGs of individuals with no infraocclusion (assessed by visual examination) were compared with 50 OPGs of individuals with infraocclusion (also assessed by visual examination). This enabled the degree of practicality of the proposed measurement technique to be determined for use on both OPGs and study models. Adobe Photoshop CS5 computer software was used on the OPGs for constructing lines between reference points and measuring distances (in mm) between reference points, taking into consideration a magnification scale of 3cm on all OPGs. This software was selected because of its many useful features, e.g. drawing lines, measurements of scales, formulation of data tables.

To check the adequacy of the Adobe Photoshop CS5 computer software package, measurements were also carried out on a small sub-sample of OPGs using another software package (ImageJ) to ensure the results between the two software packages were comparable.

Measurement techniques

Orthopantomographs (OPGs)

To determine the 'occlusal plane', a line was extended from the mesial marginal ridge of the first permanent molar to the cusp tip of the primary canine (Figure 4.1a). This was constructed to reflect the orientation of the arch (i.e. the curve of Spee). The position of the mesial marginal ridge was determined by visualising where the base of the mesiobuccal cusp ridge was located. Next, the 'occlusal table' of the primary first molars was constructed by drawing a line from the distal marginal ridge to the mesial marginal ridge of the primary first molar, using the base of the mesial and distal cusp ridges as landmarks. A similar line was constructed for the primary second molar (Figure 4.1b). Following this, a line was drawn perpendicularly, from the mid-point of the 'occlusal table' to the 'occlusal plane', and this distance was measured (mm). A magnification scale, dependent on the type of machine used to obtain the OPG, (see calculations below) was applied in order to record actual measurements.

Magnification scales:

1- Cranex dc 2, Soredex (serial number 852315), (all radiographs taken on or before the 14th March 2006) - magnification 1.3x

2- Planmega proline xc, Plandent OY (serial number xc426206), (all radiographs taken on or later than the 15th March 2006) - magnification 1.2x

Both upper and lower arches, as well as right and left sides, were measured with cases being excluded where:

- permanent first molars were partially erupted
- primary teeth to be measured had large surface restoration(s) which interfered with the measurement criteria
- there was significant loss of tooth structure due to caries and/or tooth wear
- reference teeth were missing

- it was not possible to obtain reliable measurements, e.g. OPG had significant distortion

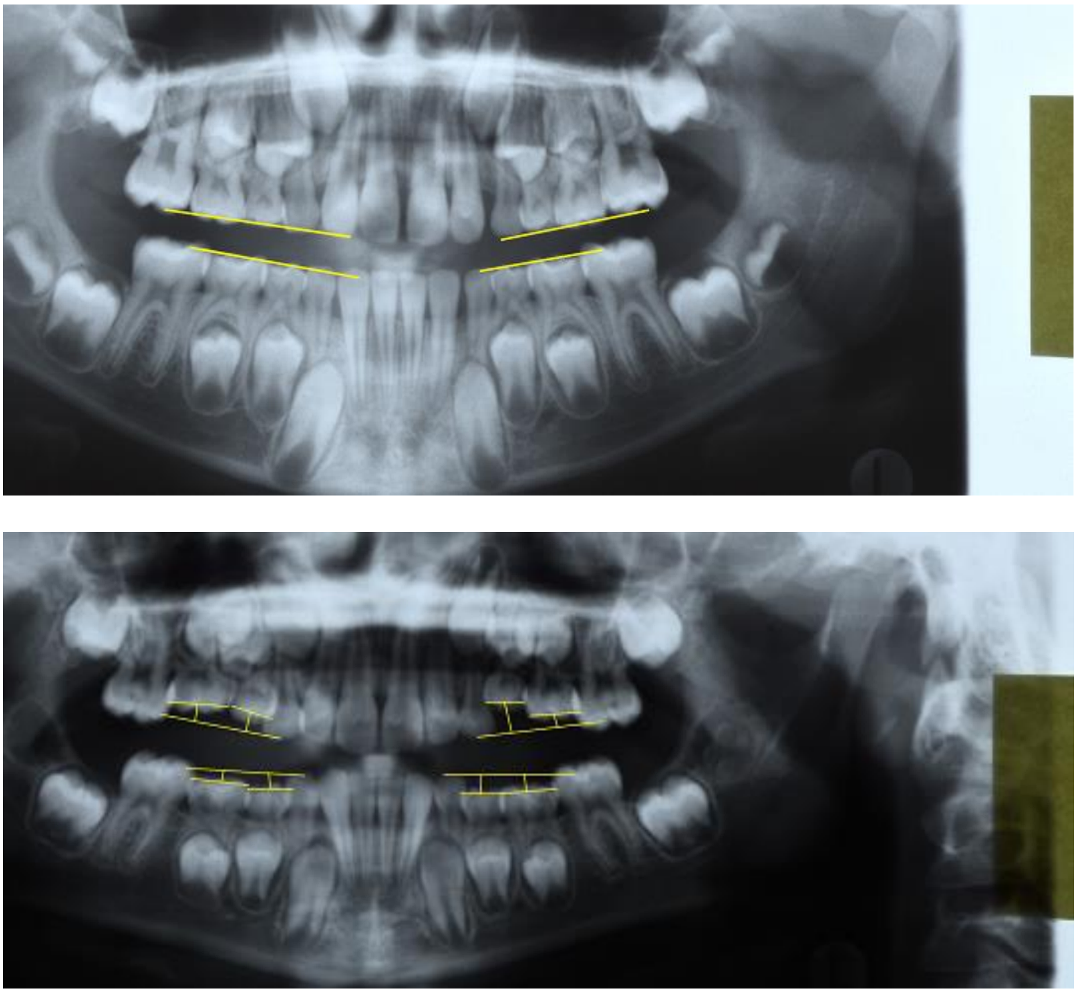


Figure 4.1.a. Construction of the occlusal plane in cases where no infraocclusion was present, to determine the reference points b. An example representing construction of lines to measure the level of infraocclusion.

Study models

The same methodology was used for data collected from the twins' study models. In order to minimise possible damage to the study models that can occur from direct measurements, a 2D scanning system was used to obtain four images per individual, that is, upper and lower images for each side of the arch. The 2D imaging system that was used in this study was adapted from the system described by (Brook et al., 1999) (Figure 4.2). This system comprised a Canon EOS D50 digital camera with a 100 mm lens *(Canon Inc, Tokyo),* a laptop and a copy stand *(Kaiser, Odenwald).* Other equipment included a portable study model holder and adjustable table used to position the study models vertically. A surveyor was used to level the occlusal plane for each study model according to specified reference points (Figure 4.3). The study models were then aligned parallel to a ruler with a 1 cm scale to determine the magnification (Figure 4.4). The camera was kept in a fixed position enabling a lateral view of the models to be obtained. The quality of the images was standardised by ensuring that the ruler and dental cast were in focus and the camera settings fixed and checked prior to every shoot (Table 4.1).

Table 4.1. Camera settings used to ensure quality and standardisation for all photographs.

F-settings	F22
Exposure time	1/20
ISO speed	125
Focal length	100
Temperature	5400K
Light setting	5 (L and R) at “15” marking

Figure 4.2. 2D imaging system used to assess infraocclusion.

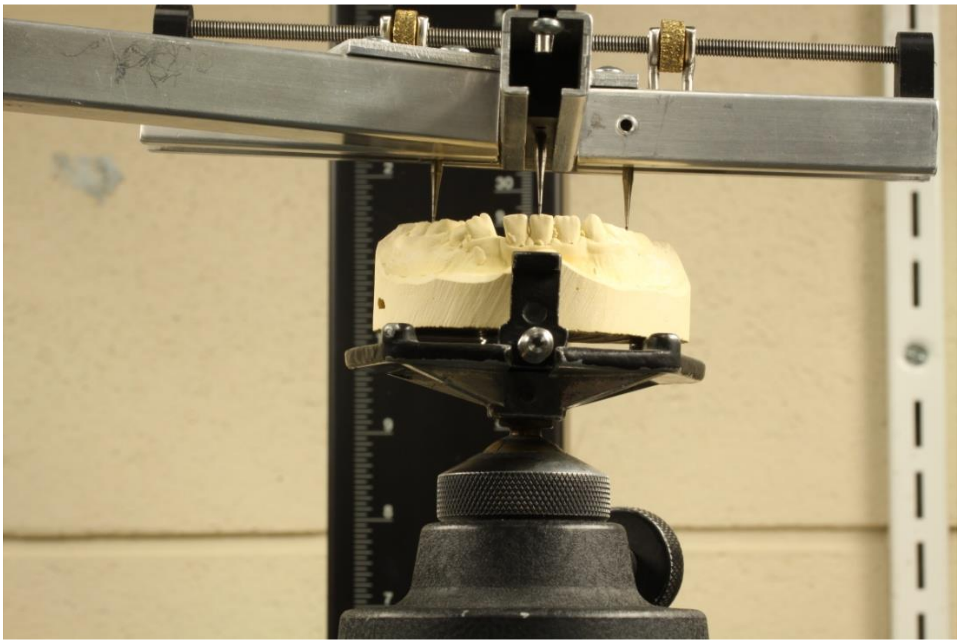


Figure 4.3. A surveyor was used to level the occlusal plane for each study model, according to specified reference points.

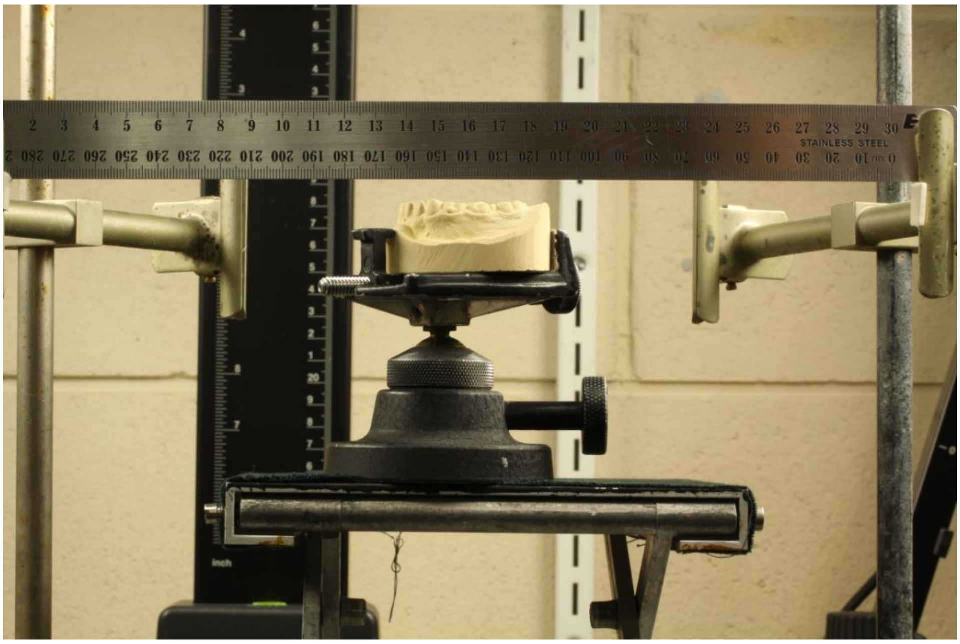


Figure 4.4. Portable study model holder and adjustable table used to position the study models vertically.

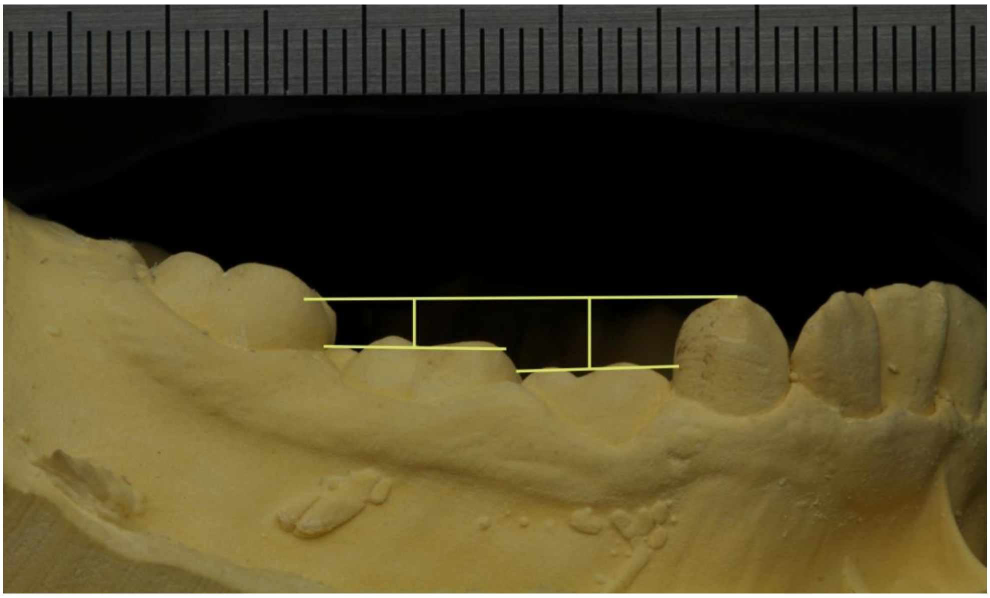
The camera was connected to a personal computer and all images were obtained by remote control. The exclusion and inclusion criteria were similar to those applied to OPGs.

In addition, cases were excluded where measurement of the models was not possible due to damage to teeth affecting one or more of the reference points.

All photographs were saved on an external hard disk in jpg format and identified by: twin ID, twin A/B, upper/lower and right/left, e.g. 342A-UL_ jpg. Measurements were then obtained from the standardised photographs using the Adobe Photoshop CS5 computer software, in the same way as described earlier for the OPGs (Figure 4.5). By using the same measurement technique for the study models as for the OPGs, it was possible to compare results obtained from two different sources of data. Examples of final 2D images of the study models are shown in Figure 4.5.

Twin 458 A

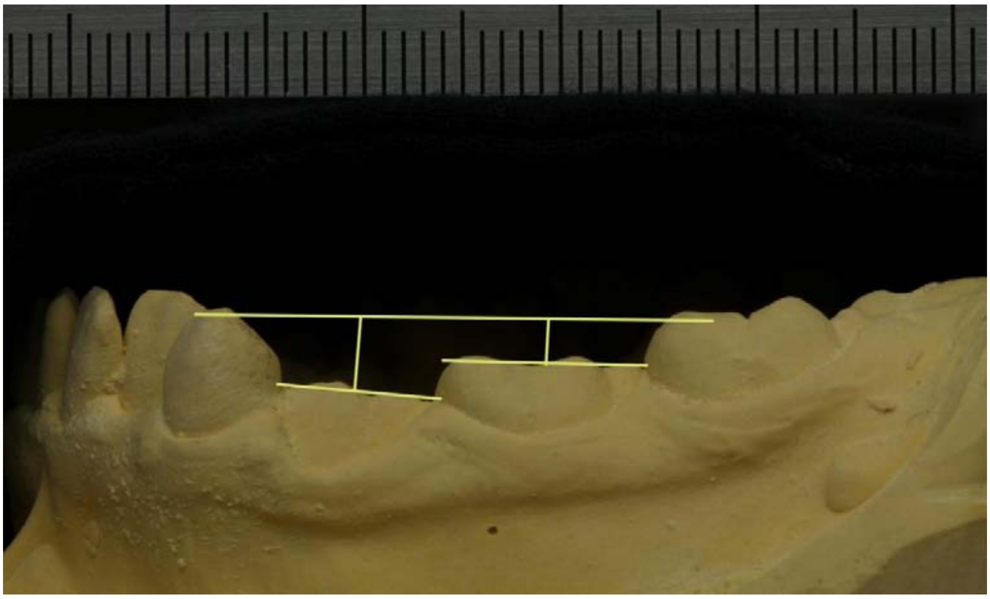
5a. 458A-lower right



Scale in mm

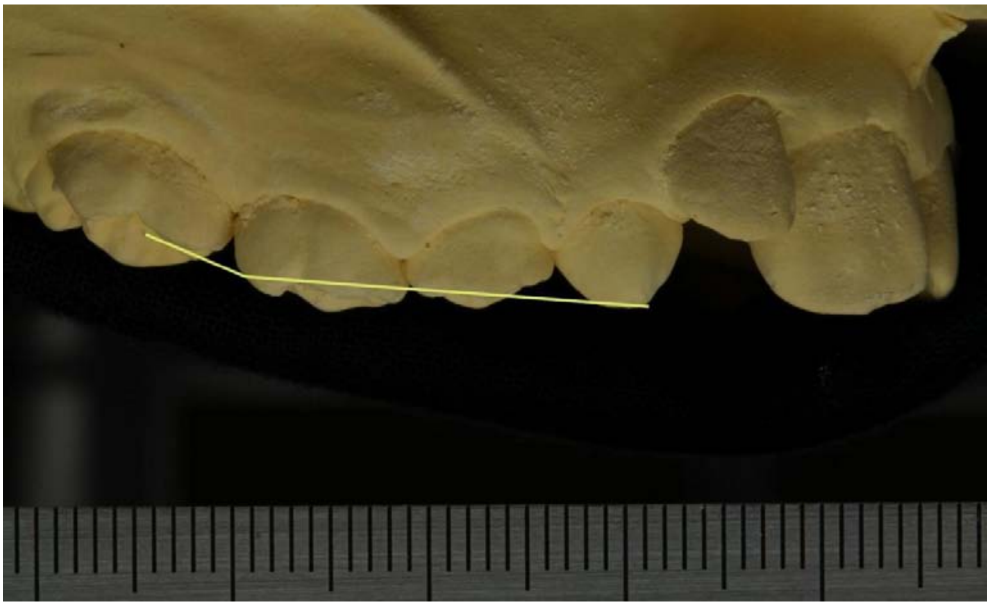
Figure 4.5. a. Photograph of study model obtained using 2D image system and then measured using the Photoshop computer software following the same criteria as the OPGs. The photograph was identified by: Twin ID, Twin A/B, upper/lower and right/left.

5b. 458A-lower left



Scale in mm

5c. 458A-upper right



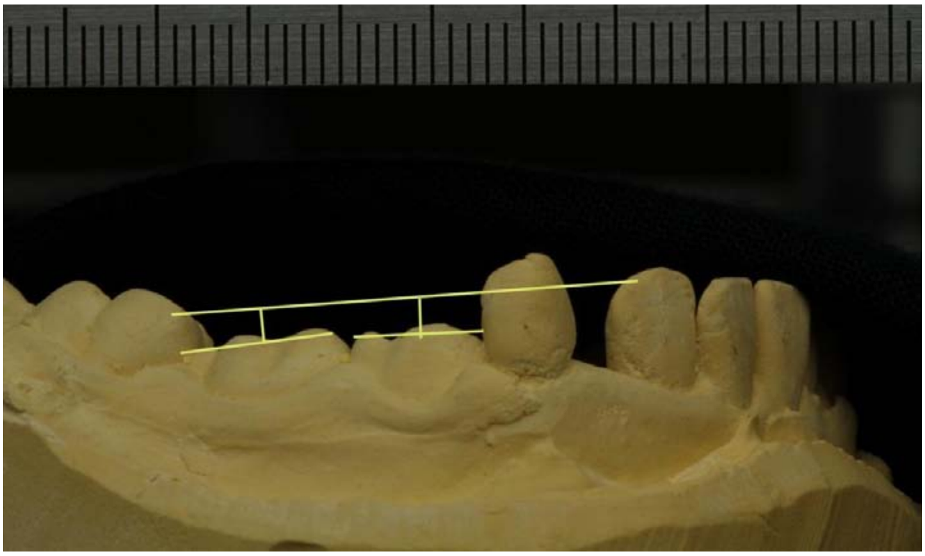
Scale in mm

Twin 458A-upper left was excluded due to absence of the primary maxillary first and second molars

Figure 4.5. (Continued) b and c. Photographs of study models obtained using 2D image system and then measured using the Photoshop computer software following the same criteria as the OPGs. The photographs were identified by: Twin ID, Twin A/B, upper/lower and right/left.

Twin 458B

5d. 458B-lower right



Scale in mm

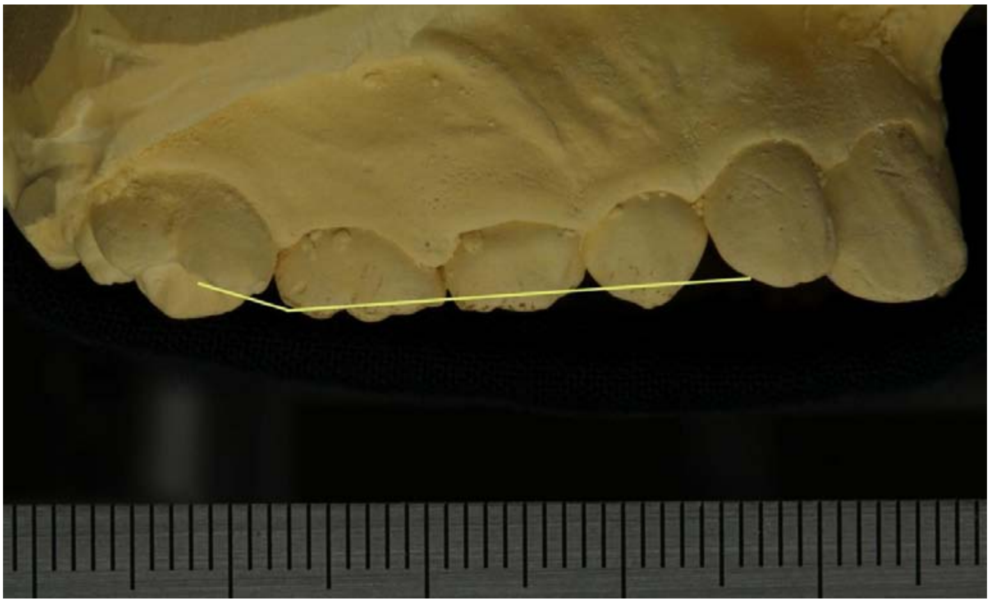
5e. 458B-lower left

Scale in mm

Figure 4.5. (Continued) d and e. Photographs of study models obtained using 2D image system and then measured using the Photoshop computer software following the same criteria as the OPGs. The photographs were identified by: Twin ID, Twin A/B, upper/lower and right /left.

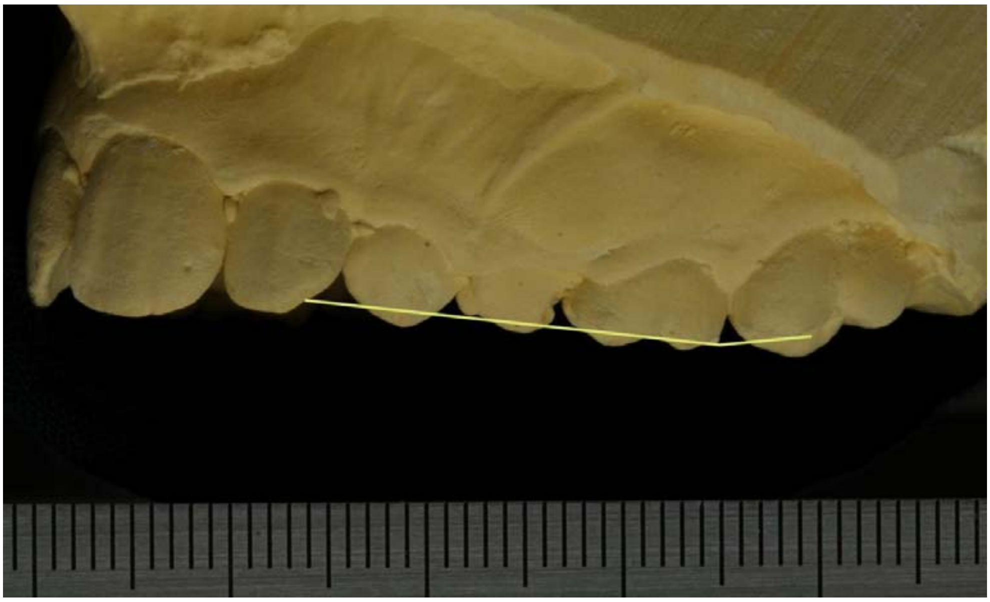
In both figures the line was extended to the mesioincisal angle of the permanent lateral incisor because the canine looked super erupted.

5f. 458B-upper right



Scale in mm

5g. 458B-upper left

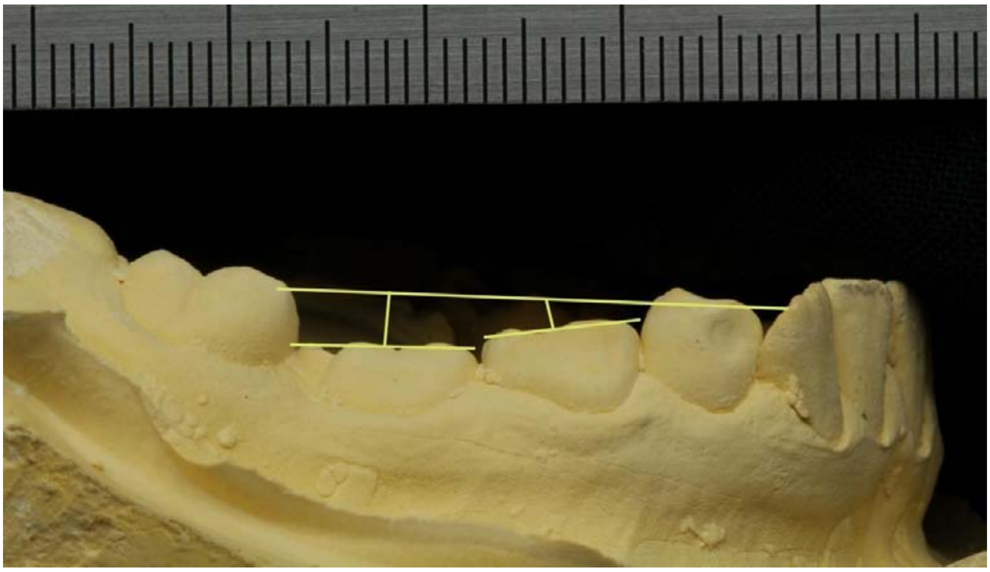


Scale in mm

Figure 4.5. (Continued) f and g. Photographs of study models obtained using 2D image system and then measured using the Photoshop computer software following the same criteria as the OPGs. The photographs were identified by: Twin ID, Twin A/B, upper/lower and right/left.

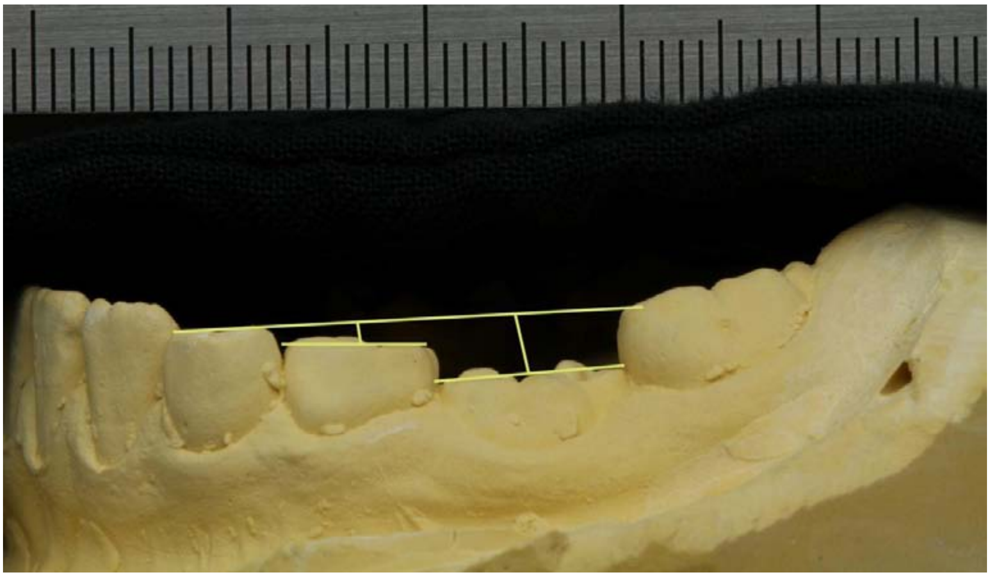
Twin 489A

5h. 489A-lower right



Scale in mm

5i. 489A-lower left

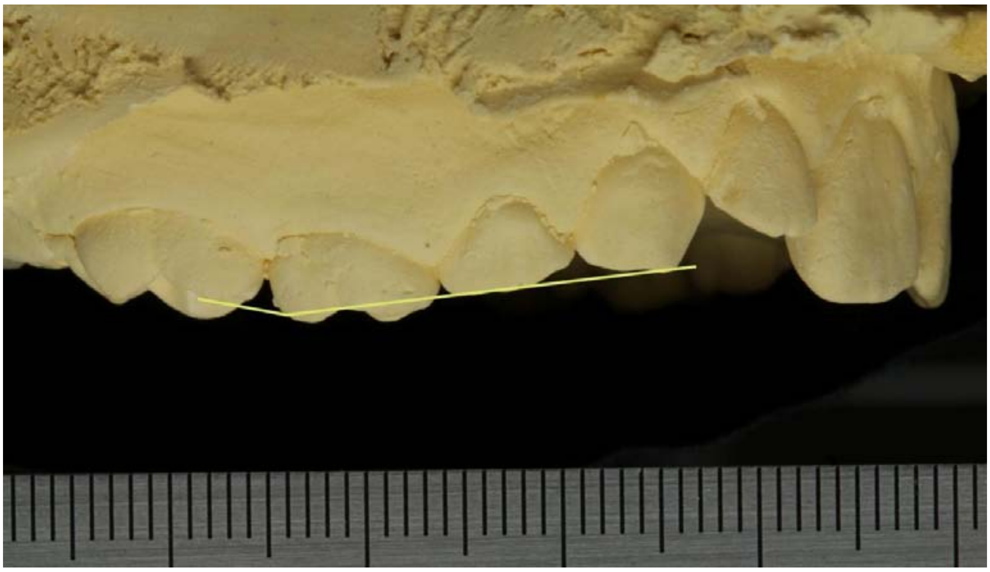


Scale in mm

Figure 4.5. (Continued) h and i. Photographs of study models obtained using 2D image system and then measured using the Photoshop computer software following the same criteria

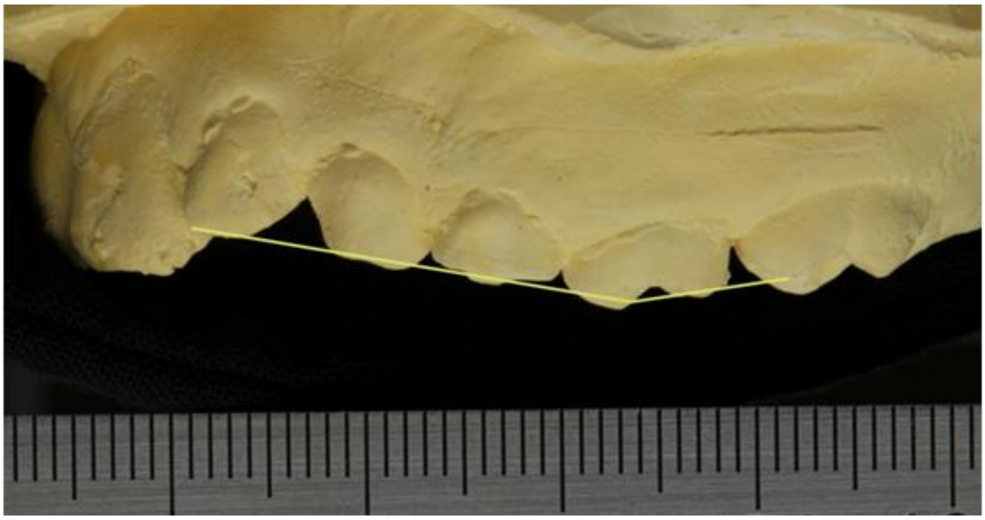
as the OPGs. The photographs were identified by: Twin ID, Twin A/B, upper/lower and right /left.

5j. 489A-upper right



Scale in mm

5k. 489A-upper left



Scale in mm

Figure 4.5. (Continued) j and k. Photographs of study models obtained using 2D image system and then measured using the Photoshop computer software following the same criteria as the OPGs. The photographs were identified by: Twin ID, Twin A/B, upper/lower and right /left.

4.3. Assessment of errors in measurement and scanning

The measurement of infraocclusion can vary when repeated measurements are carried out at different times. This variability can be reduced by carefully defining the landmarks used to produce measurements, practising identification of landmarks to ensure consistency, and preventing fatigue during data acquisition. A priority was to achieve a high level of precision (closeness of repeated measurements of the same quantity) and accuracy (closeness of measured values to true values) of the data (Figure 4.6) (Harris and Smith, 2009).

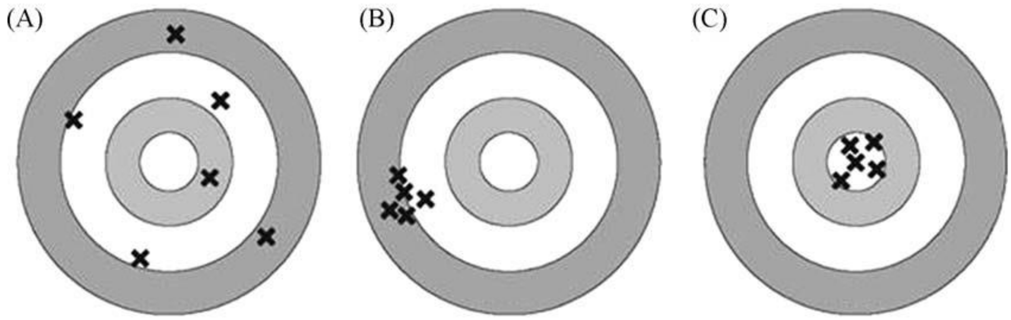


Figure 4.6. Illustration to explain precision and accuracy using a bull's eye (derived from Harris and Smith, 2009).

A. The measurements are away from the centre of the bull's eye and scattered (poor accuracy and precision) B. The measurements are away from the centre of the bull's eye (poor accuracy) but close to one another (high precision) C. The measurements are close to the centre of the bull's eye (high accuracy) and close to one another (high precision).

To try to minimise observer errors during the measurement procedures, a standard regime was followed:

- The OPGs were consistently kept to a view of 100 % enlargement, at all times, using Photoshop before constructing the lines.
- All three lines were constructed first, before the 'ruler' (in Photoshop) was used to measure the level of infraocclusion (in mm) as described previously.

- Three connecting reference points were used to level the study models according to the occlusal plane. The central fossae of the first molars and the incisal edge of the left central incisor were used to determine the occlusal plane.
- The buccal surfaces of the primary canine, first molar and second molar were kept parallel and within the same plane as a ruler placed above the buccal cusps of the teeth.
- Data were analysed to quantify the magnitude of error and these findings are included in Chapter 5.
- A fixed number of study models was measured in a single session (n=40) to avoid fatigue during data acquisition.

4.4. Methods for deciding different categories of infraocclusion

Once all measurements from the OPGs and study models were obtained, measurements were then categorised initially into four groups of ‘non-infraoccluded’, as well as ‘mild’, ‘moderate’ and ‘severe’ infraocclusion. These initial categories can be considered as relatively ‘conservative ranges’ and are defined as follows:

1. Non-infraoccluded: 0 - < 1.5 mm
2. Mild: ≥ 1.5 – < 2.5 mm
3. Moderate: ≥ 2.5 – < 3.5 mm
4. Severe: ≥ 3.5 mm

These ‘conservative’ categories tended to equate to distances that were 0.5 mm greater than those reported in the literature (Brearley and McKibben, 1973; Kurol, 1981; Koyoumdjisky-Kaye and Steigman, 1982a; Shalish et al., 2010) for defining infraocclusion, and were chosen to try to minimise false positives. However, this led to a high percentage of false negatives and also resulted in a small sample size of teeth categorised as infraoccluded. To address this, the sample was tested with a threshold set at ≥ 1mm, which increased the sample size and included some of the obviously infraoccluded cases which had been previously omitted. To finalise the categories, a validity test and double determinations were conducted.

4.5. Validity

The validity of the measurement approach to assess infraocclusion was checked by determining the degree to which different ranges of measurements could be related to observed categories of infraocclusion. In other words, after measurement, the OPGs were examined to correlate the categories derived from the measurements with the degree of infraocclusion estimated visually. This was conducted by the author selecting 20 OPGs, ten with no infraocclusion, five with infraocclusion ranging from 1 to < 2mm and another five OPGs with infraocclusion ranging from 2 to < 3mm. These OPGs were then visually examined by three different examiners (not including the author), giving the following results (Table 4.2):

Table 4.2. Scores for visual assessment of OPGs.

	No Infra score	Infra score 1to <2mm	Infra score 2 to <3mm
First examiner	10/10	4/5	6/5
Second examiner	9/10	6/5	5/5
Third examiner	9/10	7/5	4/5

The scores obtained from the examiners were interpreted as follows:

- the first examiner scored one of the 1 to <2mm infraoccluded cases as 2 to <3mm
- the second examiner scored one of the non-infraoccluded cases as 1 to <2mm (false positive)
- the third examiner scored one of the 2 to <3mm infraoccluded cases as 1 to <2mm and one of the non-infraoccluded cases as 1 to <2mm (false positive)

This exercise showed that the categories used for the measurements correlated well with the categories used for visual estimation. Hence, the approach is relevant clinically, in that it could be used when describing the degree of infraocclusion in a patient, within a clinical situation. Furthermore, the validity test revealed that there was no need to add 0.5 mm to the categories as a safety measure to avoid overestimation or errors in measurement. Based on this rationale, the categories were then revised to include infraoccluded teeth showing infraocclusion of ≥1mm below the occlusal plane.

Double determinations were carried out to assess the magnitude of the errors involved in data acquisition, comparing inter- and intra- operator measurements. The number of orthopantomographs or study models selected was 10% of the overall sample size. The results of this analysis are discussed further in Chapter 5.

Finalised categories were as follows:

1. Non-infraoccluded: 0 to <1 mm
2. Mild: ≥1 to <2 mm
3. Moderate: ≥2 to <3 mm
4. Severe: ≥3 mm

In summary, these categories were based on the following process and rationale:

- All samples were measured (in mm), and rounded to one decimal place.
- Comparisons with earlier studies were made: for example, most studies in the literature define a tooth which is more than 0.5-1 mm below the level of the adjacent teeth to be infraoccluded.
- Errors in the measurement method were carefully considered: for example, by considering only teeth ≥1mm as infraoccluded, the likelihood of including teeth that were non-infraoccluded, or only very mildly infraoccluded, was minimised. That is, it was decided to minimise the likelihood of including false positives in the study sample.
- Comparisons between measured and visual assessment of infraocclusion were made. This not only helped to validate the measurement process but also showed that the categories could be applied and were meaningful within a clinical context.

- The terms 'mild', 'moderate' and 'severe' were selected to describe the degrees of infraocclusion, the reasons being that these terms are commonly used in the dental literature and are generally understood by the public.

4.6. Methods for assessing dental anomalies and dental development in the OPG sample

1. Dental anomalies

Based on reports in the literature (Baccetti, 2000; Garib et al., 2009; Shalish et al., 2010), possible associations between infraocclusion and other dental anomalies have been proposed. Several dental anomalies were noted on the OPGs during assessment of infraocclusion. The dental anomalies included agenesis of the premolars, ectopic canines, and the lateral incisor complex anomaly (peg laterals, diminutive laterals or agenesis of the laterals).

Criteria for identifying dental anomalies:

- Agenesis of the premolars: a tooth was considered to be congenitally missing if there was no sign of tooth formation or mineralisation, either unilaterally or bilaterally, in the area of infraocclusion or in another quadrant.
- Ectopic canines: canines were considered to be ectopic if they were unerupted and showed altered positioning relative to the incisor root as defined below.

  The unerupted canine cusp tip was located relative to the lateral incisor root in one of four sectors using a modification of the method by Ericson and Kurol (1987) (Figure 4.7):

- Sector I was defined as the area distal to a line tangent to the distal heights of contour of the lateral incisor crown and root
- Sector II was mesial to sector I, but distal to a line bisecting the mesiodistal dimension of the lateral incisor along the long axis of the tooth

- Sector III was mesial to sector II, but distal to a line tangent to the mesial heights of contour of the lateral incisor crown and root
- Sector IV included all areas mesial to sector III (Lindauer et al., 1992)

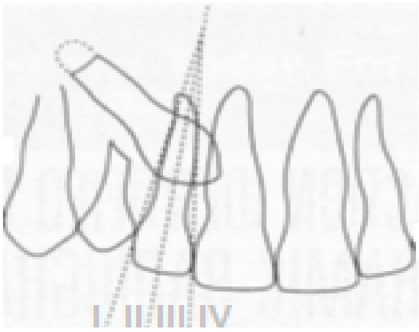


Figure 4.7. Sectors used to locate the tip of the canine in relation to the lateral incisor.

- <u>Lateral incisor complex anomaly (peg laterals, diminutive laterals or agenesis of the maxillary lateral incisors)</u>: A lateral incisor was defined to be peg-shaped if reduced in diameter from cervix to the incisal edge (Le Bot and Salmon, 1977). When a maxillary lateral incisor was compared to the contralateral incisor and appeared to be of reduced mesiodistal crown width it was scored as 'diminutive'. In cases where both lateral incisors appeared to be smaller, the lateral incisors were compared to the central incisors of the same individuals. If a lateral incisor was ≤ 60% the size of the adjacent central incisor it was considered to be diminutive. A value of 60% was chosen because this approximated values of 3 standard deviations below the mean values for the maximum mesiodistal crown dimensions of maxillary lateral incisors in males and females (Townsend et al., 1988).

All cases that were difficult to judge visually were further assessed by measurement. Using Adobe Photoshop CS5 computer software the mesiodistal width of the maxillary central incisor and lateral incisor were measured and compared.

A lateral incisor was to be considered congenitally missing if there was no sign of tooth formation or mineralization.

2. **Dental development** (n=173 males ; n=138 females)

The Demirjian and Willems systems were selected to assess the dental age of individuals in the OPG sample (Demirjian et al., 1973; Willems, 2001). First, as part of a pilot study to determine whether any relationship between infraocclusion and dental development was likely to be present, dental development was assessed in all cases of moderate to severe infraocclusion ≥2 mm (n= 33 males and females).

Following the pilot study, dental development was assessed in all cases showing infraocclusion of ≥1mm. A similar sample size of OPGs with no evidence of infraocclusion was randomly selected and also assessed for dental development using the same criteria as the infraoccluded group - this comprised the control group.

The steps of assessment of dental development by the Demirjian system were as follows:

1- The seven left permanent mandibular teeth were rated from A to H in order from the left 2nd molar to the central incisor. The rating was given after reviewing written criteria for each stage and with the aid of a standardised radiograph to help in decision making.

2- The rating was then converted into a score using tables provided for males and females.

3- The scores of all seven teeth were added and converted to maturity scores to assess dental age using tables provided for males and females (Table 4.4) (Demirjian et al., 1973).

Table 4.3. Individual maturity scores for each of the developmental stages for boys and girls.

Boys									
	Stage								
Tooth	0	A	B	C	D	E	F	G	H
2nd molar	0.0	2.1	3.5	5.9	10.1	12.5	13.2	13.6	15.4
1st molar				0.0	8.0	9.6	12.3	17.0	19.3
2nd premolar	0.0	1.7	3.1	5.4	9.7	12.0	12.8	13.2	14.4
1st premolar			0.0	3.4	7.0	11.0	12.3	12.7	13.5
Canine				0.0	3.5	7.9	10.0	11.0	11.9
Lateral incisor				0.0	3.2	5.2	7.8	11.7	13.7
Central incisor					0.0	1.9	4.1	8.2	11.8
Girls									
	Stage								
Tooth	0.0	A	B	C	D	E	F	G	H
2nd molar	0.0	2.7	3.9	6.9	11.1	13.5	14.2	14.5	15.6
1st molar				0.0	4.5	6.2	9.0	14.0	16.2
2nd premolar	0.0	1.8	3.4	6.5	10.6	12.7	13.5	13.8	14.6
1st premolar			0.0	3.7	7.5	11.8	13.1	13.4	14.1
Canine				0.0	3.8	7.3	10.3	11.6	12.4
Lateral incisor				0.0	3.2	5.6	8.0	12.2	14.2
Central incisor					0.0	2.4	5.1	9.3	12.9

(derived from Demirjian et al., 1973)

The steps of assessment of dental development using the Willems system were as follows:

1- The same first step as the Demirjian system was followed, with scoring of the teeth from A to H.

2- Using tables provided for males and females, dental age was calculated by adding the corresponding age score for each letter given directly in years for each of the seven mandibular left permanent teeth (Willems et al., 2001).

Two systems were used in order to ensure a valid estimate of dental age was obtained and to take into account possible over-estimation for dental age that may result from using the Demirjian system only, as suggested by McKenna et al. (2002).

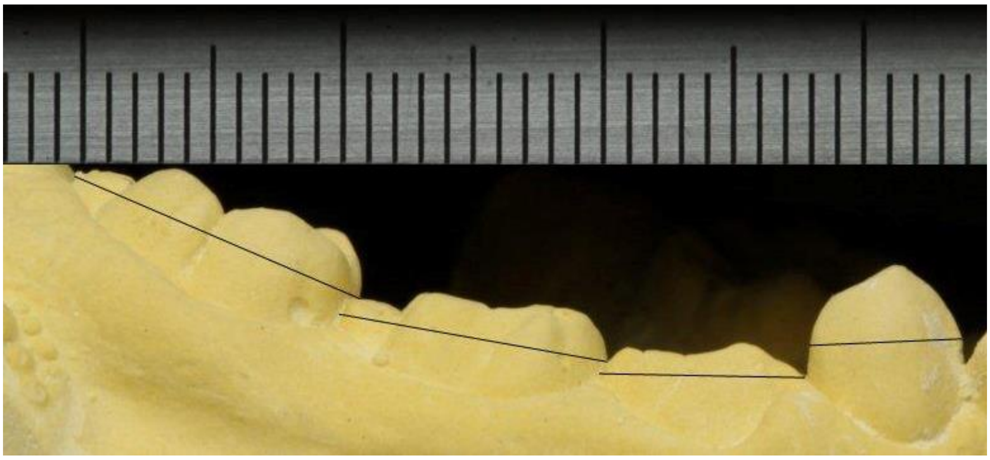
In cases where agenesis of first and/or second premolars was detected, the contralateral premolars were used for estimation of dental age. In cases where agenesis of premolars was bilateral, the subject was excluded.

A randomly selected sample (n=15) was used to perform double determinations to assess reliability in the scoring systems conducted for both the Demirjian and the Willems systems. The findings of the double determinations are reported in Chapter 5.

4.7. Methods for assessing mesiodistal crown width in the twin sample

Tooth size (n=110 males; n=92 females).

2D photographic images of the study models were used for measuring maximum mesiodistal crown widths. Measurements were performed from the buccal view of the mandibular primary canines, primary first molars, primary second molars, permanent canines, first premolars, second premolars and permanent first molars on both right and left sides. The maximum mesiodistal (MD) crown dimensions were defined as being the maximum distance between the mesial and distal proximal surfaces of the tooth crown. In cases of crowding or missing teeth, the measurements were obtained from the anatomical position where the contact occurred (Ribeiro et al., 2012). This was done using the same computer software (Adobe Photoshop CS5) that was used for the measurement of infraocclusion. The distances were measured in mm and recorded for each tooth (Figure 4.8).



Scale in mm

Figure 4.8. A 2D images of the right sides of a study model showing maximum mesiodistal width measurements from the buccal view of the primary canines, primary first molars, primary second molars and permanent first molars.

First, a small sub-sample of cases (n= 28, males and females) showing moderate to severe infraocclusion (>2 mm) was measured, to look for associations between infraocclusion and tooth size. After confirming evidence of associations in the pilot study, tooth size was assessed in all cases showing infraocclusion of ≥ 1mm. A similar sample size of study models with no infraocclusion was selected and measured for tooth size in the same manner as for the infraoccluded group - this comprised the control group.

Cases where measurements could not be obtained accurately were excluded, including instances where:

- a tooth was missing or not erupted
- a tooth was partially erupted
- a tooth was fractured or worn

To assess the validity of measurement, a digital calliper was used to measure a small, randomly selected sub-sample (n= 10) that had already been measured using the Adobe Photoshop CS5 computer software, and the results were compared. Another small, randomly selected sub-sample (n=15) was also used to assess intra-observer errors in the measurement of the mesiodistal crown widths using the 2D digital system. The findings of these double determinations are presented in Chapter 5.

4.8. Methods for assessing study models obtained at three different stages in individuals showing infraocclusion

The sample for this aspect of the study included all co-twin members who showed infraocclusion of at least one tooth. Thus, in some cases both twins were included whereas, in other cases, only one member was included. Also, cases were included if models were available at two or more of the dental development stages (n= 119). Stage one study models were obtained during the primary dentition phase; stage two study models were obtained at the mixed dentition phase (these were used for the main sample in the current study) and stage three models were obtained at either the mixed dentition or permanent dentition phase. The chronological ages of the twins in each of the three stages were approximately 4 to 9 years, 7 to 14 years and 12 to 17 years respectively.

Stage one study models were assessed by visual examination for the presence of infraocclusion. The degree of infraocclusion was not measured in mm for this part of the study as its purpose was to provide a general description of whether infraocclusion was present or whether any changes in the degree of infraocclusion had occurred over time. The classification given was either of infraocclusion being present, or not present. In cases where infraocclusion was observed to be present, the teeth affected were recorded. The same individual Stage 3 models were then examined to see if infraocclusion was present or not present, whether there was agenesis of permanent teeth or whether there was a combination of both. This was done case by case. The information was recorded and tabulated.

4.9. Methods for assessing study models of family members of individuals showing infraocclusion

For those individuals showing infraocclusion, records of their family members (brothers and sisters) (n=42), were also examined for the presence of infraocclusion and/or other dental anomalies, including missing teeth, retained primary teeth, and reduced mesiodistal width of the permanent maxillary lateral incisors.

Visual examination was conducted for the available study models. A record of the presence of infraocclusion or other dental anomalies was obtained. The information was recorded and tabulated.

4.10. Statistical analysis

Raw data representing calculated distances used to describe infraocclusion were initially recorded in mm. All measurements were plotted on bar charts. The charts for the mandibular right first and second molars are included in Chapter 6 and those for the left side are included in the Appendix. Due to a high frequency of ‘zero’ values and taking into account the skewed nature of the distribution, it was decided to set up categories as ‘non-infraoccluded’, ‘mild’, ‘moderate’ and ‘severe’. For these data, chi-square analyses were used to compare the distribution of observed values among different categories of tooth type, tooth side and sex. Contingency tables were also constructed to evaluate concordance in the expression of infraocclusion within individuals between right and left sides. Percentage concordances were also calculated to quantify the frequency of bilateral expression of infraocclusion on opposite sides of the dental arch. Where the expected numbers in any of the categories were less than five, caution was exercised in interpreting chi-square values and their associated probabilities. Where possible, the categories were collapsed, especially the moderate and severe categories to ensure expected numbers were equal to or greater than five. In some cases, Fisher’s exact tests were performed.

For continuous data, such as dental age assessments and tooth size measurements, data were tested for normality. Dental age values obtained by both Demirjian and Willems systems and chronological age were plotted and found to conform generally to symmetrical bell-shaped curves. In a small number of cases, some evidence of skewness and/or kurtosis was noted. However, for most variables, estimates of skewness and kurtosis did not differ significantly from zero ($P<0.05$). A similar approach was used to assess the distributions of tooth size data. Crown size data were plotted and they tended to follow a symmetrical bell-shaped curve for most of the data. Mean values and medians coincided and estimates of skewness and kurtosis did not differ significantly from zero ($P<0.05$). Therefore, it was deemed appropriate to summarize the data for dental age and tooth size by using mean values and standard deviations. Student’s unpaired t-tests were used to compare the means of dental ages between singletons showing infraocclusion and singletons showing no infraocclusion. Student’s paired t-tests were also performed to compare dental ages and chronological ages within individuals. Z scores of dental ages were calculated for cases showing infraocclusion together with dental agenesis, to enable comparisons to be made with the entire sample of individuals with infraocclusion. Student's unpaired t-tests were used to compare the mean measurements of maximum mesiodistal tooth widths between twins showing infraocclusion and twins showing no infraocclusion. Statistical significance was set as $P < 0.05$.

Student's paired t-tests were also applied to data obtained from double determinations for the different variables examined to assess systematic errors of measurements. Random errors were assessed by using Dahlberg's statistics for all methods used in this study.

Genetic modelling was performed to analyse data from the twin sample by testing the initiation and progression of infraocclusion in members of twin pairs. In those individuals displaying infraocclusion, progression was further categorised into mild, moderate and severe. The software package Mx (Neale et al., 2006) was used to perform the genetic modelling. Initially, data were broken down to produce a 'super-model' against which to test goodness-of-fit of nested sub-models. A model including additive genetic, unique environmental and common environmental variances was then fitted. Implicit in the model fitting were the normal assumptions of the twin method: that mating was random, that trait-related shared environmental influences on MZ and DZ twins were equal, and that there was no $G \times E$ interaction or $G \times E$ correlation (Kang et al., 1977). The parsimony of each model was indicated by Akaike's Information Criterion (AIC = chi-squared value minus twice the degrees of freedom). Narrow-sense heritability estimates (h^2) were calculated as the ratio of additive genetic variance to total phenotypic variance in the best-fitting model.

5. Errors of measurement

5.1. Introduction

Assessment of infraocclusion by direct visual examination has been the most common technique used in previous studies (Darling and Levers, 1973; Stewart and Hansen, 1974; Kurol, 1981). Because the validity and repeatability of this technique is questionable, direct measurement from study models is considered relatively more likely to be reproducible and accurate (Koyoumdjisky-Kaye and Steigman, 1982a; Shalish et al., 2010). However, holding a ruler against study models is not thought to provide a standardised technique as this allows room for error in repeated measurements. For these reasons, the recent use of 2D digital images of study models has become one of the most widely adopted methods for the measurement of dental crown dimensions (Brook et al., 1999; Ribeiro et al., 2012). This improves the reproducibility of measurements and enhances accuracy. Another way of measuring the size or position of teeth involves using radiographs. Radiographs have been used to measure enamel and dentine thickness, the size of the pulp chamber, root length, and also curvature and shape of the root (Thanyakarn et al., 1992; Grine et al., 2001; Lahdesmaki and Alvesalo, 2004). Moreover, a study by Becker and Karnei-R'em (1992b) used orthopantomographs to measure the level of infraocclusion and the amount of inclination of the adjacent teeth.

5.2. Types of measurement errors

There are two main types of measurement errors: systematic errors of measurement and random errors of measurement (Hunter and Priest, 1960; Houston, 1983; Harris and Smith, 2009). In this study, systematic errors could have resulted from magnification error due to the use of different machines to obtain the radiographs. Failure to correct for magnification can result in a systematic overestimation of measurements and lead to a bias in comparing radiographs obtained from different machines (Harris and Smith, 2009). With study models, systematic errors can be related to the material used in making the impression and also casting procedures. With radiographs, random errors can result from variations in the position of the patient and/or the machine, which can then affect the scale of magnification. Furthermore, the way in which the radiographs are processed can affect their quality, which in turn may affect the identification of landmarks. Likewise, while obtaining 2D images of study models, random errors may result from variation in the position of the models and/or camera, the level of light intensity selected and the presence of shadows. These factors, as well as the level of experience of the operator, influence how accurately landmarks can be identified.

5.3. Minimising errors of measurements

To minimise errors of the methods in the radiographic sample, magnification was accounted for by referring to a 3cm scale on all orthopantomographs. Furthermore, landmarks for the construction of the occlusal plane and the occlusal table were identified. Cases where it was difficult to identify landmarks (distortion, caries, broken teeth or partially erupted teeth) were excluded (Chapter 4). To check the adequacy of the Adobe Photoshop CS5 computer software package, measurements were also carried out on a small sub-sample of the orthopantomographs using another software package (ImageJ) to determine whether the results were comparable between the two packages. All the study models used in this study were obtained from alginate impressions that were poured immediately, using Type III Yellowstone, once the impressions were taken. Camera settings were adjusted for all 2D images to ensure quality and standardisation. Furthermore, the camera and study models were held in fixed positions. As with orthopantomographs, landmarks were identified and cases where landmarks were difficult to locate, were excluded.

5.4. Accounting for measurement error

Infraocclusion

To assess the repeatability of the measurements, duplicate measurements were compared within (intra-) and between (inter-) operators (Table 5.1). Sample sizes of approximately 10% for each sample group were selected, however, these also included cases where there were missing teeth, and non-infraoccluded teeth, so the number of remeasured cases was less than the number initially selected in most groups.

An initial repeatability test was done to assess measurement of infraocclusion in the orthopantomograph sample using the Photoshop CS5 computer software package (n=140 OPGs) (Table 5.2). To assess the reliability of Photoshop CS5, a test was conducted by measuring a sub-sample (n=50 OPGs) using both Photoshop CS5 and ImageJ software packages and comparing results (Table 5.3). Inter-operator comparisons of measurements were conducted on the orthopantomograph sample (n=50), with measurements obtained by one operator using the Photoshop CS5 software package compared to measurements obtained by a second operator using the same software package. This was done to assess the repeatability of measurements by different operators (Table 5.4). A repeatability test was also conducted for the study model sample measured from 2D images using the Photoshop CS5 software package (n=30) (Table 5.5).

Table 5.1. Summary of double determinations conducted for infraocclusion data.

Reliability	**Time from initial measurement**	**OPGs/ Study models**	**Computer program used**	**Sample size**
Intra-operator	3 months	OPGs	Photoshop CS5	140
Intra-operator	3 months	OPGs	Photoshop vs ImageJ	30
Inter-operator	3 months	OPGs	Photoshop CS5	50
Intra-operator	1 months	Study models	2D scan Photoshop CS5	30
Inter-operator	1 months	Study models	Photoshop CS5	15

To quantify intra- and inter- operator errors, paired t-tests were used to compare two sets of data and assess systematic error. Technical errors of measurement (also referred to as Dahlberg statistics (Se) were used to assess random errors of measurement, where $Se = \sqrt{\sum d^2/2n}$ (d is the difference between the first and the second measures, n is the number of pairs) (Harris and Smith, 2009).

Measuring infraocclusion from the maxillary arch was limited to a small number of cases (n=31). This is further discussed, with cases are presented in Chapter 6. Thus, only data collected from the mandibular arch were included in the overall analysis and in the double determinations.

Data were based on repeated measurements of 140 orthopantomographs, including cases with and without infraocclusion, obtained by the same operator on two separate occasions using the same software package Photoshop CS5 (Table 5.1). Both measurements included construction of the occlusal plane, occlusal tables and the perpendicular line measured in mm, taking into account the scale of magnification. Results (Table 5.2) show that there were no systematic differences between measurements (P>0.05), and the Se values were small, ranging from 0.08 to 0.14mm, indicating that random errors were small and unlikely to bias the results.

Table 5.2. Intra-operator double determinations of infraocclusion measurements on orthopantomographs, using Photoshop CS5.

Tooth	n	Mean diff	SE Mean diff	P value	Se
Mandibular right					
D	91	0.01	0.01	0.64	0.14
E	96	0.01	0.01	0.49	0.08
Mandibular left					
D	96	0.03	0.02	0.07	0.11
E	97	0.03	0.02	0.08	0.08

Values in mm, primary first molar (D), primary second molar (E)

As mentioned earlier, measurements obtained using the Photoshop CS5 software package were compared to measurements obtained using the ImageJ software package. This was done to ensure adequacy of the software package used in this study. Data were compiled from 30 orthopantomographs measured initially using the Photoshop CS5 software and remeasured on a different occasion using the ImageJ software package (Table 5.1). Both measurements involved the same steps, including construction of the lines measured in mm, as described earlier. Table 5.3 shows that there were no significant differences in the measurements obtained from the two different software packages (P>0.05), with Se values ranging from 0.05 to 0.10mm. These results prove that the software packages are highly comparable, and validates our use of Photoshop CS5 in the study.

Table 5.3. Intra-operator double determinations of infraocclusion measurements on orthopantomographs, comparing Photoshop CS5 and ImageJ.

Tooth	n	Mean diff	SE Mean diff	P value	Se
Mandibular right					
D	27	0.030	0.15	0.15	0.08
E	26	0.002	0.16	0.95	0.09
Mandibular left					
D	28	0.020	0.01	0.07	0.05
E	29	0.040	0.02	0.10	0.10

Values in mm, primary first molar (D), primary second molar (E)

To test for repeatability of the methods by different operators conducting the same measurements with the same criteria, double determinations were carried out on a small sub-sample of orthopantomographs (n= 50) (Table 5.1). Data were based on 50 orthopantomographs measured initially by one operator using Photoshop CS5 and compared to measurements obtained by another operator using the same software package. The measurements conducted by both operators followed the same criteria, including construction of the same lines, measured in mm, as described earlier. Table 5.4 shows that there were no significant differences between measurements obtained from the two different operators ($P>0.05$). Se values ranged from 0.04 to 0.10mm, indicating that random errors were small and unlikely to bias the results.

Table 5.4. Inter-operator double determinations of infraocclusion measurements on orthopantomographs, using Photoshop CS5.

Tooth	n	Mean diff	SE Mean diff	P value	Se
Mandibular right					
D	46	-0.010	0.02	0.71	0.10
E	50	0.020	0.01	0.24	0.07
Mandibular left					
D	48	0.004	0.02	0.84	0.10
E	50	0.010	0.01	0.46	0.04

Values in mm, primary first molar (D), primary second molar (E)

Similar to the orthopantomographs sample, repeatability tests were conducted for the study model sample (Table 5.1). Data were based on a random selection of 30 study models. Double determinations included obtaining 2D images of study models following the same criteria as used on the first occasion, with measurements obtained using the same software package Photoshop CS5 and following the same steps. Table 5.5 shows that there were no systematic differences between measurements (P>0.05). Se values ranged from 0.06 to 0.07mm, indicating that random errors were small and unlikely to bias the results.

Table 5.5. Intra-operator double determinations of infraocclusion measurements on study models, using Photoshop CS5.

Tooth	n	Mean diff	SE Mean diff	P value	Se
Mandibular right					
D	25	0.03	0.02	0.11	0.07
E	21	0.01	0.02	0.52	0.07
Mandibular left					
D	29	0.01	0.02	0.55	0.07
E	26	-0.02	0.02	0.40	0.06

Values in mm, primary first molar (D), primary second molar (E)

To test for repeatability of methods by different operators conducting the same measurement using the same criteria, double determinations were carried out on a small sub-sample of study models. Data were based on a random selection of 15 2D images of study models. All measurements were obtained using the same software package, Photoshop CS5, and following the same steps as the initial measurement. Table 5.6 shows that there were no systematic differences between measurements (P>0.05). Se values ranged from 0.08 to 0.10mm, indicating that random errors were small and unlikely to bias the results.

Table 5.6. Inter-operator double determinations of infraocclusion measurements on study models, using Photoshop CS5.

Tooth	n	Mean diff	SE Mean diff	P value	Se
Mandibular right					
D	11	0.04	0.03	0.25	0.08
E	9	0.02	0.04	0.69	0.08
Mandibular left					
D	12	0.09	0.04	0.29	0.10
E	10	-0.02	0.04	0.59	0.08

Values in mm, primary first molar (D), primary second molar (E)

Dental anomalies

The repeatability of scoring dental anomalies (ectopic canines, agenesis and lateral incisor complex) from the orthopantomographs was tested by comparing the scores obtained on two separate occasions. A small sub-sample of the orthopantomographs was selected (n=19) from the infraoccluded group with and without associated anomalies. Two-by-two tables were constructed to calculate the percentage concordance between the first and second scores. Since these scores were nominal (present or absent), kappa statistics were used to quantify reliability where:

$$\text{Kappa} = \frac{\text{observed agreement - chance agreement}}{\text{1- chance agreement}} \quad \text{i.e. } K = \frac{Po - Pc}{1 - Pc}$$

'Po' represents the proportion of observed agreements and 'Pc' represents the proportion of agreements expected by chance (Sim and Wright, 2005). The strength of agreement for the kappa coefficient was classified according to the scale provided by Landis and Koch (1977) as follows: poor (≥ 0), slight (0.01 – 0.20), fair (0.21 – 0.40), moderate (0.41 – 0.60), substantial (0.61 – 0.80) and perfect (0.81 – 1.0).

Duplicate recordings of ectopic canines revealed a percentage concordance of 84% and a ‘substantial’ level of agreement (kappa value of 0.69). The scores for agenesis revealed a percentage concordance of 100% and a ‘perfect’ level of agreement (kappa value of 1.0). The scores for the lateral incisor complex revealed a percentage concordance of 89% and a ‘substantial’ level of agreement (kappa value of 0.79). Unlike the agenesis scores, the ectopic canines and lateral incisor complex repeatabilities were not associated with ‘perfect’ level of agreement or kappa values > 0.81. This is thought to be related to the fact that, on the second occasion of scoring, the operator tended to score all cases that seemed questionable as absent. Thus, the difference in scoring led to a greater likelihood of false negatives rather than false positives. Nevertheless, concordance rates in the range of 84% to 89% are considered to be high and kappa scores in the ‘substantial’ range are considered to show a strong level of agreement. Overall, it was considered that the errors of methods were small and unlikely to bias the results.

Dental development

Double determinations tests were conducted to assess the repeatability of the dental age scoring systems used. A small sub-sample of orthopantomographs with infraocclusion (n=6) was selected. The selected sample size represents approximately 10% of the total sample size of orthopantomographs that were measured for assessment of dental development. For each orthopantomograph, all teeth in the left mandibular quadrant, except the third molar, were re-scored according to the Demirjian and Willems (Demirjian et al., 1973; Willems et al., 2001) systems by the same operator in a similar manner to the initial scoring.

Table 5.7. Intra-operator double determinations for dental age on orthopantomographs.

Dental age scoring	n	Mean diff	SE Mean diff	P value	Se
Demirjian	6	-0.03	0.02	0.1	0.04
Willems	6	-0.02	0.04	0.57	0.10

Age in years

Table 5.7 shows that there were no significant differences between the scores for dental age (P>0.05), with Se values at 0.04 and 0.10 years, indicating that random errors were small and unlikely to bias the results.

Tooth size

To assess errors in measurement of tooth size, repeatability tests were conducted by the same operator on a small sub-sample of study models. The re-assessment involved the same steps that were followed in the initial measurement, starting from obtaining the 2D images, constructing the lines and recording the maximum mesiodistal crown size measurements.

Table 5.8. Intra-operator double determinations for measuring the mesiodistal crown widths on study models.

Tooth	N	Mean diff	SE Mean diff	P value	Se
Mandibular right					
C	6	0.030	0.06	0.68	0.10
D	6	- 0.030	0.05	0.50	0.07
E	6	0.050	0.09	0.61	0.10
6	6	0.020	0.05	0.69	0.07
Mandibular left					
C	6	0.070	0.04	0.13	0.08
D	6	0.010	0.04	0.87	0.06
E	6	- 0.002	0.03	0.95	0.04
6	6	0.010	0.06	0.86	0.10

Values in mm, primary canine (C), primary first molar (D), primary second molar (E) and permanent first molar (6)

Table 5.8 shows that there were no significant differences between mesiodistal crown widths obtained on the first and second occasions (P>0.05). The Se values ranged from 0.04 to 0.10mm, indicating that random errors were small and unlikely to bias the results.

To assess the adequacy of the Photoshop CS5 software package used for the measurement of the mesiodistal crown widths, measurements were compared to those obtained using a set of digital callipers.

Table 5.9. Intra-operator double determinations of mesiodistal widths on study models, compared between measurements obtained using callipers and measurements obtained using Photoshop.

Tooth	n	Mean diff	SE Mean diff	P value	Se
Mandibular right					
C	10	0.08	0.07	0.43	0.07
D	10	0.08	0.07	0.96	0.07
E	10	0.01	0.06	0.47	0.06
6	10	0.02	0.10	0.70	0.10
Mandibular left					
C	10	0.18	0.07	0.92	0.10
D	10	0.10	0.04	0.75	0.08
E	10	0.12	0.05	0.13	0.11
6	10	0.08	0.04	0.96	0.08

Values in mm, primary canine (C), primary first molar (D), primary second molar (E) and permanent first molar (6)

Results of the repeatability test (Table 5.9) revealed that there were no significant differences between the different measurement methods (P> 0.05). The Se values ranged from 0.06 to 0.11mm, indicating that random errors were small and unlikely to bias the results.

5.5. Discussion

Double determination tests were conducted to assess systematic errors using paired t-tests, and random errors using Dahlberg's statistics for all methods used in this study. Results of examinations based on inter-operator (Tables 5.4 and 5.6) and intra-operator (Tables 5.2 and 5.5) duplicate measurements of infraocclusion showed no systematic significant differences. The validity of the Photoshop CS5 software package that was used to obtain measurements was also established (Table 5.3). Dental development, dental anomalies and tooth size repeatability tests were also conducted.

Scoring of dental anomalies on different occasions showed high percentages of agreement, with a kappa statistic ranging from 'substantial' to 'perfect'. Similar findings were reported by Baccetti (2000) who reported the reproducibility of assessment of dental anomalies including infraocclusion, ectopic canines, agenesis and lateral incisor complex ranged from 97% to 100%.

Blenkin and Evans (2010) assessed the accuracy of dental development determinations from radiographs using the Demirjian system and reported a kappa statistic associated with a 'substantial' level of agreement. Likewise, in this study, duplicate calculations of dental development assessment revealed no significant differences in the calculated ages compared between the first and second assessments. The double determinations of dental development that were conducted included not only scoring of the teeth using radiographs but they also involved all the steps to calculate dental age, as per the Demirjian and Willems systems (Table 5.7).

Duplicate measurement of mesiodistal crown widths conducted by the two different methods using Photoshop CS5 and by digital callipers revealed no systematic significant differences between measurements obtained at different occasions (Tables 5.8 and 5.9). These findings were comparable to those for intra-operator duplicate measurements of mesiodistal crown widths reported by Brook et al. (2009b). Likewise, the Dahlberg statistics revealed that the random errors resulting from the measurement methods were minimal and within the accepted ranges, similar to results reported in a similar sample of Australian twins by Ribeiro et al. (2013).

Overall, the methods followed in this study showed a high level of precision, based on double determinations, and accuracy, based on comparisons of the different measurement methods used and also comparisons with published data. There was no evidence of significant systematic error due to the methods of data acquisition and measurement used in this study. The random errors were shown to be small when compared to the extent of variation observed in the study variables and were, therefore, considered to be unlikely to bias the results.

6. Results of descriptive analysis of infraocclusion in singletons and twins

6.1. Introduction

The term 'infraocclusion' refers to a tooth that is positioned below the normal level of occlusion. Infraocclusion can be associated with local and/or systemic abnormalities that can lead to functional disturbances or disorders within the erupting dentition. These abnormalities are controlled by genetic and environmental factors that play a crucial role in the eruption process. The pattern of expression and the degree of severity of infraocclusion are determined by the relative contributions of genetic, epigenetic and environmental factors to observed variation (phenotypic variation) (Wise et al., 2002; Nanci and Ten Cate, 2008; Proffit and Frazier-Bowers, 2009).

Ankylosis has been proposed as the most common pathology associated with infraocclusion (Kurol, 1984; Kurol and Magnusson, 1984; Kurol, 2002; Karacay et al., 2007; Kjaer et al., 2008). Ankylosis refers to the fusion of a tooth to the surrounding alveolar bone due to disturbances in the periodontium. A high percentage of infraoccluded teeth have been reported to be ankylosed (79% to 98%) (Darling and Levers, 1973; Kurol and Magnusson, 1984).

However, ankylosis is not always present clinically or histologically (in association with incomplete eruption of teeth) (Kurol and Magnusson, 1984). The term 'primary eruption failure' (PEF) has been used to describe a non-ankylosed tooth which fails to erupt to the normal functional level due to a disturbance in the eruption process (Cobourne and Sharpe, 2013). PEF is characterised by failure of a tooth to erupt through the already cleared pathway of eruption, thus leading to infraocclusion with no associated ankylosis. PEF is distinguished from mechanical eruption failure (MEF) which is associated with ankylosis in all cases (Wise et al., 2002; Proffit and Frazier-Bowers, 2009). Infraocclusion can be associated with other factors. These factors may be evident in the affected area, such as loss of space, trauma to teeth, missing second premolars (agenesis of permanent teeth) or may be evident at a distance from the affected area such as, crowding or missing maxillary lateral incisors (Kurol and Thilander, 1984a).

The prevalence of infraocclusion reported in the literature varies widely, from 1.3% to 38%. A study in the United States reported a prevalence of infraocclusion of 1.3% among 2,342 American school children (Via, 1964). A higher prevalence of infraocclusion (8.9%) was reported by Kurol (1981) in sample of 1,059 Swedish children aged 3 to 12 years. In his study, Kurol considered a tooth to be infraoccluded if it was 1mm or more below the occlusal plane of the adjacent tooth. A prevalence of 24.8% was reported by Koyoumdjisky-Kaye and Steigman (1982b) in a sample of 1,530 Israeli children aged 2.5 to 13.5 years, with infraocclusion being determined by direct visual examination. In a literature review conducted by Andlaw (1974), a study by Steigman et al. (1973b) was referred to which included 1,042 Israeli children 3 to 6 years old. In this review, Andlaw mistakenly reported 38.5% as the prevalence of infraocclusion reported by Steigman et al. (1973b). However, the actual prevalence reported by Steigman et al. (1973b) was 9.2%. This high prevalence of infraocclusion (38.5%) has been misleading and has been used as a reference in other studies and case reports (Winter et al., 1997; Jenkins and Nichol, 2008). The prevalence of infraocclusion has not been well documented and the variation in reported estimates of the prevalence can be accounted for by the different measurement approaches, differences in sample sizes, and differences in the criteria for defining infraocclusion between studies. A variation in the prevalence of infraoccluded primary molars ranging from 14% to 35% has been reported between different ethnic groups, indicating that ethnicity may affect expression of the feature.

There has been no one standardised technique followed for measuring and categorising infraocclusion. In previous studies, infraocclusion has been identified and categorised by direct visual examination or by direct measurement from study models using a ruler (Kurol, 1981; Koyoumdjisky-Kaye and Steigman, 1982b; Sidhu and Ali, 2001). The accuracy and reliability of these techniques is questionable.

The aim of this aspect of the study was to determine the frequency of occurrence of infraocclusion in samples comprising singletons and twins. It was also aimed to determine whether there were any associations between infraocclusion and several variables including sex, arch type (maxillary/mandibular), arch side (right/left) and tooth type (primary first/second molars). Samples that were selected displayed similar age ranges and were all of European ancestry. A standardised technique was applied to measure infraocclusion from both the orthopantomographs and study models that were utilised in the study.

6.2. Materials and methods

Study samples

This study analysed data collected from two existing samples. The first sample comprised healthy singleton children, Finnish boys and girls (all of European ancestry), aged between 9-10 years. Orthopantomographs (OPGs) (n=1454) obtained during the years 1980-1997 by the Health Centre of Lapinlahti Council, Finland were made available for this study by a colleague, Dr. Raija Lahdesmaki, University of Oulu, Finland.

The other sample comprised study models of MZ and DZ twins who are part of a larger study that has been carried out over the past 30 years by the Craniofacial Biology Research Group, School of Dentistry, University Adelaide led by Professor Grant Townsend. The entire study includes records from over 1,200 pairs of twins. These are divided into three main cohorts with approximately 300 twin pairs in each of cohorts 1 and 2, and around 600 twin pairs in cohort 3. The zygosities of over 600 MZ and same-sex DZ twins have been confirmed (Hughes et al., 2013). Data available for the cohorts 1 (n=300 pairs) and 2 (n=300 pairs), which will be used in this study, include records of teeth present, dental caries, other dental problems (e.g. anomalies in the pattern of eruption), study models and intraoral photographs (Townsend et al., 2006; Hughes et al., 2013). Twins from these two cohorts were included in the present study.

A brief summary of the methods used has been included in this chapter. A more detailed description of the methods has been given in Chapter 4.

Measurement technique

A pilot study was conducted to establish the criteria for assessment of infraocclusion. Twenty OPGs of individuals considered by visual examination to display no infraocclusion were assessed, together with 50 OPGs of individuals who appeared to have infraocclusion. Adobe Photoshop CS5 computer software was used to construct the lines between reference points and to measure selected distances (in mm). The same measurement technique was used for both samples (Figure 6.1 and 6.2). For the orthopantomograph sample, a 3cm scale evident on all radiographs was then used to account for magnification.

Orthopantomographs

To determine the 'occlusal plane', a line was extended from the mesial marginal ridge of the first permanent molar to the cusp tip of the primary canine. This was constructed to reflect the orientation of the arch (i.e. the curve of Spee). Next, the 'occlusal table' of the primary first molars was constructed by drawing a line from its distal marginal ridge to its mesial marginal ridge. A similar line was constructed for the primary second molar. Following this, a line was drawn perpendicularly, from the mid-point of the 'occlusal table' to the 'occlusal plane', and this distance was measured (mm) (Figure 6.1).

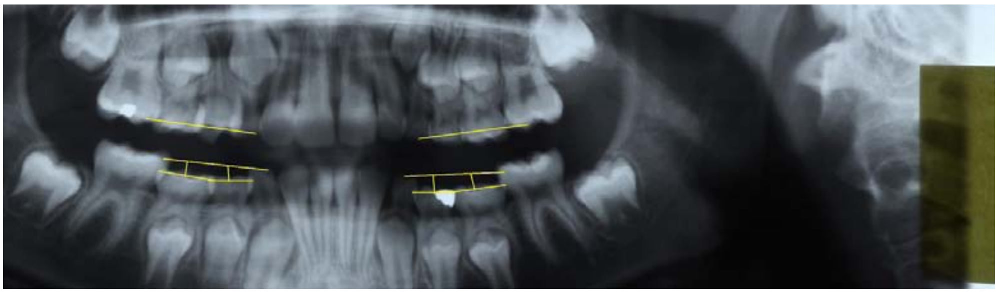


Figure 6.1. Method used to obtain infraocclusion measurements from the singleton orthopantomographs.

Study models

The same methodology was used for data collected from the twins' study models (Figure 6.2). In order to minimise possible damage to the study models that can occur from direct measuring, a 2D scanning system was used to obtain four images per individual, that is, upper and lower images for each side of the arch. The 2D imaging system that was used in this study was adapted from the system described by Brook et al. (1999).

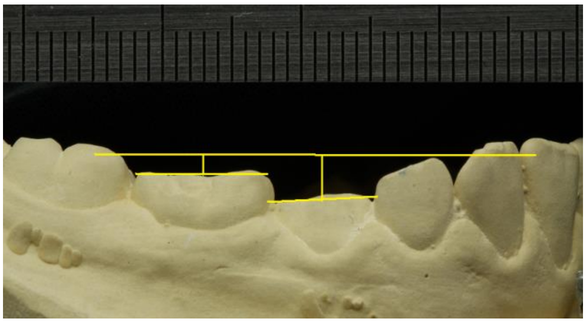


Figure 6.2. Method used to obtain infraocclusion measurements from the twin study models.

In both samples, the upper and lower arches, as well as right and left sides, were measured, with cases being excluded where: permanent first molars were partially erupted; primary teeth to be measured had large surface restoration(s) which interfered with the measurements; there was significant loss of tooth structure due to caries and/or tooth wear; reference teeth were missing; it was not possible to obtain reliable measurements, e.g. OPG(s) had significant distortion; or, in the study models, there was damage affecting one or more of the reference points.

6.3. Statistical analysis

Statistical analyses were performed using SAS Version 9.3 (SAS Institute Inc., Cary, NC, USA) and IBM SPSS Version 20. Preliminary analysis of the data obtained from the singleton sample was conducted first by plotting the data. Skewness to the right was noted associated with a high frequency of zero values, that is, 'non-infraoccluded' cases (Figures 6.3 and 6.4).

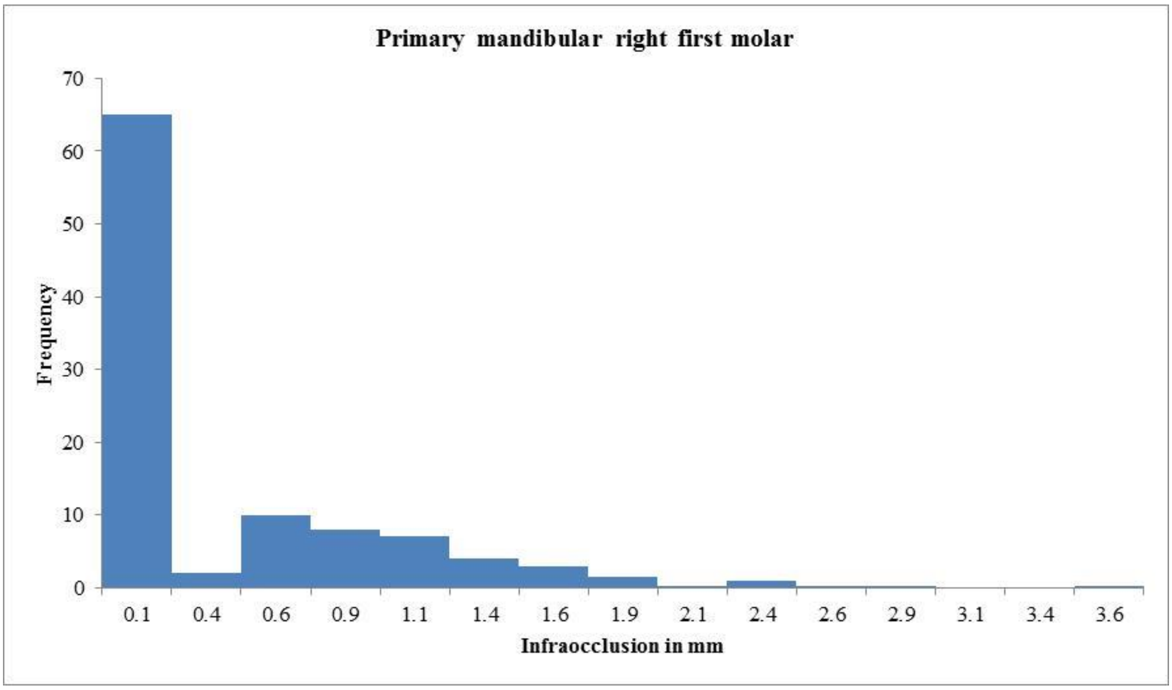


Figure 6.3. Bar chart presenting measurements obtained from the occlusal plane to the occlusal table of the primary mandibular right first molar in singletons.

Teeth were defined as being 'infraoccluded', only if they were ≥ 1mm below the occlusal plane.

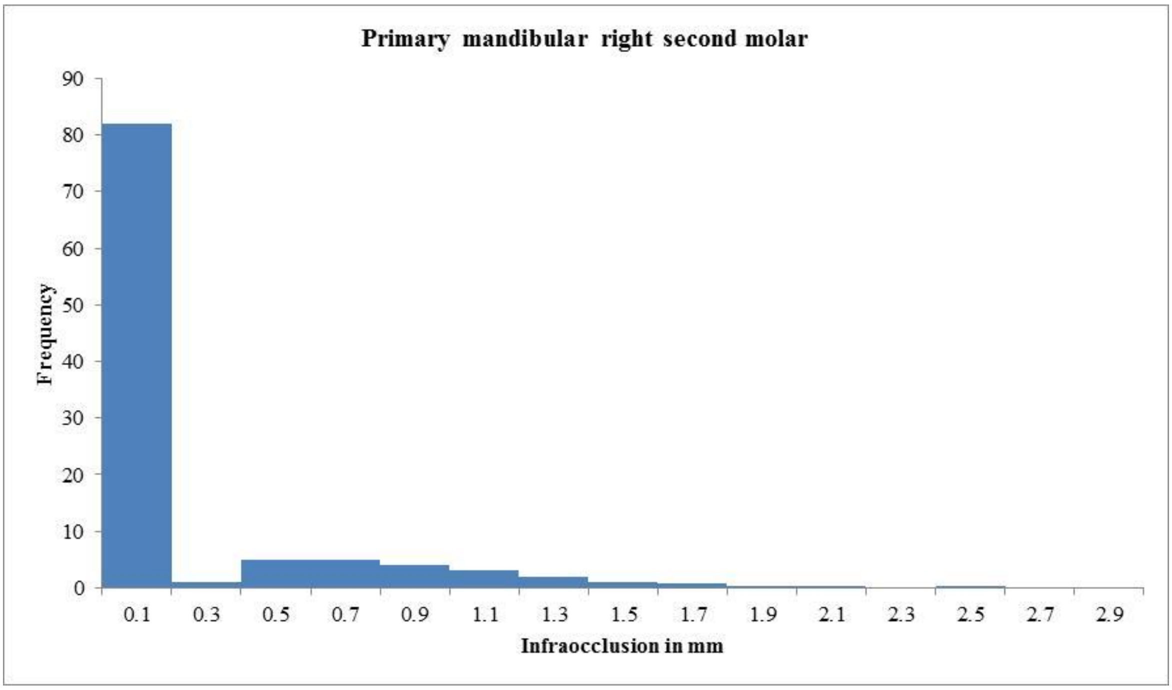


Figure 6.4. Bar charts presenting measurements obtained from the occlusal plane to the occlusal table of the primary mandibular right second molar in singletons.

Teeth were defined as being 'infraoccluded', only if they were ≥ 1mm below the occlusal plane.

Similar plots were constructed for the measurements obtained from the primary mandibular left first and second molars. These plots are included in the Appendix (Figures 1and 2). This was followed by introducing a cut-off at a measurement of 1mm, that is, only measurements that were greater or equal to 1mm were considered as being infraoccluded. The obtained measurements were then placed into categories of mild (≥1- <2), moderate (≥2 - <3), and severe (≥3). Detailed discussion of the process of selecting the categories is included in the Methodology (Chapter 4).

Chi-square tests were carried out to compare the frequency of occurrence and the degree of expression of infraocclusion according to tooth type, tooth side and sex. Four by four contingency tables were constructed to look at the distribution of infraocclusion between sides within individuals. The values falling on the diagonal represented symmetrical expression of infraocclusion, while those values that fell off the diagonal represented asymmetrical expression. Percentage concordances were also calculated to quantify bilateral occurrence of infraocclusion on opposite sides of the dental arch.

Where the expected numbers in any of the categories were less than five, caution was needed in interpreting the chi-square values and their associated probabilities. Where possible, categories were collapsed, especially the moderate and severe categories. In some cases, Fisher's exact tests were performed.

6.4. Descriptive analysis of infraocclusion in the maxillary arch

Following the measurement of all infraoccluded cases in the singleton and twin sample, analysis of the measurements was carried out, revealing that the number of cases showing infraocclusion only in the maxillary arch was very small (n=12). These findings are summarised in Tables 6.1, 6.2 and 6.3.

Table 6.1. Summary of singletons displaying infraocclusion only in the maxillary arch, including associated anomalies.

ID	Sex	Age(years)	Infraoccluded teeth	Dental anomalies
180049	F	7.53	MaxLD	agenesis + lateral incisor complex
180057	F	9.03	MaxLD	-
180103	F	9.19	MaxLD	ectopic canines
180209	F	9.65	MaxLD	-
180290	M	9.36	MaxRD, MaxLD	lateral incisor complex
180483	M	9.74	MaxLD	-
180505	M	8.93	MaxLD	lateral incisor complex
180532	M	9.59	MaxRD, MaxLE	-
180742	M	9.26	MaxLE	-
180772	M	8.91	MaxRD, MaxRE	-
191183	M	9.86	MaxRE	-
191441	M	10.16	MaxLD	ectopic canines

Female (F), Male (M), Maxillary arch (Max), Right (R), Left (L), Primary first molar (D), Primary second molar (E)

Table 6.1 shows that the most commonly affected maxillary tooth was the primary left first molar. There were five cases where associated anomalies were revealed, one of whom had two associated dental anomalies. Analysis of all the orthopantomographs revealed that a small number of individuals (n=19) displayed infraocclusion in both the maxillary and mandibular arches. These data are summarised in Table 6.2. This table shows that the most commonly infraoccluded tooth in the mandible and the maxilla was the primary first molar. Table 6.2 shows several of these cases (n=7) also had associated dental anomalies.

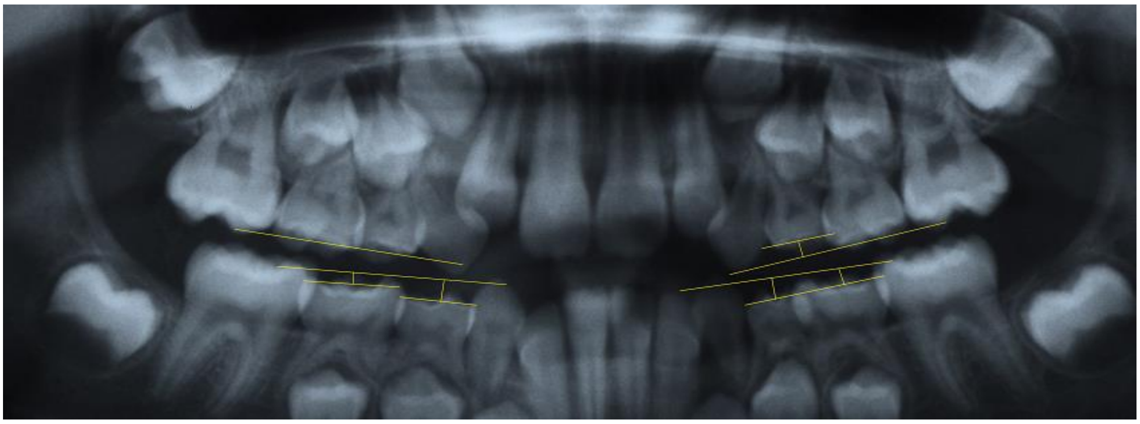
Table 6.2. Summary of singletons displaying infraocclusion in both mandibular and maxillary arches, including associated anomalies.

ID	Sex	Age(year)	Infraoccluded teeth	Dental anomalies
180004	F	10.86	ManRE, ManLE, MaxRE	-
180125	F	10.01	ManRD, ManRE, ManLD, MaxRD	-
180165	F	10.23	ManRD, ManLD, MaxLD	lateral incisor complex
180927	F	9.64	ManLD, MaxLD	lateral incisor complex
180013	F	9.66	ManLD, MaxLD	-
180037	M	10.15	ManRD, ManRE , ManLD, MaxRD	-
180282	M	9.5	ManLD, ManLE, MaxRD, MaxRE, MaxLD	-
180315	M	9.02	ManLD, ManLE, MaxRE	-
180434	M	9.05	ManRD,ManRE,ManLLD, MaxRD	-
180044	M	8.18	ManLD, MaRD, MaxLD	-
180292	M	9.47	ManRE, ManLE, MaxRD, MaxLD	agenesis
180384	M	9.06	ManRD, MaxRD	-
180447	M	9.1	ManLD, ManLE, MaxLD	-
180679	M	8.49	ManRD, ManLD, ManLE, MaxLD	-
191383	M	9.12	ManRD, MaxLD	lateral incisor complex
191386	M	9.7	ManLD, MaxLD	lateral incisor complex
180226	M	9.7	ManLD, MaxLE	lateral incisor complex
180541	M	9.47	ManLD, MaxLE	-
180634	M	9.28	ManRD, ManRE, ManLD, ManLE, MaxLE	ectopic canines

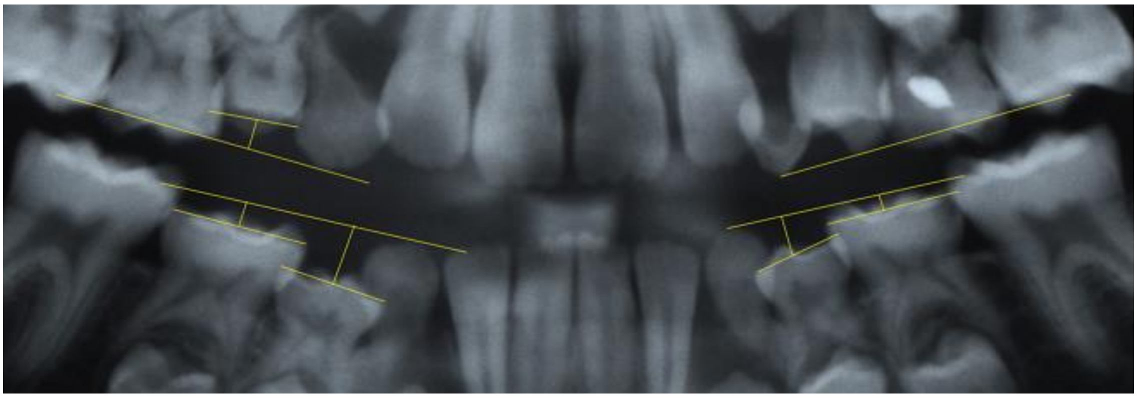
Female (F), Male (M), Mandibular acrch (Man), Maxillary arch (Max), Right (R), Left (L), Primary first molar (D), Primary second molar (E).

Different trends of expression were evident in three cases (ID 1800384, 180013, 180125) showing mild, moderate and severe degrees of infraocclusion (Figure 6.5). One of the cases also had an associated dental anomaly (Figure 6.6). Another two cases (ID 180044 and 180315) had mild and moderate infraocclusion, respectively. All other cases were mildly affected.

ID 180384



ID 180013



ID 180125

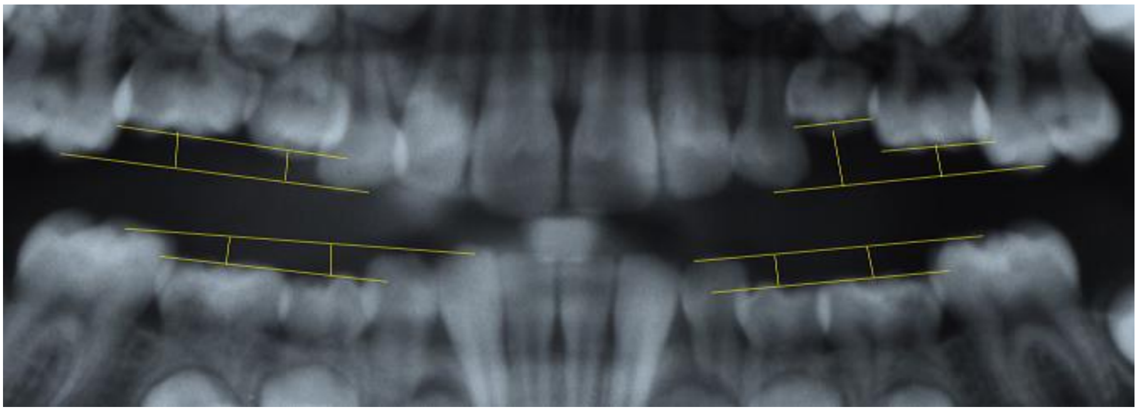
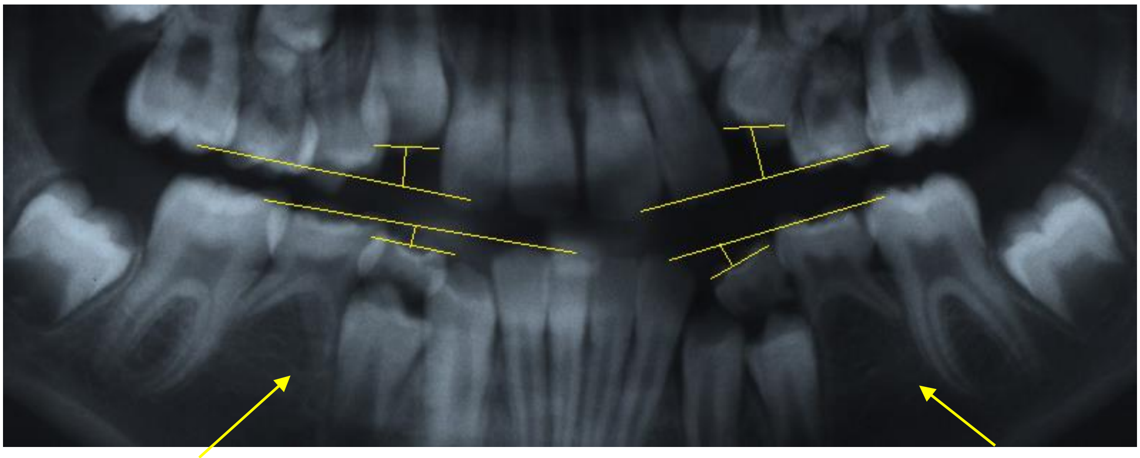


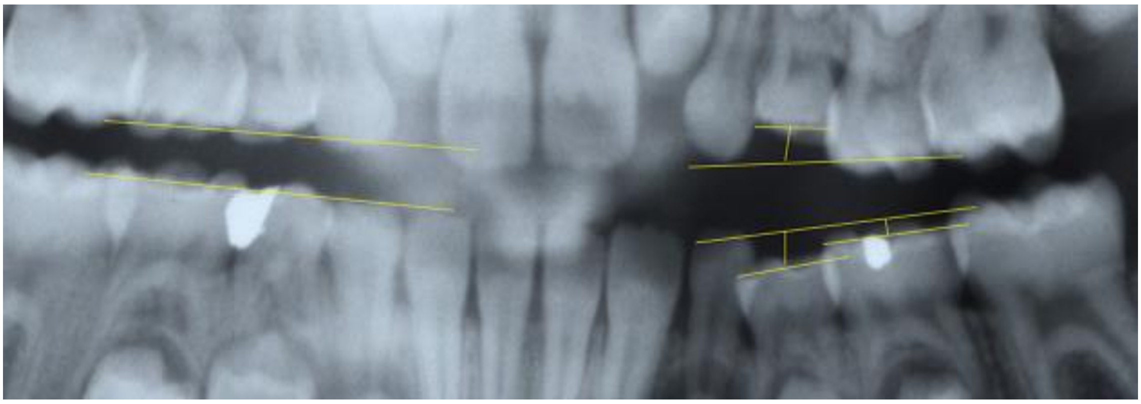
Figure 6.5.Orthopantomographic of singletons who displayed infraocclusion in both the mandible and the maxilla.

The radiographic image has been cropped for presentation and the scale is not visible.

ID180292



ID180044



ID 180315

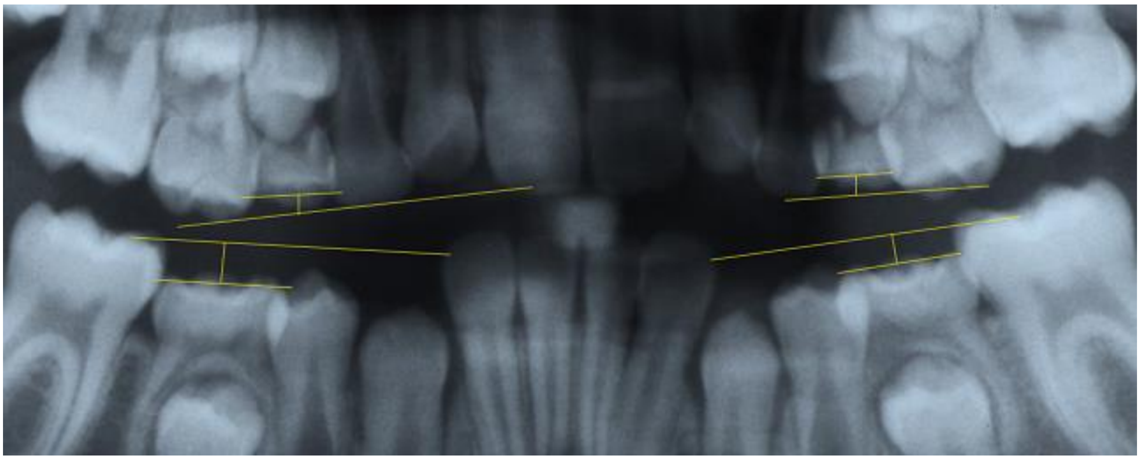


Figure 6.6.Orthopantomographic of singletons who displayed infraocclusion in both the mandible and the maxilla.

The radiographic image has been cropped for presentation and the scale is not visible.

In all cases were the primary canine was missing the line was extended to the mesioincisal angle of the permanent lateral incisor.

Table 6.3. Summary of twins displaying infraocclusion in both the mandibular and maxillary arches.

ID	Zygosity	Sex	Twin A infraoccluded teeth	Twin B infraoccluded teeth
118	MZ	M	MaxLE	ManRE, ManLE, MaxRE, MaxLE
192	DZ	F/M	ManLD	MaxRE
262	MZ	M	ManLE, MaxLE	-
269	-	F	ManRD, MaxRD	-
463	DZ	F	-	ManLD, MaxRD
488	DZ	F	ManRD, ManLD, ManLE	ManRD, ManRE, ManLD, ManLE, MaxRE
551	MZ	F	ManRD, ManLD, MaxRD	ManLE
568	DZ	M/F	ManRE, ManLE	ManRD, ManRE, ManLD, ManLE, MaxRE
596	MZ	M	ManRE, ManLE	ManRD, ManRE, ManLD, ManLE, MaxLE
808	MZ	M	ManRD, ManLD	ManRD, ManRE, ManLD, MaxRD
511	DZ	F/M	ManRD, ManRE, ManLD, ManLE, MaxLE	-

Monozygotic (MZ), Dizygotic (DZ), Female (F), Male (M), Mandibular acrch (Man), Maxillary arch (Max), Right (R), Left (L), primary first molar (D), primary second molar (E).

Nine of the 11 twin pairs who showed infraocclusion in the maxilla also had infraocclusion in the mandible (n=9), except for two cases (ID 118 and 192) (Table 6.3). The severity of infraocclusion in the maxilla ranged from mild to moderate.

The prevalence of infraocclusion in the maxillary arch was revealed to be low in both samples examined. Moreover, some difficulties occurred when obtaining measurements in the maxillary arch in both the singleton and twin samples. These included difficulties in identifying reference points radiographically or difficulties due to the curvature of study models in the maxillary arch in some cases. Thus, it was decided that those singletons who showed infraocclusion only in the maxilla (n=12) would not be included in any further analyses. For the 19 cases who showed infraocclusion in both maxillary and mandibular arches, only measurements obtained from the mandibular arch were included in further analyses, that is, it was decided to concentrate on infraocclusion in the mandible only.

Similarly, in the twin sample, those individuals who showed infraocclusion only in the maxillary arch (n=2) were not included in any further analyses, while for the remaining 9 cases who showed infraocclusion in both the maxillary and mandibular arches, only measurements obtained from the mandibular arch were included in further analyses. For the same reasons mentioned earlier and because infraocclusion data collected from the maxillary arch were excluded from further analyses, it was also decided to only include the mandibular arch in the double determinations that were conducted and that are reported in Chapter 5.

6.5. Comparing the overall prevalence of infraocclusion between singleton and twin samples

In the present study, some variations in the prevalence of infraocclusion were noted according to the study sample used, that is, singletons and twins. These data are shown in Table 6.4.

Table 6.4. Comparison of prevalence of infraocclusion between singletons and twins (males and females combined).

	Mandibular first molar				Mandibular second molar			
	right		left		right		left	
	Singleton	Twin	Singleton	Twin	Singleton	Twin	Singleton	Twin
NI	964(85.1%)	209(78.6%)	937(82.0%)	177(72.2%)	1283(95%)	250(87.1%)	1270(93.5%)	247(82.3%)
MI	153(13.6%)	40(15.0%)	190(16.6%)	54(22.00%)	60(4.4%)	30(10.5%)	82(6.00%)	43(14.3%)
MO	12(1.07%)	8(3.0 %)	14(1.22%)	9(3.7%)	9(0.6%)	4(1.4%)	6(0.45%)	7(2.30%)
SE	3(0.27%)	9(3.4%)	1(0.09%)	5(2.00%)	0(0.0%)	3(1.0%)	0(0.00%)	3(1.0%)
Total	1132(100%)	266(100%)	1142(100%)	245(100%)	1352(100%)	287(100%)	1358(100%)	300(100%)

Non-infraoccluded (NI), Mild (MI), Moderate (MO), Severe (SE).

The prevalence of infraocclusion in the singleton sample was lower than the prevalence of infraocclusion in the twin sample. Among singletons, the prevalence ranged from 4.4 % to 16.6 % in the mild category compared to a range of 10.5% to 22% among the twin sample. Moreover, in the singleton sample, the prevalence of moderate infraocclusion ranged from 0.45% to 1.22% with only 4 individuals showing severe infraocclusion. In the twin sample, moderate infraocclusion ranged from 1.4% to 3.7% and severe infraocclusion ranged from 1% to 3.4%. Therefore, the prevalence of infraocclusion for all categories was higher in the twins sample compared with the singletons sample. This finding is discussed further in the Section 6.8.

6.6. Results of descriptive analysis of the singleton sample

Chi-square tests were conducted to compare the frequency of occurrence and the degree of expression of infraocclusion between different teeth and different sides in the singleton sample. A significant difference was shown to be present in the distribution of infraocclusion categories (that is, the expression of infraocclusion) between the mandibular right first molar (MRD) and the mandibular right second molar (MRE) (P < 0.01). A greater number of cases of mild infraocclusion (MI) were noted in the MRD compared with the MRE. While there were no cases of severe (SE) infraocclusion affecting the MRE, there were three cases affecting the MRD (Table 6.5). A similar trend of expression of infraocclusion was shown on the left side (see Appendix Table 1).

Table 6.5. Comparison of the frequency of occurrence and degree of expression of infraocclusion between primary mandibular first (D) and second (E) molars (right side) - singleton sample.

	MRD		MRE	
NI	964	(85.10%)	1283	(95.00%)
MI	153	(13.60%)	60	(4.40%)
MO	12	(1.07%)	9	(0.60%)
SE	3	(0.27%)	0	(0.00%)
Total	1132	(100%)	1352	(100%)

chi-square value = 70.4, degrees of freedom (dof) =3
P < 0.001

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, chi-square value = 67.7, dof =1, P < 0.001).

No statistically significant difference was noted in the distribution of the four categories between the mandibular right first molar (MRD) and the mandibular left first molar (MLD) (Table 6.6). Similarly, Table 6.7 shows that there was not a statistically significant difference in the distribution of the four categories between the mandibular right second molar (MRE) and the mandibular left second molar (MLE). These comparisons confirm that there was no evidence of directional asymmetry in the expression of infraocclusion.

Table 6.6. Comparison of the frequency of occurrence and degree of expression of infraocclusion between mandibular right and left first molars - singleton sample.

	MRD		MLD	
NI	964	(85.00%)	937	(82.00%)
MI	153	(13.65%)	190	(16.60%)
MO	12	(1.07%)	14	(1.00%)
SE	3	(0.27%)	1	(0.00%)
Total	1132	(100%)	1142	(100%)

chi-square value = 5.5, dof = 3
P > 0.05

(When collapsed to a 2 x 3 table, with MO and SE combined, chi-square value = 4.33, dof =2, P > 0.05).

Table 6.7. Comparison of the frequency of occurrence and degree of expression of infraocclusion between mandibular right and left second molars - singleton sample.

	MRE		MLE	
NI	1283	(95.00%)	1270	(93.50%)
MI	60	(4.40%)	82	(6.00%)
MO	9	(0.60%)	6	(0.44%)
SE	0	(0.00%)	0	(0.00%)
Total	1352	(100%)	2716	(100%)

chi-square value = 4.0, dof = 2
P > 0.05

(When collapsed to a 2 x 3 table, with MO and SE combined, chi-square value = 4.06, dof =2, P > 0.05).

A comparison of the frequency of occurrence and the degree of expression of infraocclusion according to sex was carried out among the singleton sample by conducting chi-square tests. The tests were first done for each tooth separately (MRD, MRE, MLD and MLE) followed by a test combining all the examined teeth. There was not a statistically significant difference found in the distribution of the four categories of infraocclusion in any of the teeth examined between males and females. Tables 6.8 and 6.9 show comparisons between males and females for the primary mandibular right first and second molars, respectively. Results showed that there was not a statistically significant difference between right and left sides for both teeth. The left side comparisons showed a similar pattern (see Appendix Tables 2 and 3). Furthermore, there was no evidence of a statistically significant difference between males and females when samples for both teeth were combined (Table 6.10).

Table 6.8. Comparison of the frequency of occurrence and degree of expression of infraocclusion of the mandibular right first molars between males and females - singleton sample.

MRD

	M		F	
NI	521	(84.80%)	443	(85.50%)
MI	87	(14.00%)	66	(12.74%)
MO	4	(0.65%)	8	(1.54%)
SE	2	(0.32%)	1	(0.19%)
Total	614	(100%)	518	(100%)

chi-square value = 2.7, dof = 3
P > 0.05

(When collapsed to a 2 x 3 table, with MO and SE combined, chi-square value = 1.66, dof =2, P > 0.05).

Table 6.9. Comparison of the frequency of occurrence and degree of expression of infraocclusion of the mandibular right second molars between males and females - singleton sample.

MRE

	M		F	
NI	679	(95.00%)	604	(94.80%)
MI	32	(4.50%)	28	(4.40%)
MO	4	(0.55%)	5	(0.78%)
SE	0	(0.00%)	0	(0.00%)
Total	715	(100%)	637	(100%)

chi-square value = 0.30, dof = 2
P > 0.05

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, chi-square value = 0.01, dof =1, P > 0.05).

Table 6.10. Comparison of the frequency of occurrence of infraocclusion between males and females for the mandibular first and second molars combined - singleton sample.

All teeth combined

	M		F	
NI	2388	(89.20%)	2066	(89.50%)
I	290	(10.80%)	240	(10.50%)
Total	2678	(100%)	2306	(100%)

chi-square value = 0.2, dof = 1
P > 0.05

These analyses were repeated, for side, tooth type and sex, but including only subjects who displayed some level of infraocclusion, that is, MI, MO and SE. It was shown that that there was not a statistically significant difference in the distribution of infraocclusion between the mandibular first molar (MD) and mandibular second molar (ME) on either right or left sides. Also, there was not a statistically significant difference in the distribution of infraocclusion between the mandibular right and left first molar (MRD and MLD) and the mandibular right and left second molar (MRE and MLE). The results also showed that there was not a statistically significant difference in the distribution of infraocclusion in the MRD, MRE, MLD and MLE between males and females. These tables are included in the Appendix (Tables 4-11).

The expression of infraocclusion in the mandibular first and second molars was compared between right and left sides in each individual. Table 6.11 shows that in the male sample there was a significant association in expression of infraocclusion of the mandibular first molar between right and left sides within individuals (P<0.01). However, a point to be highlighted is that a large number of cases fell into the 'non-infraoccluded' category and this is the main reason for the high level of statistical significance. Additionally, there were no cases from the SE category that occurred bilaterally in individuals. Most of the affected cases were categorised in the MI category, with 39 cases having infraocclusion affecting both sides, while 85 cases (47+38) showed unilateral expression of the condition.

Percentage concordances between right and left sides within individuals were calculated for males and females separately. This calculation involved summing the values on the diagonal and dividing the sum by the grand total, then expressing as a percentage.

That is, % concordance = (sum of values on the diagonal/grand total) x 100.

A percentage concordance of 83% was found between MRD and MLD but as mentioned, this reflected a high number of cases in the non-infraoccluded group (NI).

Table 6.11. Comparison of the frequency of occurrence and degree of expression of infraocclusion of the mandibular first molar between right and left sides within individuals - male singleton sample.

	Males	MRD				
		NI	MI	MO	SE	Total
MLD	NI	448	38	0	0	486
	MI	47	39	3	0	89
	MO	2	5	0	2	9
	SE	0	0	1	0	1
	Total	497	82	4	2	585

chi-square value = 384.8, dof = 9
P <0.001, % concordance = 83.2%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, chi-square value = 117.2, dof =1, P < 0.001).

Similar to the primary mandibular first molar , Table 6.12 shows a significant association in the expression of infraocclusion for the mandibular second molar between right and left sides, within individuals (P<0.05). The percentage concordance was 92%, with 48 cases showing infraocclusion unilaterally and 12 cases showing bilateral expression.

Table 6.12. Comparison of the frequency of occurrence and degree of expression of infraocclusion of the mandibular second molar between right and left sides within individuals - male singleton sample.

	Males	MRE				
		NI	MI	MO	SE	Total
	NI	638	16	1	0	655
MLE	MI	30	12	3	0	45
	MO	1	3	0	0	4
	SE	0	0	0	0	0
	Total	669	31	4	0	704

chi-square value = 139.3, dof = 4
P < 0.001, % concordance = 92.3%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, Fisher's exact test, P < 0.001).

Like the male sample, the female sample also showed a significant association of infraocclusion between the right and left sides within individuals. Table 6.13 shows that there was a significant association in the expression of infraocclusion for the mandibular first molar between right and left sides within individuals (P<0.01). The percentage concordance was 85%, with 67 cases showing infraocclusion unilaterally and 32 cases showing bilateral infraocclusion to a similar degree. Table 6.14 shows that there was a statistically significant association in the expression of infraocclusion of the mandibular second molar between right and left sides within individuals (P<0.01). The percentage concordance was 93%, 36 cases showing infraocclusion occurring unilaterally and 11 cases bilaterally. Overall, the high percentage of concordance was influenced by the large number of cases in the 'non-infraoccluded' category. Only a few cases in the entire sample showed infraocclusion that fell into two different categories on opposite sides of the arch i.e. mild-moderate (26 cases), moderate-severe (3 cases) and mild-severe (1 case).

Table 6.13. Comparison of the frequency of occurrence and degree of expression of infraocclusion of the mandibular first molar between right and left sides within individuals - female singleton sample.

	Females	MRD				
		NI	MI	MO	SE	Total
MLD	NI	374	23	2	0	399
	MI	41	32	5	1	79
	MO	1	3	0	0	4
	SE	0	0	0	0	0
	Total	416	58	7	1	482

chi-square value = 115.1, dof = 6
P <0.001, % concordance = 84.2%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, chi-square value = 108.4, dof =1, P < 0.001).

Table 6.14. Comparison of the frequency of occurrence and degree of expression of infraocclusion of the mandibular second molar between right and left sides within individuals - female singleton sample.

	Females	MRE			
		NI	MI	MO	Total
MLE	NI	567	13	2	582
	MI	21	11	2	34
	MO	0	2	0	2
	Total	588	26	4	618

chi-square value = 134.5, dof = 4
P <0.001, % concordance = 93.5%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, Fisher's exact test, P < 0.001).

In those individuals who showed infraocclusion, contingency tables were also constructed to examine the differences in the expression of infraocclusion according to the classification used in this thesis. Infraocclusion in the mandibular first and second molars were compared between right and left sides in each individual. That is, only individuals who showed infraocclusion were included. A significant association was revealed in the expression of infraocclusion for the mandibular first molar between right and left sides in males ($P<0.01$). There were no cases in the SE or MI categories and there were only a small number of cases in the MO or MI categories (8 cases). There was not a statistically significant association in the expression of infraocclusion for the mandibular second molar between right and left sides in males, when only individuals with infraocclusion were considered. These tables are included in the Appendix (Tables 12 and13).

Similarly, in the female group there was not a statistically significant association in the expression of infraocclusion for the mandibular first molar between right and left sides, or of the mandibular second molar between right and left sides within individuals. These tables are included in the Appendix (Tables 14 and15).

6.7. Results of descriptive analysis of the twin sample

The same variables that were tested in the singleton sample were also tested in the twin sample. One member from each twin pair was randomly selected for inclusion in the study, except in cases where data were available for only one member of a pair, in which case this member was included. Chi-square tests were conducted to compare the frequency of occurrence and the degree of expression of infraocclusion between different teeth and different sides among the twin sample. A significant difference was revealed in the distribution of infraocclusion categories between the mandibular right first molar (MRD) and the mandibular right second molar (MRE) ($P < 0.05$). A greater number of cases of mild (MI), moderate (MO) and severe (SE) infraocclusion were noted in the MRD compared with the MRE (Table 6.15). A similar trend in the expression of infraocclusion was revealed on the left side. This table is included in the Appendix (Table 16).

Table 6.15. Comparison of the frequency of occurrence and degree of expression of infraocclusion between primary mandibular first (D) and second (E) molars (right side) - twin sample.

	MRD		MRE	
NI	209	(78.60%)	250	(87.10%)
MI	40	(15.00%)	30	(10.50%)
MO	8	(3.00%)	4	(1.40%)
SE	9	(3.40%)	3	(1.00%)
Total	266	(100%)	287	(100%)

chi-square value = 8.63, degrees of freedom(dof) =3
$P < 0.05$

(When collapsed to a 2 x 3 table, with MO and SE combined, chi-square value = 8.5, dof =2, $P < 0.05$).

No statistically significant difference was noted in the distribution of the four categories between the mandibular right first molar (MRD) and the mandibular left first molar (MLD) (Table 6.16). Similar to the first molar, Table 6.17 shows that there was not a statistically significant difference in the distribution of the four categories between the mandibular right second molar (MRE) and the mandibular left second molar (MLE).

Table 6.16. Comparison of the frequency of occurrence and degree of expression of infraocclusion between mandibular right and left first molars - twin sample.

	MRD		MLD	
NI	209	(78.6%)	177	(72.2%)
MI	40	(15.0%)	54	(22.0%)
MO	8	(3.00%)	9	(3.7%)
SE	9	(3.40%)	5	(2.00%)
Total	266	(100%)	245	(100%)

chi-square value = 5.08, dof = 3
$P > 0.05$

(When collapsed to a 2 x 3 table, with MO and SE combined, chi-square value = 4.17, dof =2, $P > 0.05$).

Table 6.17. Comparison of the frequency of occurrence and degree of expression of infraocclusion between mandibular right and left second molars - twin sample.

	MRE		MLE	
NI	250	(87.10%)	247	(82.30%)
MI	30	(10.50%)	43	(14.30%)
MO	4	(1.40%)	7	(2.30%)
SE	3	(1.00%)	3	(1.00%)
Total	287	(100%)	300	(100%)

chi-square value = 2.86, dof = 3
P > 0.05

(When collapsed to a 2 x 3 table, with MO and SE combined, chi-square value = 2.57, dof =2, P > 0.05).

A comparison of the frequency of occurrence and the degree of expression of infraocclusion according to sex was performed among the twin sample by conducting chi-square tests. The tests were done for each tooth separately (MRD, MRE, MLD and MLE) and then a test combining all the examined teeth was also carried out. There was not a statistically significant difference in the distribution of the four categories of infraocclusion in any of the teeth examined between males and females. Tables 6.18 and 6.19 show comparisons of primary mandibular right first and second molars between males and females. The left side comparisons are included in the Appendix (Tables 17 and 18). Also, there was not a statistically significant difference between males and females when all teeth were combined (Table 6.20).

Table 6.18. Comparison of the frequency of occurrence and degree of expression of infraocclusion of the mandibular right first molars between males and females - twin sample.

MRD				
	M		F	
NI	100	(81.30%)	99	(80.50%)
MI	17	(13.80%)	15	(12.20%)
MO	2	(1.60%)	6	(4.90%)
SE	4	(3.30%)	3	(2.40%)
Total	123	(100%)	123	(100%)

chi-square values = 2.27, dof = 3
P > 0.05

(When collapsed to a 2 x 3 table, with MO and SE combined, chi-square value = 0.73, dof =2, P > 0.05).

Table 6.19. Comparison of the frequency of occurrence and degree of expression of infraocclusion of the mandibular right second molars between males and females - twin sample.

MRE				
	M		F	
NI	121	(84.00%)	120	(86.30%)
MI	18	(12.50%)	17	(12.20%)
MO	4	(2.80%)	2	(1.40%)
SE	1	(0.70%)	0	(0.00%)
Total	144	(100%)	139	(100%)

chi-square values = 1.61, dof = 3
P > 0.05

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, Fisher's exact test, P > 0.05).

Table 6.20. Comparison of the frequency of occurrence of infraocclusion between males and females for the mandibular first and second molars - twin sample.

All teeth combined				
	M		F	
NI	447	(81.40%)	430	(80.80%)
I	102	(18.60%)	102	(19.17%)
Total	549	(100%)	532	(100%)

chi-square value = 0.06, dof = 1
P > 0.05

The previous analyses were repeated, i.e. for side, tooth type and sex, but only for subjects who displayed some level of infraocclusion, that is, MI, MO and SE. It was revealed that that there was not a statistically significant difference in the distribution of infraocclusion between the mandibular first molar (MD) and mandibular second molar (ME) on either right or left sides. Also, there was not a statistically significant difference in the distribution of infraocclusion between the mandibular right and left first molar (MRD and MLD) or the mandibular right and left second molar (MRE and MLE). The results also showed that there was not a statistically significant difference in distribution of infraocclusion in the MRD, MRE, MLD and MLE between males and females. These tables are included in the Appendix (Tables 19-26).

The expression of infraocclusion in the mandibular first and second molars was compared between right and left sides in each individual. Table 6.21 shows that in the male sample there was a significant association in the expression of infraocclusion for the mandibular first molar between right and left sides within individuals ($P<0.01$). Of the total number of cases showing infraocclusion (n=34), most of the affected cases displayed mild infraocclusion on one side only (n=19) and 10 cases displayed bilateral infraocclusion to a similar degree, while the remaining cases displayed infraocclusion of varying severity (n=5).

Similar to the singleton sample, percentage concordances were calculated between right and left sides within individuals, for males and females separately. A percentage concordance of 80% was found between MRD and MLD. This high percentage concordance can be due to the high number of cases in the non-infraoccluded category.

Table 6.21. Comparison of the frequency of occurrence and degree of expression of infraocclusion for the mandibular first molar between right and left sides within individuals - male twin sample.

Males		MRD				
		NI	MI	MO	SE	Total
	NI	87	5	1	0	93
	MI	13	7	0	1	21
MLD	MO	0	3	1	1	5
	SE	0	0	0	2	2
	Total	100	15	2	4	121

chi-square value = 103, dof = 9
P <0.001, % concordance = 80%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, Fisher's exact test, P < 0.001).

Similar to the primary mandibular first molar, Table 6.22 shows a significant association in the expression of infraocclusion for the mandibular second molar between the right and left side within individuals (P<0.05). The percentage concordance was 80 %, with 22 cases showing infraocclusion unilaterally and 9 cases bilateral expression to a similar or varying degree.

Table 6.22. Comparison of the frequency of occurrence and degree of expression of infraocclusion of the mandibular second molar between right and left sides within individuals - male twin sample.

Males		MRE				
		NI	MI	MO	SE	Total
	NI	99	10	1	0	110
MLE	MI	11	4	0	1	16
	MO	0	1	1	0	2
	SE	0	1	1	0	2
	Total	110	16	3	1	130

chi-square value = 59.7, dof = 9
P < 0.05, % concordance = 80%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, Fisher's exact test, P < 0.001).

Like the male sample, females also showed a significant association of infraocclusion between the right and left sides within individuals. Table 6.23 shows that there was a significant association in the expression of infraocclusion of the mandibular first molar between right and left sides within individuals (P<0.01). The percentage concordance was 84 %, 16 cases showing infraocclusion unilaterally and 17 cases bilaterally, to a similar or varying degree.

Table 6.23. Comparison of the frequency of occurrence and degree of expression of infraocclusion of the mandibular first molar between right and left sides within individuals - female twin sample.

	Females			MRD		
		NI	MI	MO	SE	Total
MLD	NI	86	5	2	0	93
	MI	8	9	2	0	19
	MO	1	0	2	0	3
	SE	0	1	0	3	4
	Total	95	15	6	3	119

chi-square value = 143, dof = 9
P <0.001, % concordance = 84%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, Fisher's exact test, P < 0.001).

Similar to earlier findings described for the female singleton sample, Table 6.24 shows that there was a statistically significant association in the expression of infraocclusion for the mandibular second molar between right and left sides within individuals (P<0.01). The percentage concordance between sides was 80%, with 20 cases showing infraocclusion occurring unilaterally and 12 cases bilaterally to a similar or varying degree. Overall, most of the cases occurred unilaterally, with the high percentage concordance in all groups likely to be influenced by the large number of cases in the 'non-infraoccluded' category.

Table 6.24. Comparison of the frequency of occurrence and degree of expression of infraocclusion for the mandibular second molar between right and left sides within individuals - female twin sample.

Females		MRE				
		NI	MI	MO	SE	Total
MLE	NI	87	7	0	0	94
	MI	13	8	1	0	22
	MO	0	1	1	0	2
	SE	0	0	1	0	1
	Total	100	16	3	0	119

chi-square value =76.4, dof = 6
P< 0.001, % concordance = 80%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, Fisher's exact test, P < 0.001).

In those individuals who showed infraocclusion, contingency tables were constructed to examine differences in the expression of infraocclusion. Infraocclusion in the mandibular first and second molars was compared between right and left sides in each individual. That is, only individuals who showed infraocclusion were included. A significant association in the expression of infraocclusion for the mandibular first molar between right and left sides in females was found (P<0.01). Most of the cases were in the mild category, with the remaining cases showed infraocclusion of varying severity. There was not a statistically significant association in the expression of infraocclusion for the mandibular first molar between right and left sides in males and for the mandibular second molar between right and left sides in males and females. These tables are included in the Appendix (Tables 27-30).

6.8. Discussion

In this study a standardised approach for measuring infraocclusion was followed for both singleton and twin samples. Unlike previous studies, the technique was tested as reliable, allowing precise measurements to be obtained from both samples. The definition of infraocclusion followed the most commonly-used criterion, i.e. a tooth was defined as being infraoccluded if it was 1mm below the normal plane of occlusion. A specified age group of 9 to11 years old was selected for the samples to avoid variation in the prevalence of infraocclusion that could arise due to age. In some previous studies of infraocclusion, children from approximately 3 to 12 years of age were included which is considered to be a large age range. A discrepancy in the prevalence of infraocclusion has been reported among different age groups, thus, it could be said that infraocclusion prevalence is age specific (Via, 1964; Brearley and McKibben, 1973; Kurol, 1981).

The term 'infraocclusion' in this thesis is used to describe teeth that are positioned below the level of occlusion, regardless whether those teeth that are infraoccluded have emerged and then subsequently stopped erupting before reaching the occlusal plane, or whether they have erupted to their final occlusion position before then stopped while the adjacent teeth have continued to erupt. In this study, data available from the samples do not generally enable a definite judgement to be made about the timing of infraocclusion, therefore, only the nature and extent of infraocclusion has been investigated.

Different factors that could affect the estimates of the prevalence of infraocclusion, including measurement technique, definition of infraocclusion and selection of an age group, were controlled to try to overcome previously reported variations in prevalence (Via, 1964; Kurol, 1981; Koyoumdjisky-Kaye and Steigman, 1982b; Shalish et al., 2010). However, variation in the prevalence of infraocclusion was noted in the present investigation between the two samples studied. In the singleton sample, the prevalence of infraocclusion ranged from 0.1% to 16.6%, while in the twin sample, the prevalence ranged from 1% to 22% (Table 6.4). These percentages fall within the ranges reported in the literature (Via, 1964; Brearley and McKibben, 1973; Kurol, 1981).

A large difference in the sample sizes included in this study (1454 orthopantomographs singletons compared to 323 study models of twin pairs) may have contributed to differences in the prevalences of infraocclusion observed between the samples, with the larger sample size likely to provide a better estimate of the true value in a population of European ancestry. Another reason for differences in the prevalence values between singletons and twins may relate to the different methods of data acquisition. A 2D photographic image allows for a more precise outline of the tooth structure to be visualised, as compared with the orthopantomographs, where tooth structure is less distinct due to superimposition of structures, including buccal and lingual cusps. Although double determinations confirmed that the measurement methods used for both samples displayed high reliability and precision, this may have affected the estimated prevalence of infraocclusion in some categories in the singleton sample. Moreover, the greater prevalence of infraocclusion in the twin sample may reflect a distinct feature in twins compared to singletons. For example, some dental features have been reported in the literature to be more common in twins, for example smaller tooth size. Smaller-sized primary teeth (of 2-3%) in twins with low birth weight has been reported (Apps et al., 2004). The birth weight classification followed in this study was: normal birth weight (NBW > 2500g) and low birth weight (LBW ≤ 2500g) with the maximum mesiodistal crown width compared between NBW and LBW groups. Since birth weight and tooth size have been linked, and tooth size is linked with delayed dental development, this might indirectly explain the greater frequency of infraocclusion recorded in twins. Thus, as a special feature of twinning, infraocclusion may be linked to premature birth which is associated with low birth weight and delayed dental development (Seow, 1997). However, a definite conclusion about these possible associations cannot be made at this stage. Further studies in this area are certainly warranted.

Despite variation in the estimated prevalence between the singleton and twin samples, the patterns or trends of displaying infraocclusion within each sample were similar. In the present study it has been revealed that the primary mandibular first molar was the most commonly infraoccluded tooth in both the singleton and twin samples. When compared with the primary mandibular second molar, a significant difference was found (P<0.05). However, when comparing an infraoccluded tooth to its antimere, no significant difference in the expression of infraocclusion was revealed ($P>0.05$) in either sample. Moreover, there was no significant difference in the prevalence of infraocclusion found between males and females ($P>0.05$) in either sample. These results are similar to previously reported findings (Kurol, 1981).

It has been shown that in the singleton sample there was a significant association in the expression of infraocclusion between sides (bilaterally) ($P<0.001$) when teeth in all categories were included, i.e. including the 'non-infraoccluded' category. The percentage concordance between sides varied from 83% to 94%. When the 'non-infraoccluded' category was excluded, a significant association continued to exist for the primary mandibular first molar but not for the second molar, and the percentage concordance between sides dropped to a range from 66% to 78%. Similarly, the twin sample showed a significant association in the expression of infraocclusion between sides (bilaterally) ($P<0.001$) when teeth in all categories were included, i.e. including the 'non-infraoccluded' category, with a percentage concordance varying from 80.0% to 84.0%. However, when the 'non-infraoccluded' category was excluded no significant association was revealed and the percentage concordance also dropped to a range from 55.5% to 75.0%. Except for the mandibular first molar within the female twin sample, excluding the 'non-infraoccluded' category did not result in a change to the significant association existing between right and left sides, with a consistently high percentage concordance of 82.4%. This is because in the female sub-sample most of the remaining cases displayed infraocclusion bilaterally.

6.9. Conclusion

Findings reported in this phase of the study revealed that the frequency of occurrence of infraocclusion was higher in twins compared to singletons, although these results need to be interpreted with caution due to the differences in sample sizes and in the records used to record infraocclusion. It was also revealed that the mandibular arch showed a higher prevalence of infraocclusion compared to the maxillary arch in both samples. Moreover, the primary mandibular first molar was found to be the most commonly infraoccluded tooth in singletons and twins. No sex predilection was reported in association with infraocclusion and no evidence to show an association between the degree of expression of infraocclusion and arch side (right/left) was found.

7. Genetic influences on infraocclusion in twins

7.1. Introduction

Tooth eruption is influenced by genetic, epigenetic and environmental factors which determine the timing and direction of the process. More than 300 genes have been identified in the process of dental development (Thesleff, 2006; Brook, 2009), and a series of intracellular and extracellular interactions, mediated by these genes, occurs in the ectoderm and mesenchyme during dental development (Cobourne and Sharpe, 2013). These interactions regulate initiation, morphogenesis and differentiation of the developing teeth (Brook, 2009). Factors that influence the expression of genes, known as 'epigenetic factors', play a role in the dental developmental process. These factors may be the result of variation in methylation of DNA or acetylation of their histones, or may be due to changes in the responses of odontogenic cells to variations in local signalling molecules transmitted between cells during dental development (Townsend et al., 2009). A small epigenetic variation at the local level during tooth formation can result in distinct differences in the final clinical expression (Townsend et al., 2005; Hughes and Townsend, 2013).

Infraocclusion can occur in some human syndromes affecting the oro-facial region, such as cleidocranial dysplasia, osteopetrosis, ectodermal dysplasia and Down syndrome (Suri et al., 2004; Ahmad et al., 2006). For most of these syndromes, the causative genes and modes of inheritance have been identified (Proffit and Frazier-Bowers, 2009). Infraocclusion can also occur in the absence of associated syndromes and, in this form, a genetic predisposition to infraocclusion has been proposed (Kurol, 1981; Winter et al., 1997; Karacay et al., 2007). However, only a few cases of infraocclusion have been reported in twins. Similarities in the distribution, pattern and severity of infraoccluded teeth have been recorded in monozygotic (MZ) twin pairs, suggesting that genetic factors are important (Stewart and Hansen, 1974; Kurol, 1981; Helpin and Duncan, 1986; Dewhurst et al., 1997; Cozza et al., 2004; Zengin et al., 2008). A genetic predisposition to infraocclusion has also been postulated because one or more other dental anomalies have been identified to be associated with the condition (Baccetti, 2000; Garib et al., 2009; Peck, 2009; Shalish et al., 2010). However, a strong genetic predisposition to infraocclusion has not been confirmed so far.

The relative contributions of genetic and environmental factors to variation in a variety of traits, including dental phenotypes, can be estimated by investigating data collected from twins. Two types of twins have been identified, monozygotic (MZ) where the co-twins are assumed to share 100% of their genes, and dizygotic (DZ) who share 50% of their genes on average, so the theoretical maximum correlation between MZ co-twins will be 1.0 and that between DZ co-twins will be 0.5. (Townsend et al., 2009). In studies using the traditional twin model, the assumption is that similar environmental factors are acting on both MZ and DZ twins. Shared or common environmental factors between co-twins tend to increase the DZ correlation above half the MZ value, while dominance genetic factors (dominance effect between alleles at the same locus) will decrease it below this value (Morris-Yates et al., 1990; Neale and Cardon, 1992). Thus, if DZ correlations are found to be less than half the corresponding MZ values, then dominance genetic factors are likely to be the main source of variance rather than shared environmental factors (van Dongen et al., 2012). Moreover, MZ twins allow the estimation of unique environmental factors that are not shared with a co-twin (Hopper, 1993). The extent of genetic variation contributing to variation in the expressed phenotype, known as the heritability estimate (h^2), has been found to be high for most tooth size variables, for Carabelli trait and for dental arch dimensions. Moderate heritability estimates have been reported for intercuspal distances and low estimates for occlusal traits (Townsend et al., 2009).

Genetic modelling of data derived from MZ and DZ twin pairs has been used to investigate the relative contribution of genetic and environmental factors to variation of several dental phenotypes, such as Carbelli trait (Townsend and Martin, 1992), primary and permanent tooth size (Dempsey et al., 1999; Hughes et al., 2000; Dempsey and Townsend, 2001), and timing of primary tooth eruption (Hughes et al., 2007). Genetic models can be fitted to data to test how different combinations of variance components, including additive genetic factors (A), non-additive genetic factors (D), common or shared environmental factors (C) and environmental factors that are unique to the individual, including measurement error (E), contribute to overall phenotypic variation.

A simple model can be seen in Figure 7.1. Genetic factors and common environmental factors are correlated between twins, while unique environmental effects are not. The expected maximum correlations of genetic and environmental variances for MZ co-twins are 1.0, while the expected maximum correlations of additive genetic variance, common environmental variance and dominance genetic variance for DZ co-twins are 0.5, 1.0 and 0.25 respectively.

For twins reared together, it is not possible to model ACDE simultaneously as the model is under-identified (Dempsey et al., 1999). Normally, the model is ACE and/or ADE and then comparisons are carried out using Akaike's Information Criterion (AIC). Model-fitting methods to analyse twin data allow estimation of the strength of genetic and environmental contributions within calculable confidence intervals (Townsend et al., 2009). The best models fitted to data on primary incisor emergence, derived from Australian twins, included additive genetic variance (A) and unique environmental variance (E). In contrast, the best models for some intercuspal distances of molars included only unique environmental variance (E) or a combination of common and unique environment (CE). Models that best fit data for the mesiodistal crown diameters of permanent first molars and maxillary canines were ACE and ADE models respectively (Townsend et al., 2009).

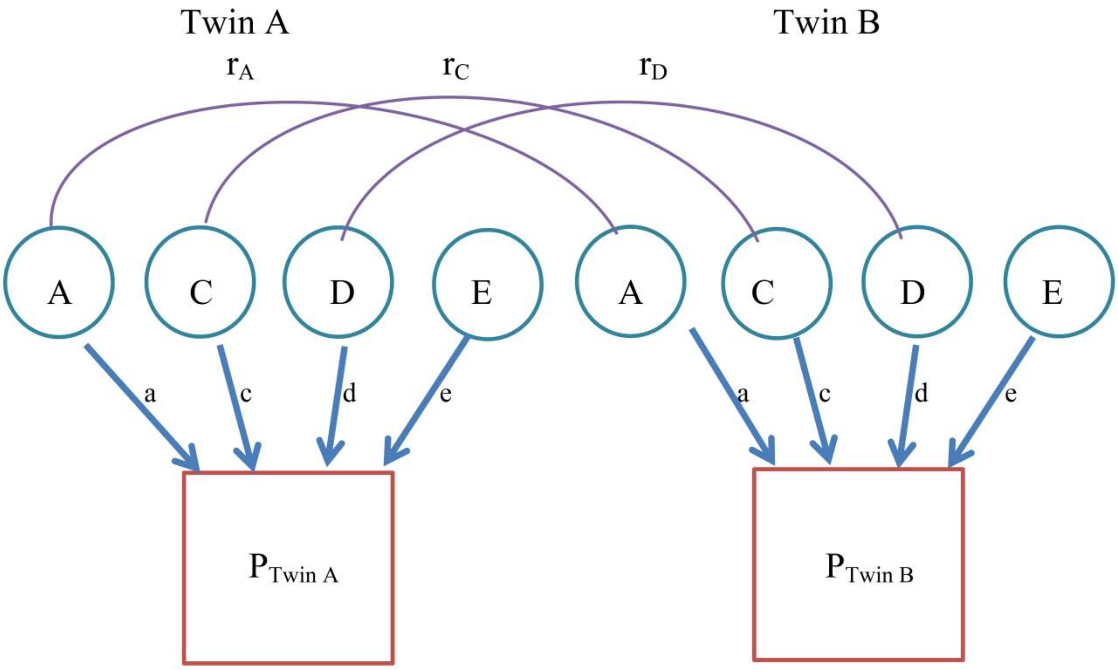


Figure 7.1.Univariate model of the twin relationship.

The latent or hidden factors contributing to phenotypic variation of each twin are postulated to be: additive genetic factors (A), common environment factors (C), dominance genetic factors (D) and unique environment factors (E). Arrows pointing to the phenotype in each twin ($P_{Twin\ A}$ and $P_{Twin\ B}$) represent the contribution of the variances (a,c,d and e) to the phenotype. Correlations between Twin A and Twin B for additive genetic factors, common environment factors and dominance genetic factors are denoted by r_A, r_C and r_D respectively and their theoretical values are 1 for MZ twin pairs. While for the DZ twin pairs the theoretical values for the r_A is 0.5, 1 for the r_C and 0.25 for the r_D.

Genetic modelling can be applied to categorical data and also to continuously variable data. For example, Townsend and Martin (1992) classified Carabelli trait on an eight-grade categorical scale, then applied genetic modelling to clarify genetic and environmental contributions to variation of the trait on permanent first molar teeth in a sample of 448 South Australian twin pairs. The fitted model included additive genetic effects together with both a general environmental component and an environmental effect specific to each side. An estimate of heritability of around 90% indicated that there was a very strong genetic contribution to observed variation.

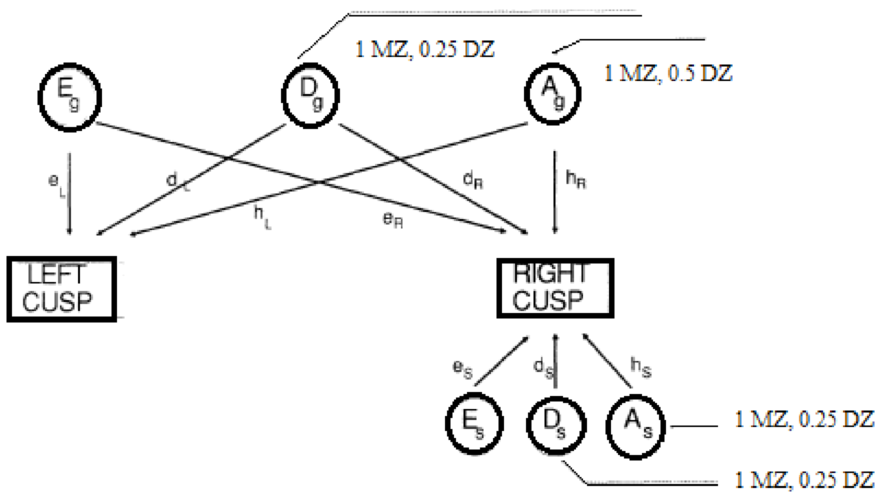


Figure 7.2. Gene-environment modelling of Carabelli trait for one member of a pair of MZ twins, consisting of general additive genetic factor (Ag), general dominance genetic factor (Dg) and general unique environmental factor (Eg) (derived from Townsend and Martin, 1992).

The arrows pointing to the right and left cusps represent the amount of contribution of variance to occurrence of the trait on each side, that is, the h_R, d_R and e_R arrows represent the contribution of genetic and environmental factors to the right side. While the h_L, d_L and e_L arrows represent the contribution of genetic and environmental factors to the left side. The specific variance contributing to the expression of the trait on the right side consisting of unique environmental, dominance, and additive genetic influences (E_S, D_S and A_S). The arrows pointing to the right cusp represent specific variance influences on the right cusp denoted by e_S, d_S and h_S (derived from Townsend and Martin (1992)).

A full genetic model was fitted to correlate the genetic and environmental influences on the expression of the trait between Twin A and Twin B on both sides. A model for one of the twins is shown in Figure 7.2. A general additive genetic factor (A_g) influences the expression of the trait on the right and left sides (h_L and h_R), and a general dominance genetic factor (D_g) influences the expression of the trait on both sides (d_L and d_R) (Townsend and Martin, 1992).

Another example of genetic modelling fitted to a continuous variable, such as tooth size, is presented in a study conducted by Hughes et al. (2000). Data of primary dental crown sizes from 220 twin pairs and 160 singletons were subjected to univariate genetic analysis with the structural equation modelling package Mx, using the normal assumptions of the twin model. Estimates of heritability ranged from 62% to 90% indicating that variation in primary tooth crown size has a strong genetic component.

From an extensive review of the literature no paper has been found investigating a possible genetic contribution to infraocclusion by applying genetic modelling to a large sample of twins. As mentioned earlier, only a few case reports of twins with infraocclusion have been presented. Moreover, an accurate and reliable technique for measuring infraocclusion has been lacking in previous reports and the application of a sophisticated genetic modelling approach should add to our current knowledge by enabling the contributions of genetic and non-genetic factors to variation in infraocclusion to be quantified.

In this phase of the study, the classical twin method was used to clarify whether a significant genetic contribution to variation in expression of infraocclusion could be identified in the study sample. Initially, similarities in infraocclusion among MZ twin pairs were compared with similarities in infraocclusion among DZ twin pairs, assuming similar environmental factors, to determine whether there was any evidence of a genetic influence on variation in infraocclusion. More sophisticated genetic modelling was also performed.

The main aim of preliminary comparisons was to assess if genetic associations existed in relation to the occurrence of infraocclusion in members of a twin pair. This was followed by model fitting to quantify genetic and environmental contributions to infraocclusion and to provide estimates of heritability.

7.2. Materials and methods

Study samples

This study analysed data collected from study models of MZ and DZ twins who are part of a larger study that has been carried out over the past 30 years by the Craniofacial Biology Research Group, School of Dentistry, University Adelaide led by Professor Grant Townsend. The entire study includes records from over 1,200 pairs of twins. These are divided into three main cohorts with approximately 300 twin pairs in each of cohorts 1 and 2, and around 600 twin pairs in cohort 3. The zygosities of over 600 MZ and same-sex DZ twins have been confirmed (Hughes et al., 2013). Information available for cohorts 1 (n=300 pairs) and 2 (n=300 pairs) includes records of teeth present, dental caries, other dental problems (e.g. anomalies in the pattern of eruption), study models and intraoral photographs (Townsend et al., 2006; Hughes et al., 2013). Twins from these two cohorts were included in the present study.

A brief summary of the methods used has been included in this chapter. A more detailed description of the methods has been given in Chapter 4.

Measurement technique

A 2D scanning system was used to obtain four images per individual, that is, images of both sides of the upper and lower dental arches. The 2D imaging system that was used in this study was adapted from the system described by Brook et al. (1999). Photoshop CS5 computer software was then used to construct the lines between reference points and to measure selected distances (in mm). To determine the 'occlusal plane', a line was extended from the mesial marginal ridge of the first permanent molar to the cusp tip of the primary canine. This was constructed to reflect the orientation of the arch (i.e. the curve of Spee). Next, the 'occlusal table' of the primary first molars was constructed by drawing a line from its distal marginal ridge to its mesial marginal ridge. A similar line was constructed for the primary second molar. Following this, a line was drawn perpendicularly, from the mid-point of the ‘occlusal table’ to the ‘occlusal plane’, and this distance was measured (mm). After completion of all measurements from study models, ranges of measurements were categorised initially into the groups of ‘non-infraoccluded’, as well as ‘mild’, ‘moderate’ and ‘severe’ infraocclusion. A more detailed description of the process of determining the categories of infraocclusion has been included in Chapter 4.

7.3. Statistical analysis

Statistical analyses were performed using IBM SPSS Version 20. Chi-square tests were carried out to compare the frequency of occurrence and the degree of expression of infraocclusion between members of twin pairs. Percentage concordances were also calculated to quantify the occurrence of infraocclusion in both members of a twin pair.

Where the expected numbers in any of the categories were less than five, caution was exercised in interpreting the chi-square values and their associated probabilities. Where possible, categories were collapsed, especially the moderate and severe categories. In cases where expected counts in the cells were less than five, Fisher’s exact tests were performed.

Genetic modelling performed in this phase of the study followed an approach that was developed by Neale et al. (2006). This model allows testing the initiation and progression of infraocclusion in members of twin pairs, taking into consideration that those individuals who do not show infraocclusion, that is, do not have the condition at the initial examination (initiation), will also not show any progression of the feature. In those individuals displaying infraocclusion, progression was further categorised into mild, moderate and severe (due to the small sample size the moderate and severe categories were combined for the final model). The path diagram generated to display the inter-relationships between latent variables and observed phenotypes within and between twin pairs is shown in Figure 7.3.

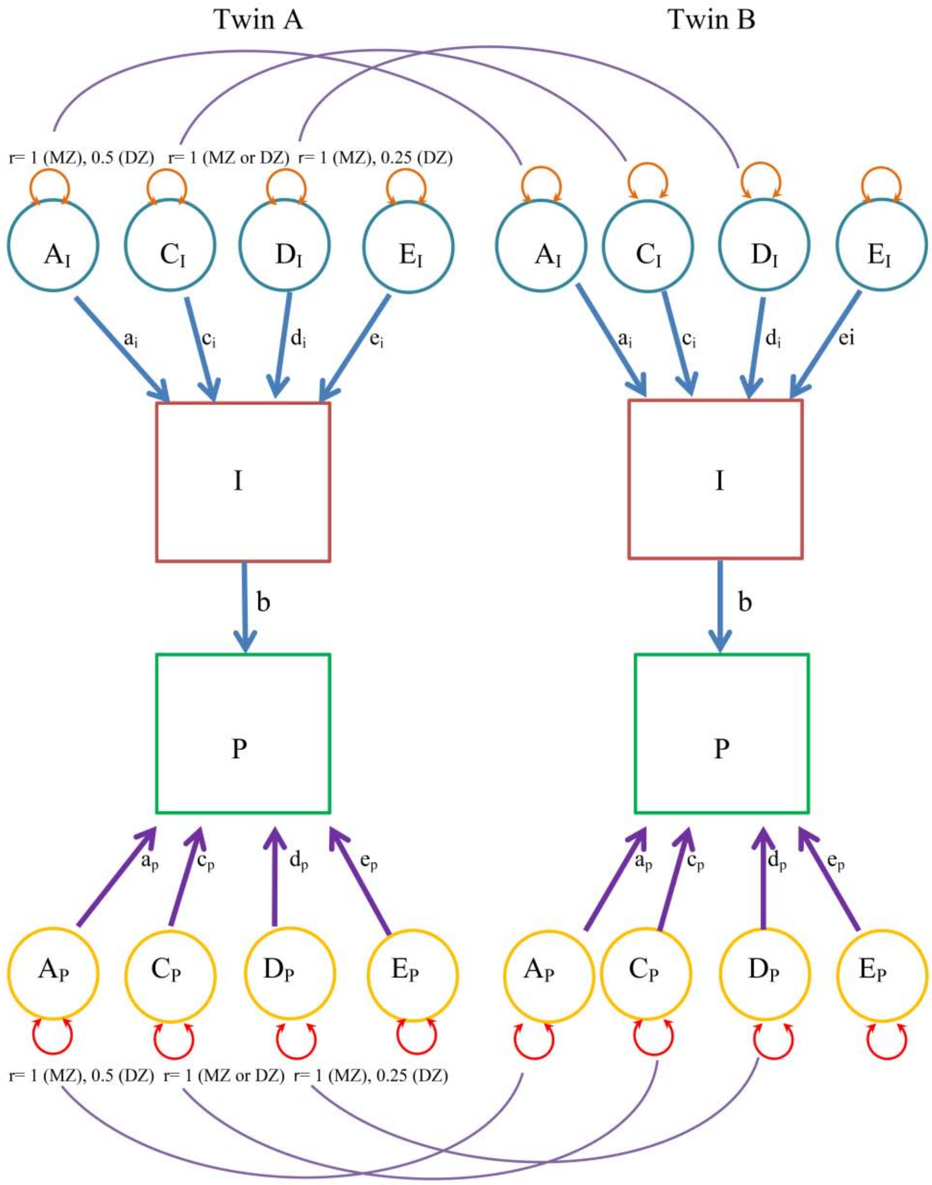


Figure 7.3. Full genetic modelling path diagram for Twin A and Twin B showing the relationship between initiation and progression. The model includes specifications for MZ and DZ twins.

The general factors contributing to initiation and progression of phenotypic variation of each twin are: additive genetic factors (A_I), common environment factors (C_I), dominance genetic factors (D_I) and unique environment factors (E_I). The value of the (b) estimates (arrows pointing down) represents the proportion of the variance in initiation that is transported to progression. The specific factor estimates represent the contribution to the residual progression that is not explained by the general factors: additive genetic factors (A_P), common environment factors (C_P), dominance genetic factors (D_P) and unique environment factors (E_P).

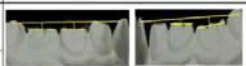
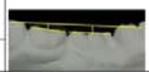
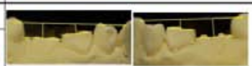
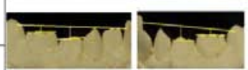
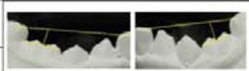
The software package Mx (Neale et al., 2006) was used to perform the genetic modelling. Initially, data were broken down to produce a 'super-model' against which to test goodness-of-fit of nested sub-models. A model including additive genetic, unique environmental and common environmental variances was then fitted. Implicit in the model fitting were the normal assumptions of the twin method: that mating was random, that trait-related shared environmental influences on MZ and DZ twins were equal, and that there was no G × E interaction or G × E correlation (Kang et al., 1977).

The process of model-fitting included an estimation of the path coefficients (a, c, and e) and chi-squared statistics for goodness-of-fit of the models. The parsimony of each model was indicated by Akaike's Information Criterion (AIC = chi-squared value minus twice the degrees of freedom). Narrow-sense heritability estimates (h^2) were calculated as the ratio of additive genetic variance to total phenotypic variance in the best-fitting model. When estimated heritability values are near 100% this indicates that most of the phenotypic variation can be explained by additive genetic effects, whereas when estimated heritability values are near zero this indicates that variation in the phenotype are due mainly to environmental effects.

7.4. Comparisons of expression of infraocclusion among MZ and DZ twin pairs

An overall presentation of the twin sample, including all cases displaying infraocclusion in one or both members of a twin pair, is provided in Tables 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, and 7.8. These tables display 2D images that were obtained and measured according to the criteria discussed earlier. Twin A and Twin B refer to the original designation of the twins when they were included as part of the larger ongoing study, with no relevance to the presence or absence of infraocclusion. Table 7.1 includes male MZ twin pairs where both members of a pair had infraocclusion: there were 17 male twin pairs where both members of the pair displayed infraocclusion. Among these MZ twin pairs a similarity in the expression of infraocclusion was noted in most cases, however, the severity of infraocclusion varied. Of the male MZ pairs, 10 showed similar expression of infraocclusion (ID 163, 228, 326, 402, 489, 596, 609, 648, 808) and there were six male MZ pairs where one member of the pair had unilateral infraocclusion while the other pair had bilateral infraocclusion (ID 118, 208, 243, 337, 398, 502). There was one male MZ pair who showed a mirror image pattern of infraocclusion (ID 324) (Table 7.1).

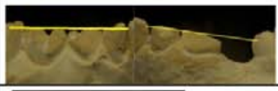
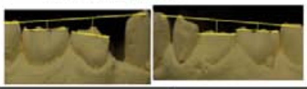
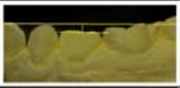
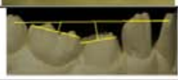
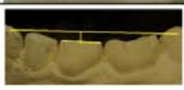
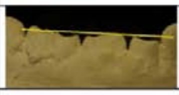
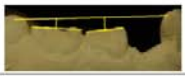
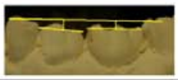
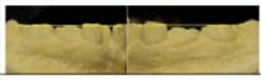
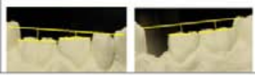
Table 7.1. MZ male twins where both members of the pair showed infraocclusion.

Twin ID	MZ Male twins both members of the pair showing infraocclusion					
	Twin A			Twin B		
	Teeth affected		2D Images	Teeth affected		2D Images
118		E(MO)		E (MI)	E(MI)	
				E(MO)	E(SE)	
163						
	E(MI)	E(MI)		E(MI)	E(MI)	
208						
		E(MI)		E(MI)	E(MI)	
228						
		E(MI)			E(MI)	
243						
	D,E(MI)	D,E(MI)		D,E(MI)		
324						
	E(MI)				E(MO)	
326						
	D(MI)	D(MO)		D(MI)	D(MI)	
328						
		D,E(MI)			D(MI)	
337						
		D(MI)		E(MO)	D,E(MI)	
398						
	E(MI)	E(MI)			E(MI)	
402						
		D(MI)			D(MI)	
489						
	D(MI)E(MO)	D(MI)E(MO)		D,E (MO)	D(MI)E(MO)	
502						
	D(MI)	D(MI)		D(MI)		
596						
	E(MO)	E(SE)		D(SE)E(MO)	D,E(SE)	
609						
	D(SE)E(MI)	D(MI)E(MO)		D,E(MI)	E(MI)	
648						
	D(MO)E(MI)	D(MO)E(MI)		D(SE)E(MO)	D,E (MO)	
808						
	D,E (MO)	D,E (MO)		D(MO)E(MI)	D(MO)	

Mild (MI), moderate (MO), severe (SE), primary first molar (D), primary second molar (E).

For 13 male MZ pairs, one member only of the pair showed infraocclusion. Most of these cases were examples of mild infraocclusion present unilaterally, except for three cases where infraocclusion was present bilaterally (ID 372, 519 and 599) (Table 7.2). For two of the cases presented in Table 7.2 (ID 184 and 262) the models of the co-twin were not available, so these are not presented.

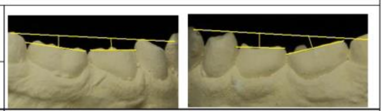
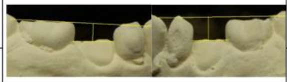
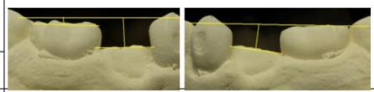
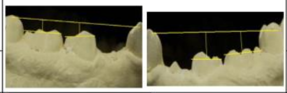
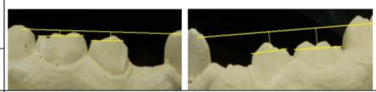
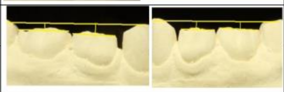
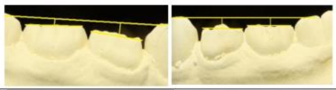
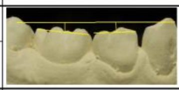
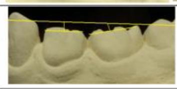
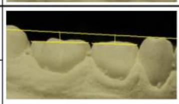
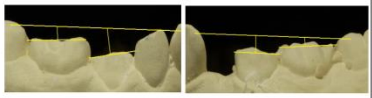
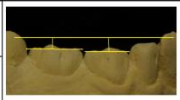
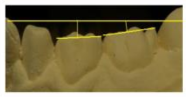
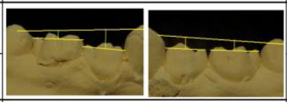
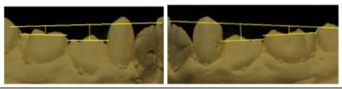
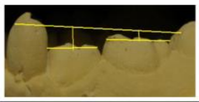
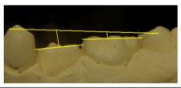
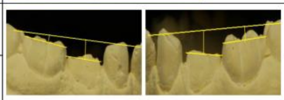
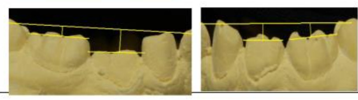
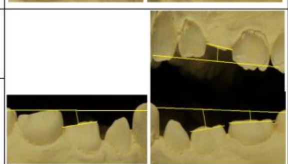
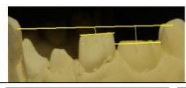
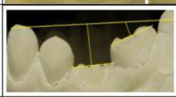
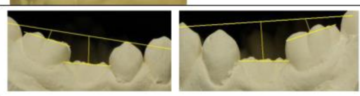
Table 7.2. MZ male twins where one member of the pair showed infraocclusion.

Twin ID	Twin A			Twin B		
	Teeth affected		2D Images	Teeth affected		2D Images
174	D(MI)					
184		E(MI)				Models not available
254	D(MI)					
262		E(MI)				Models not available
372				D(MI)	D(MI)	
374		D(MI)				
429				D(MI)		
441		D(MI)				
447				D,E(MI)		
449		D ,E(MI)				
477				D(MI)		
514		D,E(MI)				
519				D,E(MI)	D,E(MI)	
550					D(MI)	
599	D,E(MI)	D(MI)				

Mild (MI), moderate (MO), severe (SE), primary first molar (D), primary second molar (E).

Among the female MZ pairs (Table 7.3), 12 pairs showed infraocclusion in both members. Six twin pairs displayed bilateral infraocclusion (ID 205, 212, 317, 379, 440, 515) and four had unilateral infraocclusion in one member of the pair while the other member had bilateral infraocclusion (ID 65, 320, 551, 624). The remaining two pairs had different expression of infraocclusion that can be described as mirror image phenomena (ID 319 and 368).

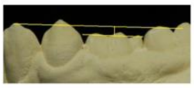
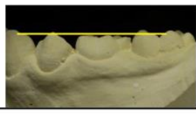
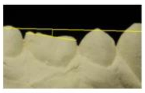
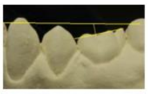
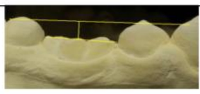
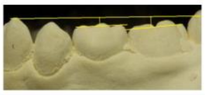
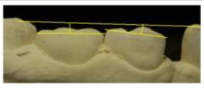
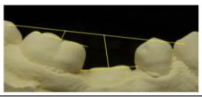
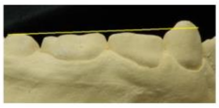
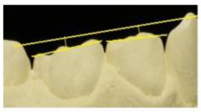
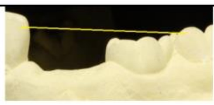
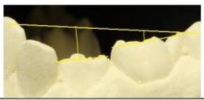
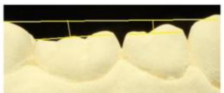
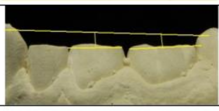
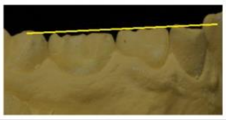
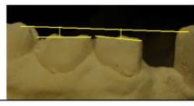
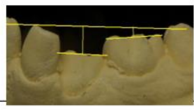
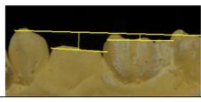
Table 7.3. MZ female twins where both members of the pair showed infraocclusion.

Twin ID	Twin A Teeth affected			Twin B Teeth affected		
65		D,E (MI)		D,E(MI)	D,E (MI)	
205	D(SE)	D(SE)		D(SE)	D(SE)	
212	D(MI)E(MO)	D,E(SE)		D (MI)	D,E(MO)	
317	D(MI)	D(MI)		D(MI)	D,E (MI)	
319	E(MI)				D(MI)	
320	D(MI)			D(MO)E(MI)	D(MO)	
368	D,E(MI)				D,E(MI)	
379	D,E(MI)	D,E (MI)		D,E(MI)	D,E (MI)	
440		D(MO)			D(MI)	
515	D(MI)	D(MO)E(MI)		D(MO)	D,E(MI)	
551	D(MI)	D(MI) D,E(MI)			E(MI)	
624		D(SE)E(MI)		D(SE)	D(SE)E(MI)	

Mild (MI), moderate (MO), severe (SE), primary first molar (D), primary second molar (E).

Of the female MZ twin pairs, eight pairs displayed unilateral infraocclusion in one member of the pair (Table 7.4). Cases where models were not available for a co-twin were not included (ID 151, 217, 219, 292 and 421).

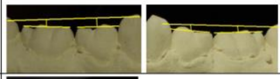
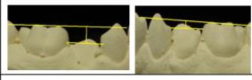
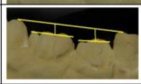
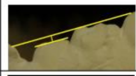
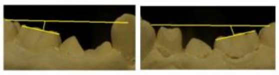
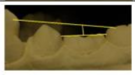
Table 7.4. MZ female twins where one member of the pair showed infraocclusion.

Twin ID	Twin A Teeth affected			Twin B Teeth affected		
71		E(MI)				
151	E(MI)	E(MI)	 			Models not available
217	E(MO)					Models not available
219		E(MI)				Models not available
234	E(MI)					
292			Models not available	D(SE)		
312				E(MI)		
315					D(MO)	
316	E(MI)					
321		D,E (MI)				
370				D,E(MI)		
400					D(MO)	
421			Models not available		D(MI)	

Mild (MI), moderate (MO), severe (SE), primary first molar (D), primary second molar (E).

The male DZ twin pairs displaying infraocclusion in both members of the pair are included in Table 7.5. Two had a similar pattern of bilateral infraocclusion (ID 323 and 363), while the third pair displayed unilateral infraocclusion in one member and bilateral infraocclusion in the other member (ID 367).

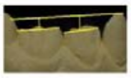
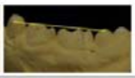
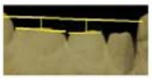
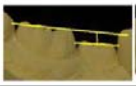
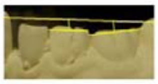
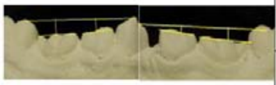
Table 7.5. DZ male twins where both members of the pair showed infraocclusion.

Twin ID	**Twin A**			**Twin B**		
	Teeth affected		2D Images	Teeth affected		2D Images
323	D,E(MI)	D(MI)		D(MO)	D(MI)	
363	D,E(MI)				D(MI)	
367	E(MI)	E(MI)			E(MI)	

Mild (MI), moderate (MO), severe (SE), primary first molar (D), primary second molar (E).

Of the male DZ twin pairs, seven pairs displayed infraocclusion in one member as presented in Table 7.6. Four co-twins displayed unilateral infraocclusion (ID 424, 499, 562, 574) and the remaining three co-twins displayed bilateral infraocclusion (ID 351, 404, 591). For two pairs, models were not available for one member of the pair, so these are not presented.

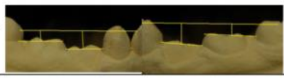
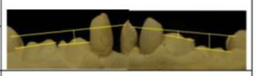
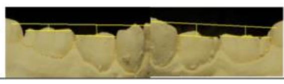
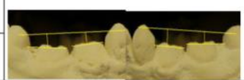
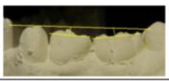
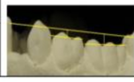
Table 7.6. DZ male twins where one member of the pair showed infraocclusion.

Twin ID	Twin A			Twin B		
	Teeth affected			Teeth affected		
172			Models not available		E(MI)	
351			 	D,E(MI)	D,E(MI)	 
404			 	D(MI)	D(MI)	 
424	D, E(MI)					
470	D(MI)	D(MI)	 			Models not available
499					D,E(MI)	
562		D,E(MI)				
574		D,E(MI)				
591	D,E(MI)	E(MI)				 

Mild (MI), moderate (MO), severe (SE), primary first molar (D), primary second molar (E).

Similar to the males, three pairs in the female DZ twin sample displayed infraocclusion in both members (Table 7.7). A similar pattern of bilateral infraocclusion was expressed in the three pairs; however, the severity varied among members of the pair.

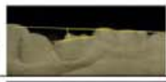
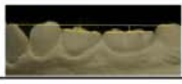
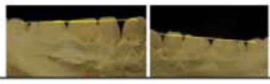
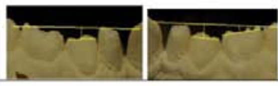
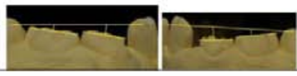
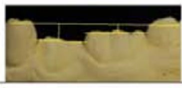
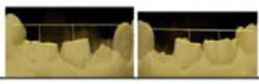
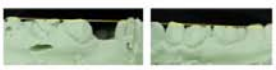
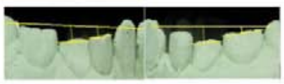
Table 7.7. DZ female twins where both members of the pair showed infraocclusion.

Twin ID	Twin A			Twin B		
	Teeth affected		2D Images	Teeth affected		2D Images
458	D(MO)E(MI)	D(SE)E(MO)		D, E(MO)	D(MI)E(MO)	
488	D(MI)	D,E(MI)		D,E(MI)	D(MO)E(MI)	
566		E(MI)			D,E(MI)	

Mild (MI), moderate (MO), severe (SE), primary first molar (D), primary second molar (E).

The female DZ twin pairs who displayed infraocclusion in one member are presented in Table 7.8. Eight co-twins displayed unilateral infraocclusion (ID 340, 347, 350, 369, 483, 505, 531, 543), while the other five co-twins displayed infraocclusion bilaterally (ID 162, 393, 463, 538, 804). Models were not available for one member of the pair, so these are not included in the comparison.

Table 7.8.DZ female twins where one member of the pair showed infraocclusion.

Twin ID	Twin A			Twin B		
	Teeth affected		2D Images	Teeth affected		2D Images
162	D,E(MI)	D,E(MI)				
341		D,E(MI)				
347	D(MI)					
349					D(MI)	
350		D(MI)				
369	D(MI)					
393				D(MI)	D(MI)	
463				D(MI)	D(MI)	
483	D(MO)					
505		D(MO)E(MI)				
531	D(MI)					
538	D,E(MI)	D,E(MI)				
543		D,E(MI)				
804				D(MI)	D(MI)	

Mild (MI), moderate (MO), severe (SE), primary first molar (D), primary second molar (E).

7.5. Comparisons of concordances for infraocclusion among MZ and DZ twin pairs

As mentioned earlier, to compare the frequency of occurrence and degree of expression of infraocclusion between co-twins, that is, Twin A and Twin B, contingency tables were constructed, chi-square tests were carried out and percentage concordances were calculated. Comparisons of the expression of infraocclusion were carried out among monozygotic (MZ) and dizygotic (DZ) co-twins (denoted twin A and twin B) for males and females separately.

A significant association of infraocclusion in the primary mandibular right first molars was revealed between Twin A and Twin B of the male MZ twin pairs (P< 0.001), and a percentage concordance of 79% was calculated (Table 7.9). Although the high percentage concordance indicates similarities in the expression of infraocclusion between Twin A and Twin B, it needs to be pointed that a large number of concordant cases were in the non-infraoccluded category (n= 31), as this is mainly responsible for these results.

Table 7.9. Comparison of the frequency of occurrence and degree of expression of infraocclusion in the primary mandibular right first molars among members of male MZ twin pairs.

	MRD	Twin A				
		NI	MI	MO	SE	Total
Twin B	NI	31	1	0	0	32
	MI	3	3	0	1	7
	MO	1	2	0	0	3
	SE	0	0	1	0	1
	Total	35	6	1	1	43

Chi-square value = 64, dof =9
P < 0.001, % concordance=79%
Excluding NI % concordance = 25%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, Fisher's exact, P < 0.001).

The male MZ twin pairs also showed a significant association (P <0.05) when infraocclusion of the primary mandibular right second molar was compared between Twin A and Twin B. Percentage concordances of 87% was revealed (Tables 7.10).

Table 7.10. Comparison of the frequency of occurrence and degree of expression of infraocclusion in the primary mandibular right second molars among members of male MZ twin pairs.

	MRE	Twin A				
		NI	MI	MO	SE	Total
	NI	36	1	0	0	37
	MI	4	2	0	0	6
Twin B	MO	1	0	2	0	3
	SE	0	0	0	0	0
	Total	41	3	2	0	46

Chi-square value = 38, dof = 4
P < 0.001, % concordance = 87%
Excluding NI % concordance = 40%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, Fisher's exact, P < 0.001).

The left side comparisons of infraocclusion in the primary mandibular left first and second molars in male MZ twin pairs showed similar results to the right side. These are included in the Appendix (Tables 31 and 32).

When cases of infraocclusion in the primary mandibular right and left first molars in Twin A were compared to Twin B among male DZ twins, no association was revealed. High percentage concordances of 87% for the right side and 75% for the left side were revealed, although these percentages reflect the fact that most of the concordant cases were in the non-infraoccluded category. Table 7.11 represents the right side, and the results of the left side are included in the Appendix (Table 33).

Table 7.11. Comparison of the frequency of occurrence and degree of expression of infraocclusion in the primary mandibular right first molars among members of male DZ twin pairs.

	MRD	Twin A		
		NI	MI	Total
Twin B	NI	28	2	30
	MI	2	0	2
	Total	30	2	32

Fisher's exact, P > 0.05
% concordance = 87.5%
Excluding NI % concordance = 0%

Similar to the first molar, no statistically significant association was found when comparing infraocclusion of the primary mandibular right and left second molars between Twin A and Twin B in male DZ twin pairs. High percentage concordances of 94% and 85% respectively were noted, reflecting the large number of concordant cases in the non-infraoccluded category. Table 7.12 shows results of the right side, while results for the left side are included in the Appendix (Table 34).

Table 7.12. Comparison of the frequency of occurrence and degree of expression of infraocclusion in the primary mandibular right second molars among members of male DZ twin pairs.

	MRE	Twin A		
		NI	MI	Total
Twin B	NI	35	2	37
	MI	0	0	0
	Total	35	2	37

Fisher's exact, P > 0.05
% concordance = 94.6%
Excluding NI % concordance = 0%

Comparisons of the expression of infraocclusion in the primary mandibular right first molars were carried out between Twin A and Twin B of female MZ twins, showing a significant association (P < 0.001) (Table 7.13). A percentage concordance of 87% was revealed. A large number of concordant cases were in the non-infraoccluded category (n=32), which is mainly responsible for these results.

Table 7.13. Comparison of the frequency of occurrence and degree of expression of infraocclusion in the primary mandibular right first molars among members of female MZ twin pairs.

	MRD	Twin A				
		NI	MI	MO	SE	Total
	NI	32	1	0	0	33
	MI	1	3	0	0	4
Twin B	MO	0	2	0	0	2
	SE	0	0	0	1	1
	Total	33	6	0	1	40

Chi-square value = 66, dof =6
P < 0.001, % concordance=87%
Excluding NI % concordance = 50%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, Fisher's exact, P < 0.001).

Similar to the first molar, comparisons were carried out for the primary mandibular right second molar between Twin A and Twin B of female MZ twin pairs. There was no significant association when infraocclusion in the primary mandibular right second molars were compared between Twin A and B (Table 7.14). The high percentage concordance of 84% is because most of the cases are in the non-infraoccluded category.

Table 7.14. Comparison of the frequency of occurrence and degree of expression of infraocclusion in the primary mandibular right second molars among members of female MZ twin pairs.

	MRE	Twin A		
		NI	MI	Total
	NI	36	4	40
	MI	3	2	5
Twin B	MO	0	0	0
	Total	39	6	45

Fisher's exact , P > 0.05
% concordance = 84.5%
Excluding NI % concordance = 22%

The left side comparisons of infraocclusion in the primary mandibular left first molars in female MZ twin pairs showed similar results to the right side. These are included in the Appendix (Table 35). Different to the right side, comparison of infraocclusion in the primary mandibular left second molar revealed a significant association between Twin A and Twin B. These are also included in the Appendix (Table 36).

Similar to the male twin pairs, comparisons were also carried out for the female DZ twin pairs. It was revealed that there was a significant association of infraocclusion of the primary mandibular right first molars between Twin A and Twin B (P < 0.001) (Table 7.15). A high percentage concordance (81%) was found, reflecting the large number of concordant cases in the non-infraoccluded category (n=22).

Table 7.15. Comparison of the frequency of occurrence and degree of expression of infraocclusion of the primary mandibular right first molars among members of female DZ twin pairs.

	MRD	Twin A				
		NI	MI	MO	SE	Total
Twin B	NI	22	4	0	0	26
	MI	2	3	0	0	5
	MO	0	0	1	0	1
	SE	0	0	0	0	0
	Total	24	7	1	0	32

Chi-square value = 37, dof =4
P < 0.001, % concordance=81%
Excluding NI % concordance = 40%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, Fisher's exact, P < 0.05).

When infraocclusion of the primary mandibular left first molars between Twin A and Twin B were compared, no significant association existed. The percentage concordance was 63% which is lower than that reported for the other teeth. These results are included in the Appendix (Table 37).

The comparisons carried out to compare infraocclusion of the primary mandibular second molars between Twin A and B of female DZ twin pairs revealed that there was no significant association when the primary mandibular right second molars were compared (Table 7.16). When the primary mandibular left second molars were compared a significant association existed ($P < 0.05$). The percentage concordance was high for both teeth on the right and left sides 94% and 86% respectively. The reason is due to the great number of cases in the non-infraoccluded category. These are also included in the Appendix (Table 38).

Table 7.16. Comparison of the frequency of occurrence and degree of expression of infraocclusion of the primary mandibular right second molars among members of female DZ twin pairs.

	MRE	Twin A		
		NI	MI	Total
Twin B	NI	31	2	32
	MI	1	1	1
	Total	31	3	33

Fisher's exact, $P > 0.05$
% concordance = 94%
Excluding NI % concordance = 50%

7.6. Results of genetic modelling in the twin sample

From the previous analyses based on this data set (sections 7.4 and 7.5) and based on previous analyses associated with hard tissue variables (Hughes et al., 2007), it was decided to focus on common environment as a source of phenotypic variance, rather than genetic dominance (as mentioned earlier these two components of variances are partially confounded in data from twins reared together). Nested AE, CE and E models were formally tested (χ^2) against the full ACE model for all variables. Non-nested AE and CE models were compared using AIC.

There was no significant difference in model specification between sides. Consequently, all results are reported for the right side only. There was a significant difference in model specification between the primary mandibular first molar and the primary mandibular second molar. For both teeth, the parameter that associated the variance components of initiation with those of progression (b) converged close to its theoretical upper limit (1), with any residual variation in progression associated with the unique environment (E). As a consequence, reporting of variance components has been simplified to the overall effects of A, C or E on infraocclusion.

For the primary mandibular first molar, the most parsimonious model (Figure 7.4) incorporated an additive genetic effect (A) and a unique environment effect (E). For the primary mandibular second molar, the most parsimonious model (Figure 7.5) incorporated a common environment effect (C) and a unique environmental effect (E).

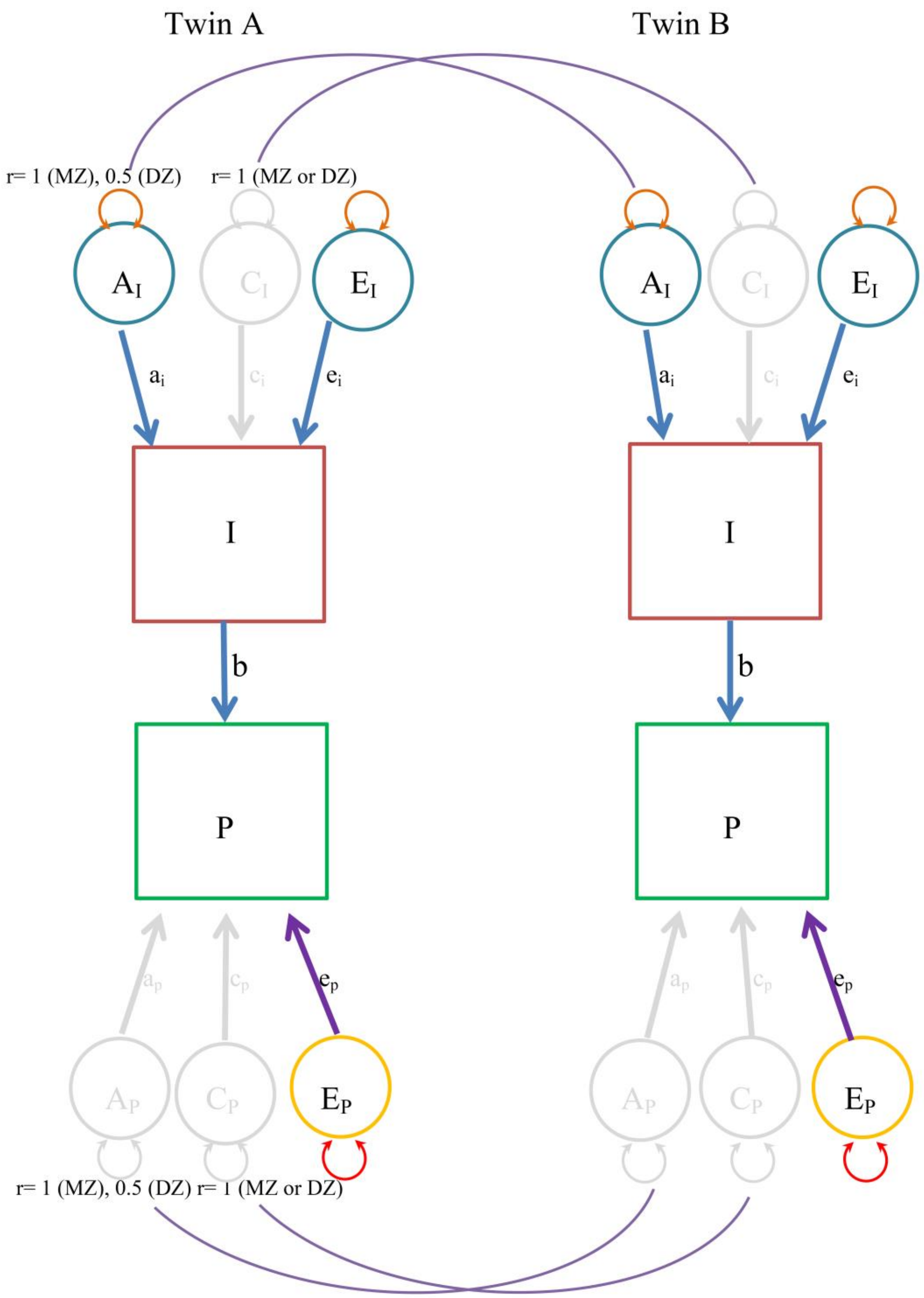


Figure 7.4. Final fitted genetic modelling path diagram for Twin A and Twin B showing the relationship between initiation of infraocclusion in primary mandibular first molar and its progression. The model includes specifications for both MZ and DZ twins.

(Explanation of symbols is included in Figure 7.3. The grey colour indicates that the general environmental factors contributing to initiation and progression (C_I) and the specific additive genetic and common environmental factors contributing to the residual progression (A_P and C_P) are not included in the final model for the primary mandibular first molar).

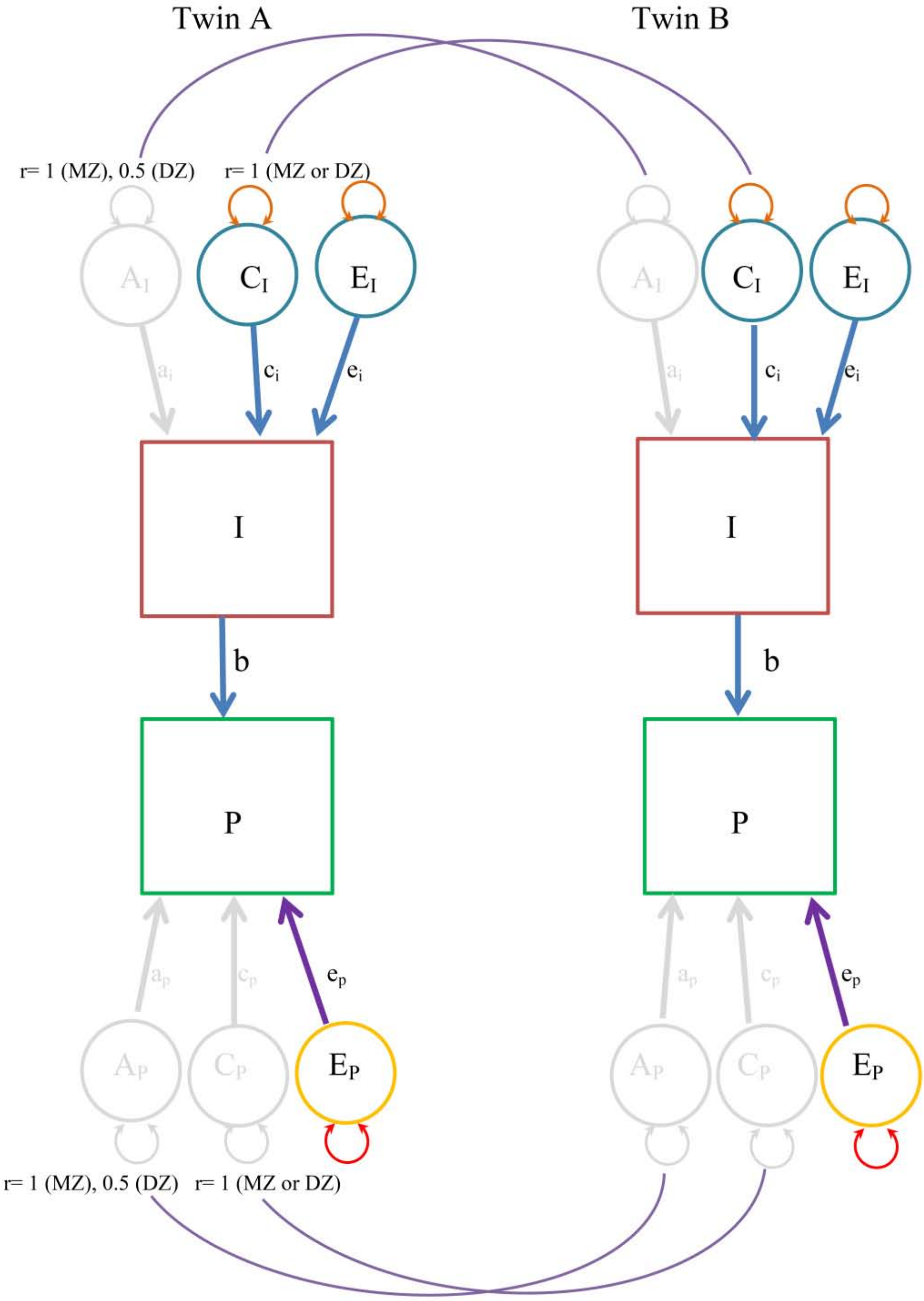


Figure 7.5. Final fitted genetic modelling path diagram for Twin A and Twin B showing the relationship between initiation of infraocclusion in primary mandibular second molar and its progression. The model includes specifications for both MZ and DZ twins.

(Explanation of symbols is included in Figure 7.3. The grey colour indicates that the general additive genetic factors contributing to initiation and progression (A_I) and the specific additive genetic and common environmental factors contributing to the residual progression (A_P and C_P) are not included in the final model for the primary mandibular second molar).

A significant genetic contribution (93%) to the observed variation in infraocclusion of the primary mandibular first molar was noted. This is a 'narrow sense' estimate of heritability. There was also a small contribution of the unique environment. A significant common environment contribution (66%) to the observed variation in infraocclusion of the primary mandibular second molar was noted. There was also a modest contribution (34%) of the unique environment (Table 7.17).

Table 7.17. Results of fitting the conditional causal model to data on initiation and subsequent progression of infraocclusion.

	Initiation			b	Progression		
Tooth	a_i	c_i	e_i		a_p	c_p	e_p
D	0.93	-	0.07	0.95	-	-	1.00
95% CI	0.80-0.99	-	0.01-0.20	0.48-0.99	-	-	1.00-1.00
E	-	0.66	0.34	0.98	-	-	1.00
95% CI	-	0.42-0.82	0.26-0.58	0.78-0.99	-	-	1.00-1.00

Primary mandibular first molar (D), primary mandibular second molar (E), confidence interval (CI), additive genetic factors (a_i), common environmental factors (c_i), unique environmental factors (e_i). Unique factors contributing to phenotypic variation denoted by a_p, c_p and e_p respectively. Coefficient of common factors shared between initiation and progression (b).

7.7. Discussion

The frequency of occurrence of infraocclusion in both members of a MZ twin pair was higher (20% - 29/141) than the frequency in only one member of an MZ pair (15% - 21/141). Of the DZ twin pairs, 4% (6/154) displayed infraocclusion in both members of a pair compared with 13% (20/154) where only one member of DZ pair showed the feature. A similar pattern of expression of infraocclusion, that is, similar in the affected sides and number of teeth, was noted in 11% of the MZ twin pairs compared with only 3% of the DZ twin pairs. Interestingly, three MZ twin pairs showed differences in expression of infraocclusion in the form of mirror imaging, and this will be discussed in more detail in Chapter 9.

A significant association was revealed between Twin A and Twin B of the male MZ twin pairs when conducting a comparison of the frequency of occurrence and degree of expression of infraocclusion of the primary mandibular first and second molars on both sides ($P<0.001$). On the other hand, the male DZ twin pairs revealed no significant association when a similar comparison was conducted between Twin A and Twin B. A slightly different pattern was revealed in the female MZ twin sample. A significant association was revealed between Twin A and Twin B when examining the frequency of occurrence and the degree of expression of infraocclusion of the primary mandibular right and left first molars and also for the primary mandibular left second molar ($P<0.001$) in the MZ female sample. However, no association was revealed between Twin A and Twin B when infraocclusion was compared between primary mandibular right second molars. Among the female DZ twin pairs, a significant association of infraocclusion for the primary mandibular right first molars and primary mandibular left second molars was revealed when Twin A and Twin B were compared ($P<0.001$), and no significant association between Twin A and B was revealed when comparing the primary mandibular left first molars and the primary mandibular right second molars. The calculated percentage concordance excluding the non-infraoccluded category ranged between18% to 40% for the male MZ twin pairs and from 0% to 11% for the male DZ twin pairs. The percentage concordance for the female MZ pairs ranged from 22% to 50% and for the female DZ twin pairs ranged from 0% to 50%. The high percentage concordances of the female DZ twin pairs were because, when the non-infraoccluded group was excluded, the percentage of infraoccluded cases compared to the overall sample size was a small ratio, e.g. one of two cases that is 50%.

MZ twin pairs share 100% of their genes compared to DZ twin pairs who share 50% of their genes on average. This is reflected by the expression of infraocclusion, where MZ twin pairs showed more similarity in the expression of infraocclusion (20% in MZ twin pairs compared to 4% in DZ twin pairs). Moreover, the number of DZ twin pairs where one member of the pair showed infraocclusion only was relatively high at 13%. Thus, among the DZ twin pairs, it was more likely that only one member of the pair would display infraocclusion, unlike the MZ twins where both members of the pair were more likely to show infraocclusion.

The genetic model that was adopted was chosen to suit the nature of the data; that is, since most of the cases showed no infraocclusion, the data were first categorised into presence or absence of infraocclusion, and then, for those cases where infraocclusion was present, further analysis of the progression was classified according to the severity (mild, moderate and severe). Results of univariate genetic modelling of infraocclusion, using an initiation/progression structure, suggested that the parameter that associated the variance components of initiation with those of progression (b) converged close to its theoretical upper limit (1). This indicates that most of the variance components that explained initiation also explained progression. The small amount of residual variation in progression was associated with unique environment (E). The results also showed that variation in infraocclusion of the primary mandibular first molar was primarily determined by genetic factors (93%), with only a small influence of unique environment. Conversely, variation in infraocclusion of the primary mandibular second molar was primarily accounted for by environmental factors, both common and unique.

These findings suggest that the occurrence of infraocclusion may be related to the timing of tooth development and eruption of the primary mandibular molars, which occurs at an earlier stage for the primary mandibular first molar than that of the primary mandibular second molar (Liversidge and Molleson, 2004). When the primary mandibular first molar is associated with infraocclusion, this in turn may be associated with variations in the dental development process such as local space loss, reduced alveolar bone height and changes to dental arch length (Becker and Karnei-R'em, 1992b; 1992a; Becker et al., 1992). These associated disturbances could prevent the normal eruption path of the developing primary mandibular second molar and subsequently result in infraocclusion of this tooth too. This may explain why variation in infraocclusion of the primary mandibular second molar could be explained by a roughly equal mix of common and unique environmental factors. However, this theory does not adequately address the presence of infraocculsion in the second molar, in the absence of infraocculsion in the first molar. Sample size limitations may also have played a role in the dichotomy between the first and second molars, although confidence intervals on the parameter estimates are relatively narrow. Multivariate modelling of the primary mandibular first and second molars, with associated relevant environmental factors, may further elaborate the complex aetiology of infraocclusion in the primary dentition.

It can be postulated that infraocclusion can occur in three different forms, one form where infraocclusion occurs in association with syndromes, another form where infraocclusion occurs in association with other dental anomalies, and a third form infraocclusion presents as a single anomaly. It appears that genetic, epigenetic and environmental factors all contribute to the occurrence of infraocclusion. However, it seems that the contribution of factors contributing to infraocclusion of the primary mandibular first molar differs from the contribution of factors contributing to infraocclusion of the primary mandibular second molar.

The causative genes in some of the syndromic forms of infraocclusion have been identified, for example, cleidocranial dysplasia is caused by mutation in the RUNX2 gene, encoding transcription factor CBFA1, on chromosome 6p21 and ectodermal dysplasia is caused by mutation in the gene encoding ectodysplasin-A (O'Connell and Torske, 1999; Suri et al., 2004; Ahmad et al., 2006). These genes may have a role in the occurrence of other forms of infraocclusion. However, to date specific genes associated with infraocclusion, either with other dental anomalies or as a single anomaly, are still to be identified.

7.8. Conclusion

Analysis of data from the twin sample indicated that percentage concordances for infraocclusion in MZ twin pairs (males and females) tended to exceed those in DZ twin pairs. Moreover, univariate genetic modelling revealed that a genetic association exists with infraocclusion of the primary mandibular first molar. However, when the primary mandibular second molar displayed infraocclusion, common and unique environmental factors accounted for most of the observed variation.

8. Infraocclusion and associated dental anomalies

8.1. Introduction

Dental anomalies may affect the human dentition in various forms, such as anomalies in the number, size, shape, mineralisation and eruption of teeth (Brook, 2009; Koch et al., 2009b; Cobourne and Sharpe, 2013). Although it may appear that an anomaly is present alone in many cases, it may be that other anomalies have either been missed by a clinician or are yet to develop, as it has been well documented that dental anomalies are strongly associated in a number of cases (Brook, 1984; Peck et al., 1996; Garib et al., 2009; Peck, 2009). A combination of two or more different anomalies in the same patient, one of which may be infraocclusion, was described by Peck (2009) as the dental anomaly pattern (DAP). An increased prevalence of other dental anomalies has also been reported to be associated with infraocclusion (Baccetti, 1998; Garib et al., 2009; Shalish et al., 2010). Moreover, the prevalence of infraocclusion in the presence of other dental anomalies is higher when compared to the prevalence of infraocclusion alone in the general population (Baccetti, 1998; Shalish et al., 2010).

In a small sample of Israeli and American 8 to 15 year-old patients with infraocclusion (n=99), increased prevalence of dental anomalies was revealed when compared to the prevalence of the investigated anomalies in the general population (Shalish et al., 2010). Agenesis of the second premolar was reported in 20.2% (20/99) of cases (Shalish et al., 2010), a relatively higher prevalence when compared to a 5% prevalence (53/1,064) in the general population (Grahnén, 1956). A higher prevalence of small maxillary lateral incisor, 15.5% (15/99) (Shalish et al., 2010) was shown to be present in the same group of patients with infraocclusion, higher than that reported in the general population, 4.7% (47/1,000) (Baccetti, 1998). Likewise, a higher prevalence of palatally displaced canines 12.1% (12/99) (Shalish et al., 2010) in association with infraocclusion was revealed when compared to a prevalence of 1.7% (25/1,450) in the normal population (Dachi and Howell, 1961).

Baccetti (1998) investigated patterns of associations between seven dental anomalies, including infraocclusion, agenesis of the second premolars, small size lateral incisors and ectopic canines, in 3,980 Italian patients in the range of 7 to 14 years. It was found that the prevalence of infraocclusion in association with other dental anomalies ranged between 14% and 22% when compared with a prevalence of infraocclusion of 5.6% in the control group (n=1000) (Baccetti, 1998). Other dental anomalies that have been reported to be associated with infraocclusion include agenesis of other teeth, ectopic eruption of first molars, enamel hypoplasia and supernumerary teeth (Bjerklin et al., 1992; Baccetti, 1998). The fact that these features are not related by anatomical position, and generally develop during different stages of an individual's dental developmental process, suggests it is unlikely that local environmental factors are the cause, but instead, supports the proposition that the underlying causative factor may be of common genetic origin.

The association of infraocclusion of a primary tooth with agenesis of its permanent successor has been highlighted previously, with 17% of cases being reported to show this association (Steiner-Oliveira et al., 2007). In the literature, associations have also been reported between dental age and the occurrence of dental anomalies. Specifically, cases that display dental anomalies have been shown to have delayed dental development (Becker and Chaushu, 2000). For instance, an investigation of dental age in patients with agenesis revealed a significant delay in dental age (Tunc et al., 2011). The difference can reach up to 1.5 years and the severity of the agenesis can affect the magnitude of the delay, with teeth adjacent to the area associated with agenesis being more affected (Uslenghi et al., 2006). Likewise, as patients with maxillary canine displacement are often observed to have delayed dental development, this has prompted studies into the investigation of associations between the two conditions (Zilberman et al., 1990; Becker and Chaushu, 2000; Naser et al., 2011; Rozylo-Kalinowska et al., 2011). However, to the author's knowledge, there has been no proposition of an association between infraocclusion and delayed dental development, or that infraocclusion in the presence of other dental anomalies results in delayed dental development.

Delayed dental development has been reported in cases showing agenesis (Garn and Lewis, 1970; Brook et al., 2009a; Brook et al., 2009b). Furthermore, agenesis has also been associated with smaller tooth size (microdontia). Indeed, a direct relationship has been found, that is, the greater the number of missing teeth, the greater the chance of having associated microdontia in the same individual (Garn and Lewis, 1970; Brook et al., 2009a; Brook et al., 2009b). A direct relationship between infraocclusion and tooth size has not been proposed thus far, hence further investigation in this area is warranted, given the implications in term of clinical management and treatment planning.

In this part of the study, it is aimed to report the prevalence of selected dental anomalies in association with infraocclusion. These include ectopic maxillary canines, lateral incisor complex (agenesis, diminutive or peg-shaped lateral incisors), and agenesis of the premolars. It is also aimed to investigate whether there is any association between infraocclusion and delayed dental development by comparing dental age with chronological age in singletons with and without infraocclusion. Further, it is aimed to investigate whether infraocclusion is associated with reduced tooth size, by measuring maximum mesiodistal crown diameters in twins who showed infraocclusion and comparing the values with twins who did not display infraocclusion.

8.2. Materials and methods

Methods for assessing dental anomalies and dental development in the singletons sample

1. **Dental anomalies** (n = 1,454 orthopantomographs)

The dental anomalies recorded were: agenesis of premolars, ectopic canines and lateral incisor complex. Agenesis of premolars was identified when there was no sign of tooth formation or mineralisation of a premolar. Ectopic canines were identified as unerupted canines (with complete root development) or when the contralateral tooth was erupted for at least six months (with complete root development), while the tooth under examination was still unerupted (Lindauer et al., 1992). The lateral incisor complex anomaly included peg laterals, diminutive laterals and agenesis of the laterals. The maxillary lateral incisor was defined as peg shaped if it was reduced in diameter from cervix to the incisal edge (Le Bot and Salmon, 1977). When a maxillary lateral incisor was compared to the contralateral incisor and appeared to be of reduced mesiodistal width, it was scored as 'diminutive'. In some cases where it was difficult to distinguish between true diminutive and rotated teeth, these were excluded. More detailed criteria for identifying dental anomalies are described in Chapter 4.

2. **Dental development** (n=311 orthopantomographs showing infraocclusion)

The Demirjian and Willems systems were selected to assess dental age of individuals in the singleton sample (Demirjian et al., 1973; Willems et al., 2001). Dental development was assessed in all cases that showed infraocclusion of ≥ 1mm. A similarly-sized sample of OPGs with no evidence of infraocclusion was randomly selected and also assessed for dental development using the same criteria as the infraoccluded group - this comprised the control group. The steps in the assessment of dental development by the Demirjian system and the Willems system are described in Chapter 4 in greater detail.

Two systems were used to try to ensure a valid estimate of dental age was obtained and to take account possible over-estimation that may result when using the Demirjian system as suggested in the literature (McKenna et al., 2002).

In cases where agenesis of first and/or second premolars was detected, the contralateral premolars were scored for estimation of dental age.

Methods for assessing mesiodistal crown width in the twin sample

Tooth size (n=202 members of twin pairs).

2D photographic images of the study models were used for measuring maximum mesiodistal crown widths. Data were collected from the mandibular arch only, because the maxillary arch was not included in the overall analysis as discussed in Chapter 6. Measurements were performed from the buccal view of the primary mandibular canines, primary first molars, primary second molars, permanent canines, first premolars, second premolars and permanent first molars on both right and left sides. This was done using the same computer software that was used for the measurement of infraocclusion - Adobe Photoshop CS5 computer software. The distances were measured in mm and recorded for each tooth. Tooth size was assessed in all cases showing infraocclusion of ≥ 1mm. A similarly-sized sample of study models with no infraocclusion was selected and measured for tooth size in the same manner as for the infraoccluded group - this comprised the control group.

8.3. Statistical analysis

Statistical analyses were performed using SAS Version 9.3 (SAS Institute Inc., Cary, NC, USA) and IBM SPSS Version 20. Chi-square tests were carried out to examine the associations of dental anomalies with infraocclusion. Dental age was compared within and between groups with and without infraocclusion by conducting Student’s t-tests. Paired t-tests were used to compare dental ages of individuals in the ‘infraoccluded’ group with their chronological ages, and the values of dental ages of individuals in the ‘non-infraoccluded’ group with their chronological ages. This was done for males and females separately. Unpaired t-tests were used to compare the mean values of the dental ages between 'infraoccluded' and 'non-infraoccluded' groups, for males and females separately. Also, unpaired t-tests were carried out to compare average tooth size between the 'infraoccluded' group and the 'non-infraoccluded' group in the twin sample. Statistical significance was set as $P < 0.05$.

8.4. Results

Association of dental anomalies and infraocclusion

Chi-square tests were conducted to examine the association of dental anomalies with infraocclusion in the singleton sample. The three dental anomalies being examined were scored as present or absent in the 'non-infraoccluded' group and in the 'infraoccluded group'. The frequency of dental agenesis in the infraoccluded sample was similar to that of the non-infraoccluded sample, that is, 7% compared to 5.6% respectively. Results did not reveal any statistically significant association between infraocclusion and dental agenesis (Table 8.1).

Table 8.1. Association between infraocclusion and dental agenesis in singletons.

	NI	Infra
No agenesis	1079 (94.4%)	289 (93.0%)
Agenesis	64 (5.6%)	22 (6.8%)
Total	1143 (100%)	311 (100%)

chi-square value =0.95, dof = 1
p-value > 0.05

Greater frequencies of ectopic canines (14%) were noted in the infraoccluded sample when compared to the non-infraoccluded sample (4%). The difference between the two samples was significant ($P < 0.001$) (Table 8.2).

Table 8.2. Association between infraocclusion and ectopic canines in singletons.

	NI	Infra
No ectopic canines	1098 (96.0%)	267 (85.8%)
Ectopic canines	45 (4.0%)	44 (14.1%)
Total	1143 (100%)	311 (100%)

chi-square= 44.35, dof = 1
p-value < 0.001

Likewise, greater frequencies of lateral incisor complex (17%) were noted in the infraoccluded sample when compared to the non-infraoccluded sample (3%). A significant association was also shown to be present between the occurrence of infraocclusion and lateral incisor complex (P < 0.001) (Tables 8.3).

Table 8.3. Association between infraocclusion and lateral incisor complex in singletons.

	NI	Infra
No lateral complex	1104 (96.6%)	257 (82.6%)
Lateral complex	39 (3.4%)	54 (17.4%)
Total	1143 (100%)	311 (100%)

chi-square= 79.4, dof = 1
p-value = < 0.001

In the present sample, dental anomalies existed as a single anomaly or in combination with other anomalies in both infraoccluded and non-infraoccluded groups. The prevalence of dental anomalies in the infraoccluded sample was higher than its prevalence in the non-infraoccluded sample. When the dental anomalies were combined according to their occurrence in individuals, it was also revealed that in those subjects showing infraocclusion the prevalence of more than one dental anomaly was higher than the prevalence in subjects with no infraocclusion. The frequencies of occurrence of dental anomalies with or without the presence of infraocclusion is summarised in Table 8.4. Examples of orthopantomographs showing the associations of infraocclusion with other dental anomalies are shown in Figures 8.1, 8.2 and 8.3.

Table 8.4. Comparisons of findings of associated dental anomalies between infraoccluded and non-infraoccluded groups in the singleton sample.

Dental anomalies	Infraocclusion			Non-infraocclusion		
	n	% total	% infra	n	% total	% non-infra
Agenesis	22	1.51%	7.07%	64	4.40%	5.59%
Ectopic canines	44	3.03%	14.14%	45	3.09%	3.93%
Lateral complex	54	3.71%	17.36%	39	2.68%	3.41%
Agenesis + Ectopic canines	4	0.28%	1.28%	2	0.14%	0.17%
Agenesis + Lateral complex	7	0.48%	2.25%	11	0.76%	0.96%
Ectopic canines + Lateral complex	9	0.62%	2.89%	2	0.14%	0.17%
Agenesis + Ectopic canines + Lateral complex	1	0.07%	0.32%	1	0.07%	0.08%

n_I= number of individuals with infraocclusion also showing dental anomaly(s)
% total sample= (n_I/1454)x100
% infraoccluded group=(n_I/311)x100
n_{NI}=number of individuals without infraocclusion but showing dental anomaly(s)
% total sample= (n_{NI}/1454)x100
% infraoccluded group=(n_{NI}/1143)x100

ID 180217

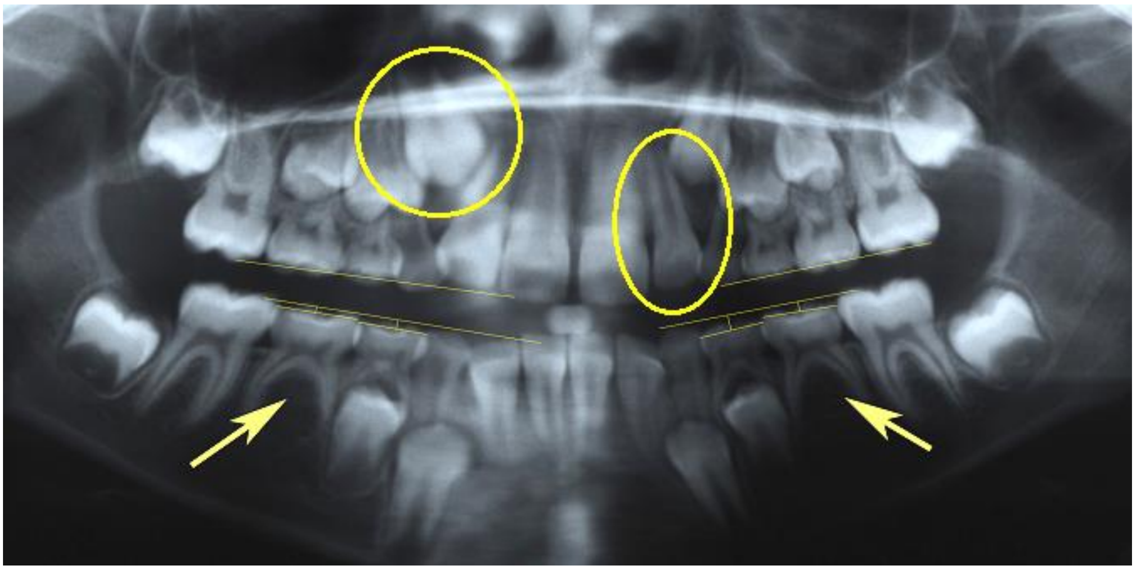


Figure 8.1. Orthopantomograph showing an example of infraocclusion, agenesis of the mandibular left and right second premolars, ectopic canine (13) and small size lateral incisor (22).

ID191067

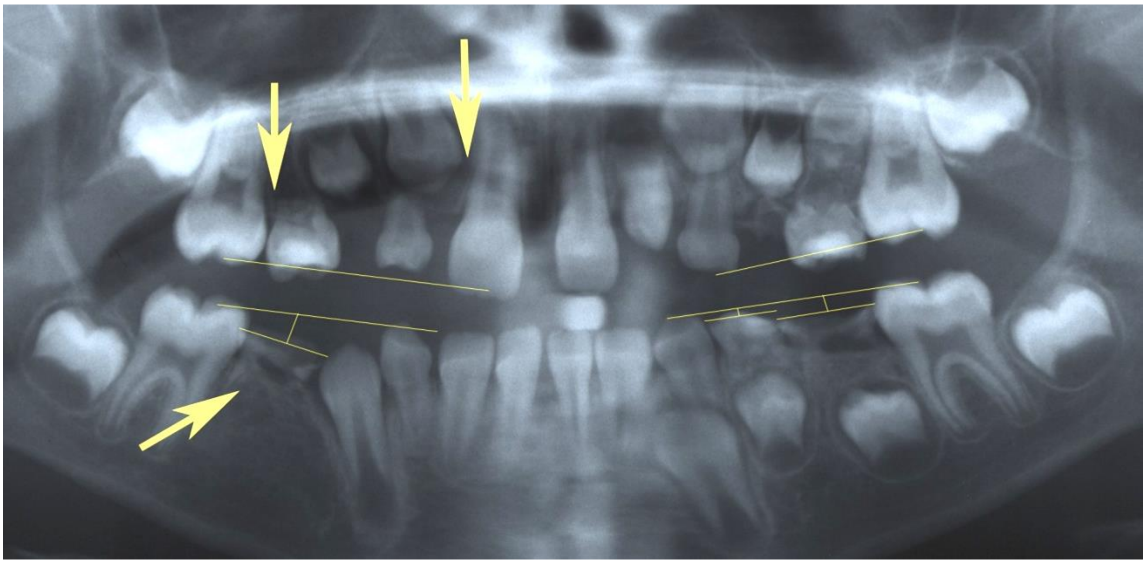


Figure 8.2. Orthopantomograph showing an example of infraocclusion, agenesis of the mandibular right first and second premolars, agenesis of the maxillary right second premolar and agenesis of the maxillary right lateral incisor.

There is evidence of attrition associated with primary mandibular second right and left molars but these teeth are clearly infraoccluded.

ID 180398

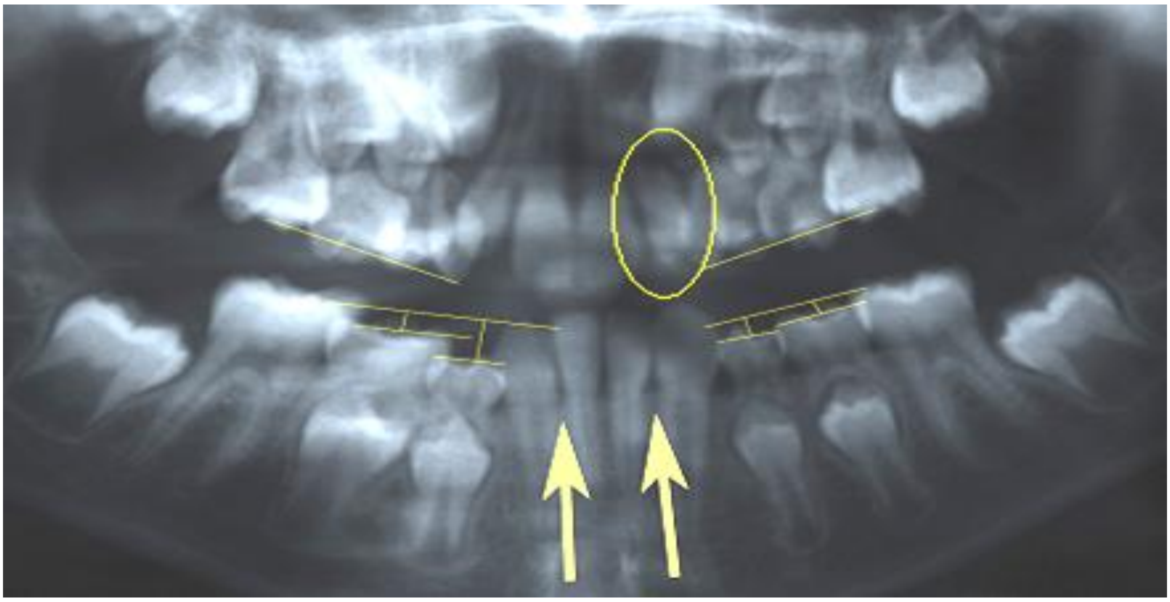


Figure 8.3. Orthopantomograph showing an example of infraocclusion and agenesis of the mandibular left and right lateral incisor. A small maxillary left lateral incisor was also noted.

Association of dental development and infraocclusion

Dental development in singletons showing infraocclusion was assessed to clarify the proposition, that there may be an association between infraocclusion and delayed dental development. To compare dental age and chronological age within individuals, paired t-tests were carried out for both the infraoccluded and non-infraoccluded groups. Table 8.5 shows the mean difference (x) between chronological age (CA) and dental age obtained by the Demirjian system (DA). The mean difference was calculated by subtracting the chronological age from the dental age (x= DA - CA). It can be seen that dental age was significantly greater when compared to chronological age in each group (P< 0.001). This is probably due to the Demirjian system overestimating dental age in the study sample. However, the mean dental age of the non-infraoccluded group was more advanced when compared to the mean dental age of the infraoccluded group. Moreover, when the average dental age of the infraoccluded group was compared with the average dental age of the non-infraoccluded group by conducting an unpaired t-test (Table 8.6), a significant difference in dental age was revealed (P< 0.001). The infraoccluded group showed a reduction in average dental age compared with the non-infraoccluded group.

Table 8.5. Comparison of dental age obtained using the Demirjian system with chronological age in the infraoccluded and non-infraoccluded groups - singleton males.

	n	x diff	SD	P
I	173	0.93	1.11	<0.001
NI	173	1.40	1.11	<0.001

Age in years
Paired t-tests

Table 8.6. Comparison of dental age obtained using the Demirjian system between infraoccluded and non-infraoccluded groups - singleton males.

	n	x	SD	P
I	173	10.35	1.22	< 0.001
NI	173	10.84	1.24	

Age in years
Unpaired t-test

The Willems dental age assessment system also overestimated the dental age of the infraoccluded and non-infraoccluded groups, but to a lesser extent. Despite the variation in dental age estimation obtained by the Willems system compared with the Demirjian system, the pattern of results was similar, i.e. the mean difference between dental age calculated by the Willems system and chronological age was significant for both groups (Table 8.7) and a significant difference in average dental age was revealed between both groups ($P < 0.001$), with the infraoccluded group showing a significant reduction in average dental age compared with the non-infraoccluded group ($P < 0.001$) (Table 8.8).

Table 8.7. Comparison of dental age obtained using the Willems system with chronological age in the infraoccluded and non-infraoccluded groups - singleton males.

	n	x diff	SD	P
I	173	0.49	1.01	< 0.001
NI	173	1.02	1.08	< 0.001

Age in years
Paired t-tests

Table 8.8. Comparison of dental age obtained using the Willems system between infraoccluded and non-infraoccluded groups - singleton males.

	n	x	SD	P
I	173	9.91	1.12	< 0.001
NI	173	10.46	1.23	

Age in years
Unpaired t-test

To ascertain whether there was any significant difference between the chronological age of the two groups, which could account for the significant difference in infraoccluded and non-infraoccluded calculated dental ages, a comparison of chronological age between the infraoccluded and non-infraoccluded groups was conducted by performing an unpaired t-test (Table 8.9). No significant difference was revealed between the chronological ages of infraoccluded and non-infraoccluded groups.

Table 8.9. Comparison of the chronological age between infraoccluded and non-infraoccluded groups - singleton males.

	n	x	SD	P
I	173	9.42	0.59	> 0.05
NI	173	9.44	0.61	

Age in years
Unpaired t-test

Similar to the male singleton sample, paired t-tests were carried out to compare dental age and chronological age in the infraoccluded group and the non-infraoccluded group of the female singleton sample. Unpaired t-tests were also carried out to compare dental age between infraoccluded and non-infraoccluded groups. The results of the t-tests were also similar to those revealed in the male group. The females showed that there was a significant difference between chronological age and dental age calculated by the Demirjian system in both groups (Table 8.10). A significant difference was also revealed when the dental age obtained by the Demirjian system of the infraoccluded group was compared to that of the non-infraoccluded group in the female singleton sample (Table 8.11).

Table 8.10. Comparison of dental age obtained following the Demirjian system to chronological age in the infraoccluded and non-infraoccluded groups - singleton females.

	n	x diff	SD	P
I	138	0.96	0.99	< 0.001
NI	138	1.31	0.96	< 0.001

Age in years
Paired t-tests

Table 8.11. Comparison of dental age obtained following the Demirjian system between infraoccluded and non-infraoccluded groups - singleton females.

	n	x	SD	P
I	138	10.34	1.14	< 0.05
NI	138	10.65	1.06	

Age in years
Unpaired t-test

Overestimations of dental ages by the Willems system were also revealed when compared to the chronological ages of both infraoccluded and non-infraoccluded groups, in the female singletons (Table 8.12). Moreover, when the Willems dental ages of the infraoccluded and non-infraoccluded samples were compared, a significant difference was revealed (< 0.001) (Table 8.13).

Table 8.12. Comparison of dental age obtained following the Willems system to chronological age in the infraoccluded and non-infraoccluded groups - singleton females.

	n	x diff	SD	P
I	138	0.24	1.25	< 0.05
NI	138	0.74	0.89	< 0.001

Age in years
Paired t-tests

Table 8.13. Comparison of dental age obtained following the Willems system between infraoccluded and non-infraoccluded groups - singleton females.

	n	x	SD	P
I	138	9.62	1.33	< 0.05
NI	138	10.08	1.04	

Age in years
Unpaired t-test

Similar to the male sample, a comparison of the chronological age between the infraoccluded and non-infraoccluded group was conducted by performing an unpaired t-test (Table 8.14). A non-significant difference between the chronological ages of infraoccluded and non-infraoccluded groups was also revealed.

Table 8.14. Comparison of chronological age between infraoccluded and non-infraoccluded groups - singleton females.

	n	x	SD	P
I	138	9.38	0.60	> 0.05
NI	138	9.34	0.67	

Age in years
Unpaired t-test

Dental age assessment was conducted in cases where agenesis was present. In cases where there was an absent tooth, the antimeric tooth was used as a substitute for scoring. The dental ages of the infraoccluded cases with agenesis were compared with the dental ages of infraoccluded cases without agenesis by calculating Z-scores. This was done due to the small number of cases of infraocclusion with agenesis in the present sample, and to compare on a case-by-case basis the dental age of individuals showing infraocclusion with agenesis in relation to the mean dental age of all individuals infraocclusion for males and females separately.

Z-scores were calculated by taking the dental age of individuals with infraocclusion and agenesis (x), and then subtracting the mean dental age of all individuals showing infraocclusion (x). The result was then divided by the standard deviation for dental age in the total infraoccluded group (SD) (Tables 8.15 and 8.16).

$$Z = \frac{x - x}{SD}$$

Results of the Z-scores calculated for both systems, Demirjian and Willems, were negative for all of the males showing infraocclusion with agenesis, i.e. the dental age of subjects showing infraocclusion with agenesis were all below the mean age for those showing infraocclusion. The Z-scores for the females were also negative for most of the cases, indicating that their dental ages, whether using Demirijian or Willems systems, tended to fall below the mean value for the total infraoccluded sample.

Table 8.15. Z-scores of dental age for singleton males showing infraocclusion with agenesis.

ID	Agenesis	CA	DDA	WDA	DZ-score	WZ-score
180248	2nd premolar	9.28	7.9	7.75	-2.01	-1.93
180389	2nd premolar	9.64	9.4	9.19	-0.78	-0.64
191106	2nd premolar	8.5	8.9	8.8	-1.19	-0.99

Chronological age (CA), Demirjian dental age (DDA), Willems dental age (WDA).

Age in years

Table 8.16. Z-scores of dental age for singleton females showing infraocclusion with agenesis.

ID	Agenesis	CA	DDA	WDA	DZ-score	WZ-score
180049	lateral incisor	7.53	7.70	7.21	-2.32	-1.81
180128	1st, 2nd premolars	10.54	10.30	10.28	-0.04	0.50
180136	2nd premolar	9.67	9.50	9.53	-0.74	-0.07
180181	2nd premolar	9.28	8.50	8.03	-1.61	-1.20
180213	laterla incisor	8.05	9.00	8.01	-1.18	-1.21
180285	2nd premolar	9.63	10.10	9.87	-0.21	0.19
180323	2nd premolar	9.83	10.30	9.41	-0.04	-0.16
191067	lateral incisor, 2nd premolar	8.44	8.40	7.92	-1.70	-1.28
191149	2nd premolar	8.34	8.90	8.74	-1.26	-0.66

Chronological age (CA), Demirjian dental age (DDA), Willems dental age (WDA).

Age in years

Association between tooth size and infraocclusion

Maximum mesiodistal crown widths of the twin sample were measured and compared among infraoccluded and non-infraoccluded groups for males and females separately, to clarify whether infraocclusion was associated with reduced tooth size. Measurements were performed from the buccal view of the primary mandibular canines (C), primary first molars (D), primary second molars (E), permanent canines, first premolars, second premolars and permanent first molars (6) on both right and left sides. However, only data for the primary canine, primary mandibular first and second molars and permanent mandibular first molar were included because the permanent canines, and the first and second premolars, were not present in most cases and so the mesiodistal crown width data collected from these teeth represented only a very small sample size.

Unpaired Student's t-tests were carried out to compare the mean values of mesiodistal crown widths in the infraoccluded group with those in the non-infraoccluded group. Results revealed a significant reduction in size of the primary canines in the male group on both right and left sides when the infraoccluded group was compared with the non-infraoccluded group (Tables 8.17 and 8.18). There were no significant differences when the remaining teeth were compared. In the female sample, it was also revealed that the primary canines were significantly reduced in size in the infraoccluded group compared with the non-infraoccluded group on both right and left sides (Table 8.19 and 8.20). Moreover, in the female group it was revealed that the primary mandibular first molar was also significantly reduced in size when the infraoccluded group was compared to the non-infraoccluded group.

Table 8.17. Comparison of mesiodistal crown widths between the infraoccluded group and non-infraoccluded group in the twin male sample - right side.

Males

Right Teeth	n	Infraoccluded x	SD	Non-infraoccluded x	SD	P
C	33	5.83*	0.29	6.05	0.39	0.01
D	42	8.13	0.56	8.11	0.53	0.91
E	49	10.13	0.55	10.16	0.46	0.84
6	49	10.78	0.66	10.63	0.55	0.17

Measurements in mm

(n is equal for both infraoccluded and non-infraoccluded groups)

*Mean value significantly different in infraoccluded group (P<0.05)

Table 8.18. Comparison of the mesiodistal crown widths between the infraoccluded group and non-infraoccluded group in the twin male sample - left side.

Males

Left Teeth	n	Infraoccluded x	SD	Non-infraoccluded x	SD	P
C	35	5.86*	0.31	6.04	0.38	0.03
D	41	8.10	0.51	8.18	0.46	0.42
E	51	10.16	0.48	10.21	0.45	0.39
6	51	10.86	0.62	10.61	0.54	0.11

Measurements in mm

(n is equal for both infraoccluded and non-infraoccluded groups)

*Mean value significantly different in infraoccluded group (P<0.05)

Table 8.19. Comparison of the mesiodistal crown widths between the infraoccluded group and non-infraoccluded group in the twin female sample - right side.

Females

Right Tooth	n	Infraoccluded x	SD	Non-infraoccluded x	SD	P
C	26	5.65*	0.39	5.97	0.35	0.003
D	38	7.94*	0.56	8.17	0.37	0.036
E	45	10.05	0.58	9.97	0.44	0.476
6	44	10.48	0.63	10.45	0.53	0.851

Measurements in mm

(n is equal for both infraoccluded and non-infraoccluded groups)

*Mean value significantly different in infraoccluded group (P<0.05)

Table 8.20. Comparison of the mesiodistal crown widths between the infraoccluded group and non-infraoccluded group in the twin female sample - left side.

Females

Left Tooth	n	Infraoccluded x	SD	Non-infraoccluded x	SD	P
C	32	5.71*	0.34	5.88	0.34	0.053
D	40	7.90*	0.50	8.22	0.33	0.001
E	44	10.00	0.48	10.06	0.37	0.531
6	43	10.47	0.61	10.44	0.52	0.821

Measurements in mm

(n is equal for both infraoccluded and non-infraoccluded groups)

*Mean value significantly different in infraoccluded group (P<0.05)

8.5. Discussion

Often when a dental anomaly is identified in a patient, the diagnosis and treatment options tends to focus on that specific anomaly. Our results highlight the increased prevalence of other dental anomalies in association with infraocclusion. This should attract the attention of clinicians, and provoke the need for appropriate long-term management not only in respect to existing infraocclusion, but to other associated dental anomalies.

In the present study, the prevalence of dental agenesis was 7% in the infraoccluded singleton group compared with 5.6% in singletons without infraocclusion. The difference between these two frequencies was not statistically significant. However, a prevalence of 7% was less than that reported in previous studies. In a study by Baccetti (1998), the prevalence of agenesis of the second premolar in association with infraocclusion was reported to be 14% in a sample of 100 individuals. The same study also revealed prevalences of 13% for small lateral incisors and 13% for ectopic canines in association with infraocclusion. The prevalence of ectopic canines in the present study was 14.2%, close to the 13% prevalence reported by Baccetti (1998). The prevalence of the lateral incisor complex in the present study was 17.2%. Although this value is higher than the 13% prevalence of small lateral incisors reported by Baccetti, these values are not directly comparable as the lateral incisor complex anomaly in the present study also included agenesis of the lateral incisors. When compared to estimates of the prevalence of dental anomalies reported in the literature, that is, 5% for tooth agenesis (Grahnén, 1956), 4.7% for small lateral incisors (Baccetti, 1998) and 1.7% for ectopic canines (Dachi and Howell, 1961), a strong association between infraocclusion and other dental anomalies was indicated in this study. The fact that these features are not related by anatomical position supports a common genetic origin.

A total of 214 (66.8%) out of the total 320 individuals displaying infraocclusion showed infraocclusion with no associated dental anomalies compared with 106 individuals (33.2%) expressing at least one associated anomaly with infraocclusion. There is evidence, therefore, that infraocclusion can be an early indicator of other dental anomalies that may not be revealed at the time of initial diagnosis. Thus, in clinical practice, cases displaying infraocclusion with no apparent associated dental anomalies should be treated with caution. A protocol involving regular recall visits and continuous screening is essential for early diagnosis and treatment planning.

Delay in dental development has been shown to be associated with a number of dental anomalies, such as agenesis and ectopic maxillary canines (Peck, 2009; Rozylo-Kalinowska et al., 2011; Tunc et al., 2011). In the present study, a relationship between infraocclusion and dental development was investigated in the singleton sample. The Demirjian and Willems dental age assessment systems were used to conduct the investigation. The estimated dental ages based on the Demirjian and Willems systems study were greater than the chronological ages for all subjects, i.e. both infraoccluded and non-infraoccluded groups. The Demirjian system has been reported to result in an overestimation of dental age by 1.6 years in a similar ethnic group to the one included in the present study (McKenna et al., 2002) and an overestimation of 0.5 to 1 year in other ethnic groups (Willems, 2001; Blenkin and Evans, 2010). To a small extent, the Willems dental age assessment system also resulted in a greater estimated dental age when compared to chronological age. However, this overestimation may be due to a different ethnic group being examined in the present study rather than the Belgian sample on which the Willems system was based. In this study, the main aim was not to assess the accuracy of the dental age assessment systems used. The aim was to compare the difference in dental age between the infraoccluded and the non-infraoccluded groups. Thus, although both systems used resulted in overestimation in dental ages for both groups, the focus was on the difference in years between both samples.

An association was found between infraocclusion and dental development. In the male infraoccluded sample, a significant difference ($P < 0.05$) in dental age was revealed when comparing the infraoccluded group to the non-infraoccluded group. Delayed dental development in association with infraocclusion was also revealed in the female infraoccluded sample, with a significant difference between the infraoccluded and non-infraoccluded groups ($P < 0.001$). Moreover, the dental ages of subjects showing infraocclusion with agenesis was consistent with a delay in dental development when compared with subjects showing infraocclusion only.

Assessment of dental development is a common practice in orthodontics and paediatric dentistry. It is essential in treatment planning for different types of malocclusions in relation to maxillofacial growth. Thus, dental age influences decision making in regards to the timing/type of treatment, and helps clinicians answer questions regarding the timing of extraction if required and to determine when orthodontic treatment should be implemented. From a clinical viewpoint, dental development should be assessed routinely in cases showing infraocclusion before the commencement of any further treatment.

Agenesis has also been associated with reduced tooth size (Rantanen, 1956; Garn and Lewis, 1970; Baum and Cohen, 1971; Brook et al., 2009b). The association between agenesis and tooth size suggests a common aetiological basis (Brook, 2009), which provoked an investigation of tooth size in relation to infraocclusion in the present study. The male twin sample with infraocclusion showed a significant reduction in tooth size of the mandibular primary canine on the right and left sides ($P< 0.05$). However, the female twin sample with infraocclusion showed a significant reduction in tooth size of the mandibular primary canine and also the first molar on the right and left sides ($P<0.05$). No significant differences in the size of the primary mandibular second molar or the permanent first molar were revealed between the infraoccluded and non-infraoccluded groups.

Nevertheless, from a clinical perspective, the notion that a tooth or teeth of a patient showing infraocclusion tend to be smaller in size should be placed in context with the general treatment planning process. Thus, reduction in the size of the mandibular primary canines may be the result of a gene that influences multiple dental phenotypic traits, a concept known as ‘pleiotropy”. This effect is presented in many different body structures, and, some pleiotropic gene effects have been reported in dental structures, for example, amelogenesis imperfecta, dentinogenesis imperfecta and dentin dysplasia (Calcagno and Gibson, 1988). This suggests the possibility of other dental anomalies arising in the future in a patient showing infraocclusion, such as agenesis, ectopic canines and delayed dental development.

8.6. Conclusion

The findings of this part of the study showed that infraocclusion is not always an isolated condition. It may occur in association with other dental anomalies. It was also revealed that dental age tends to be delayed in cases of infraocclusion. Dental crown size of some primary teeth is also reduced in cases of infraocclusion.

9. Selected cases which illustrate aspects of study and raise future research questions

In this chapter, several cases showing infraocclusion will be highlighted. These cases were revealed during examination and data acquisition using the twins' study model sample, as well as study models of their family members. They are reported in the form of case reports. The purpose of these cases was to reflect on different aspects that were raised in previous chapters of this thesis, highlighting patterns of expression of infraocclusion between members of a twin pair and their family. This adds to our current state of knowledge and enhances our understanding and management of the condition. The first selection of cases will represent MZ twin pairs showing similar and dissimilar patterns of expression of infraocclusion. The next selection of cases will report the longitudinal observation of infraocclusion among MZ and DZ twin pairs. Another interesting case which is presented is a case of triplets all of whom display infraocclusion of varying severity. The last selection of cases shows expression of infraocclusion and associated dental anomalies among family members of twin pairs who displayed infraocclusion.

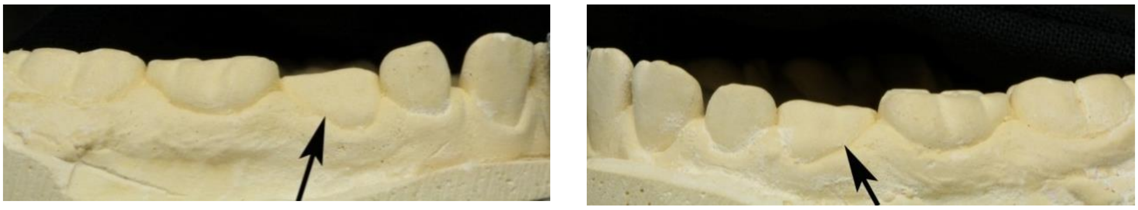
9.1. Range of expressions of infraocclusion in MZ twin pairs

Example of similar expression of infraocclusion in an MZ twin pair

Most MZ twin pairs were found to have a similar pattern of expression of infraocclusion with varying severity. An example of close similarity in the pattern and severity of expression of infraocclusion among an MZ twin pair is represented in Figure 9.1. Moderate infraocclusion affecting the primary mandibular first molar on the right and left sides of Twin A and Twin B was detected.

Mandibular right quadrant Mandibular left quadrant

Twin A



Twin B

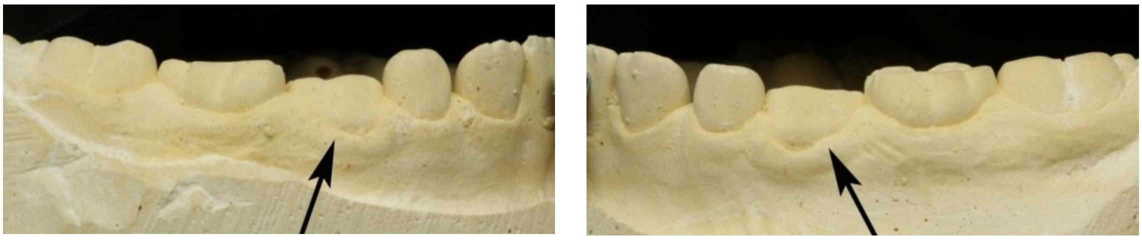


Figure 9.1.Similar expression of infraocclusion in an MZ Twin pair (ID 808).

Example of dissimilar expression of infraocclusion in an MZ twin pair

In contrast to the previous case, Figure 9.2 represents a case of an MZ twin pair showing distinct variation of expression of infraocclusion. Twin A had no infraocclusion diagnosed on both sides of the mandibular arch, while Twin B displayed mild infraocclusion affecting both primary mandibular first and second molars on the right and left sides.

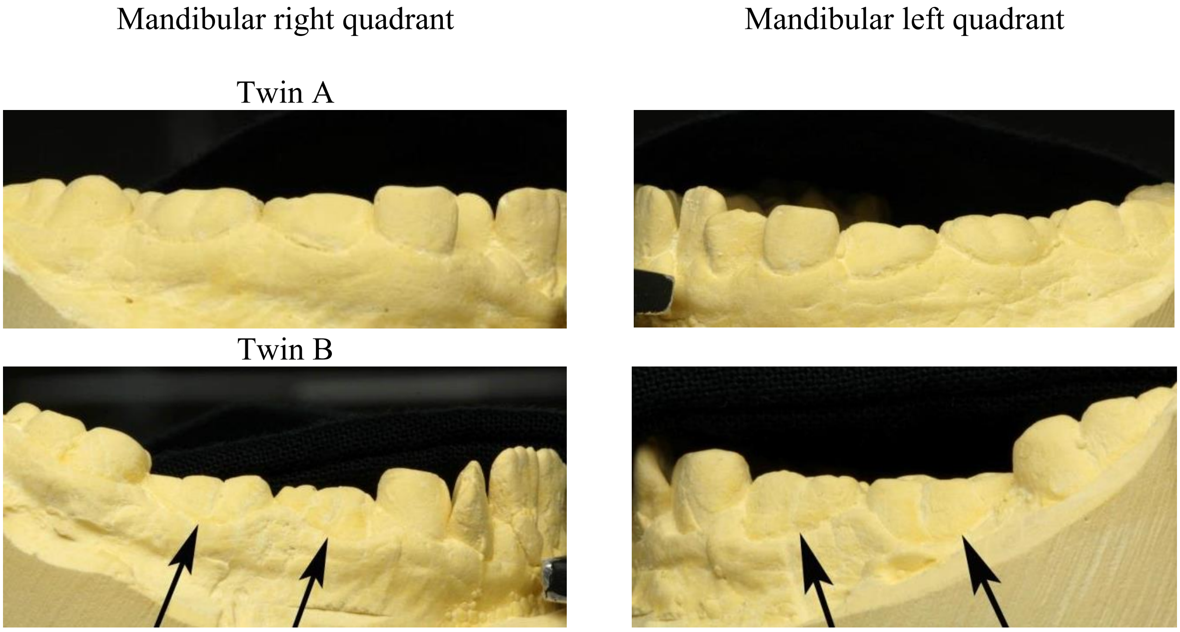


Figure 9.2.Dissimilar expression of infraocclusion in an MZ Twin pair (ID 808).

Example of mirror imaging of infraocclusion in an MZ twin pair

In most of the cases examined, the pattern of expression of infraocclusion was similar in both members of an MZ twin pair, but with some variation in severity. However, an interesting variation in the expression of infraocclusion is the phenomenon of mirror imaging, where one member of a twin pair 'mirrors' the other for one or more features (Townsend et al., 2009). The explanation behind this feature has been that the original embryo divides into two halves to form two separate embryos one from each side. The absent part is completed by subsequent development of the embryos (Townsend and Martin, 1992). Previously, this effect has been reported in association with agenesis (Townsend et al., 2005). In the present study, mirror image presentation was shown by an MZ twin pair (ID 324) (Figure 9.3). It can be seen that the primary mandibular right second molar is infraoccluded in Twin A but not in Twin B, whereas the primary mandibular left second molar is infraoccluded in Twin B but not in Twin A. In the maxillary arch, Twin A showed moderate infraocclusion of the primary right second molar, which is not expressed in Twin B.

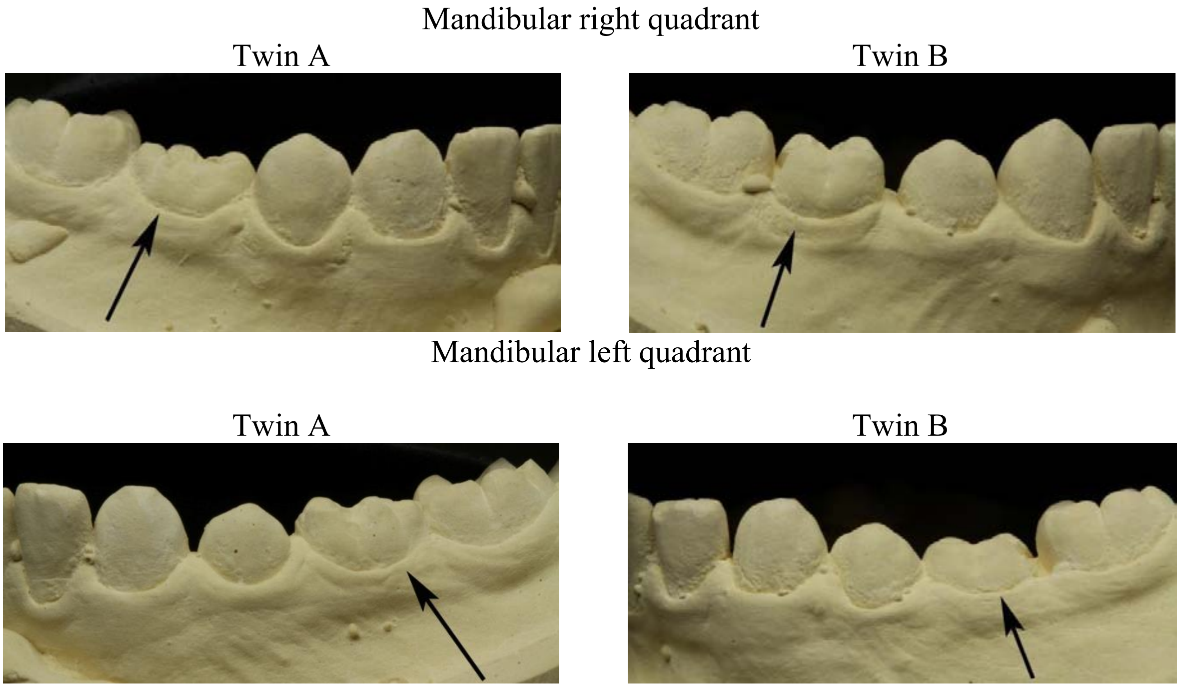


Figure 9.3.Mirror image presentation of infraocclusion in an MZ twin pair (ID 324).

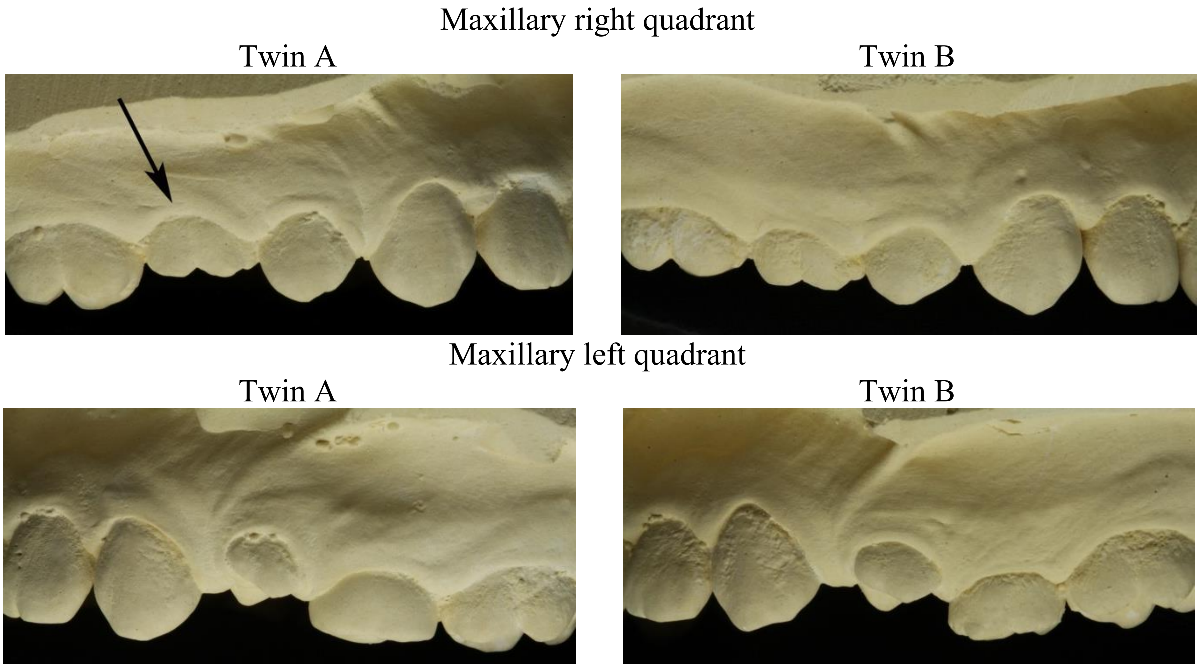


Figure 9.3. (continued) Mirror image presentation of infraocclusion in an MZ twin pair (ID 324).

9.2. Longitudinal observations of infraocclusion in MZ and DZ twins

Presentation of these cases provides an opportunity to look at how infraocclusion develops over time, and to point out some of the associated consequences of the condition. Infraocclusion may occur at an early stage (primary dentition) and then proceed to a later stage (mixed dentition). In the permanent dentition, infraocclusion may persist or an infraoccluded tooth may be exfoliated (or extracted). Moreover, in the permanent dentition stage, the region previously showing infraocclusion may show the consequences of the condition, such as crowding, rotation, and displacement of the permanent teeth and/or infraocclusion of permanent teeth. In the present study, models were available at three different stages for some of the twin pairs. However, some cases had a missing study model at one stage or another or one member of the twin pair may have had full set of models while the other did not. This was a limitation of this phase of the study and reduced the number of cases that could be reported. Findings of the examination are summarised in Table 9.1 and cases are reported below.

Table 9.1. Summary of twins' study models examined at three stages in twins showing infraocclusion.

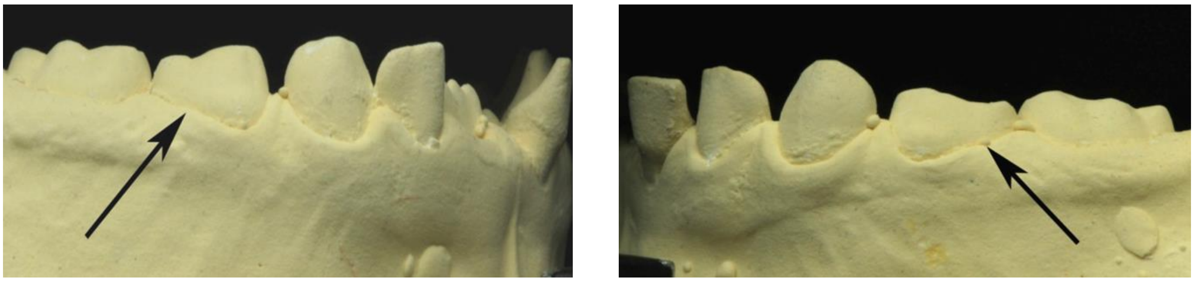
	Stage 1 or 2	Stages 1-2 or 2-3	Stages 1,2 and 3	missing premolars	crowding
Twin A+B	14	26	2	8	9
Twin A	15	15	1	4	2
Twin B	18	12	5	3	4

(n=119) study models of individuals.

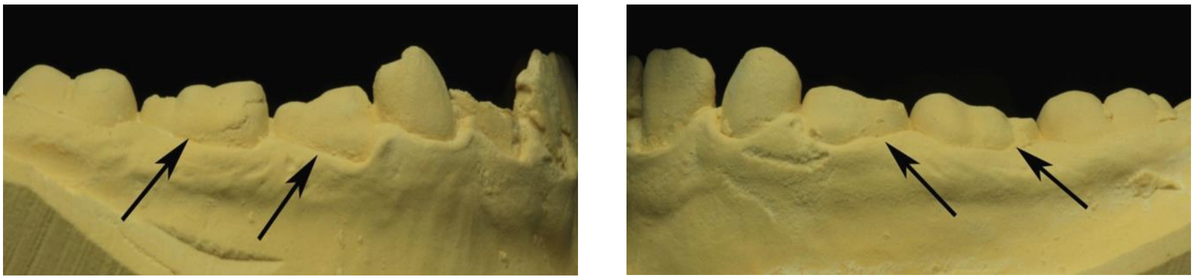
The first case involved a DZ twin pair (ID 451B). This case displayed the progression of infraocclusion through different stages of dental development. The twin pair were of opposite sex, with models being available for one member (the female who was Twin B) (Figure 9.4). Infraocclusion was mildly expressed in Stage 1 associated with the primary mandibular right and left first molars. In Stage 2, infraocclusion was more prominent than Stage 1, on both sides. However, it was categorised as being 'mild' when measured from the mesial marginal ridge of the first permanent molar. Progressing into the Stage 3, it appears that the primary mandibular right and left first molars had exfoliated but the primary mandibular second molar on both sides had become more severely infraoccluded. Stage 3, presented in Figure 9.4, shows that the primary maxillary right and left second molars were super-erupted beyond the occlusal plane as a consequence of infraocclusion of the opposing tooth.

Twin B Mandibular right quadrant Mandibular left quadrant

Stage 1



Stage 2



Stage 3

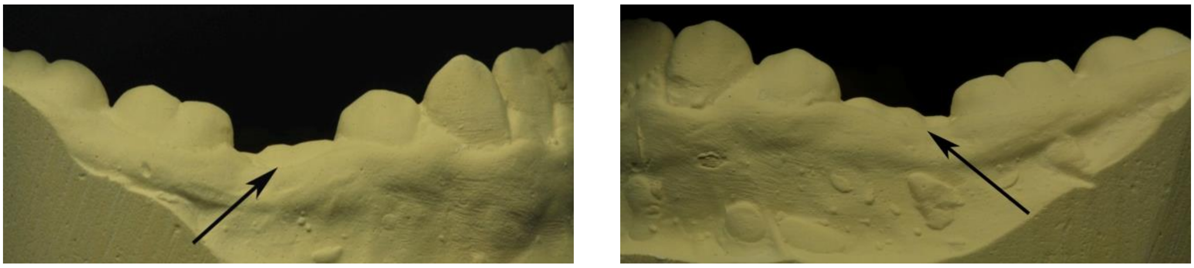
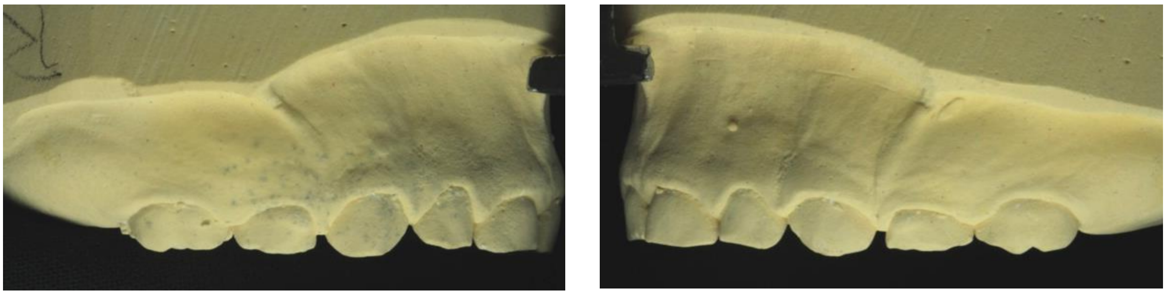


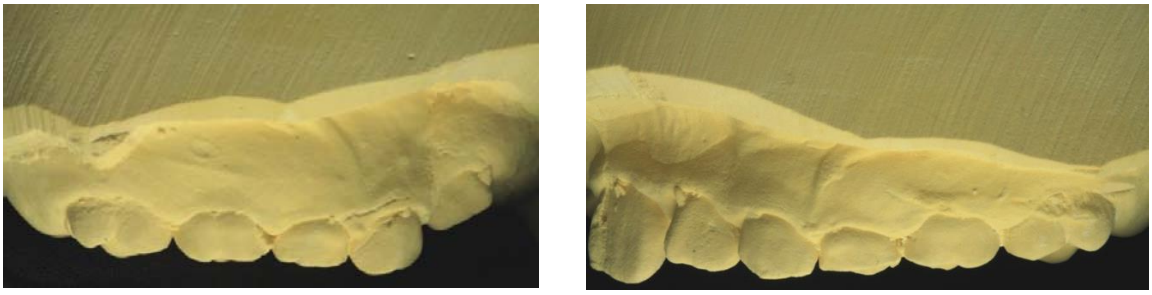
Figure 9.4.The pattern of progression of infraocclusion through different stages of dental development (ID 451).

Twin B Maxillary right quadrant Maxillary left quadrant

Stage 1



Stage 2



Stage 3

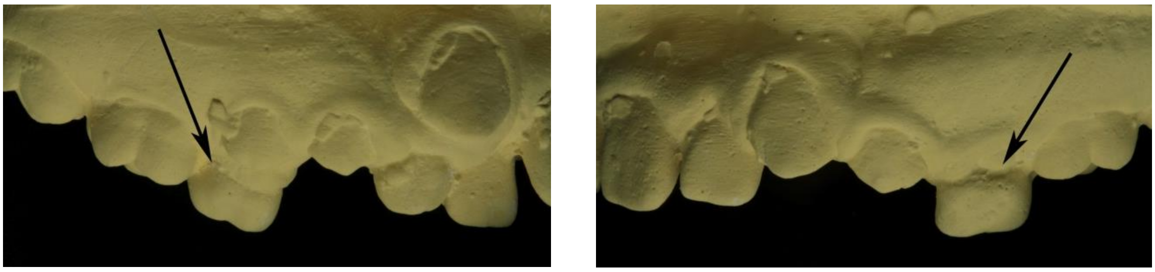


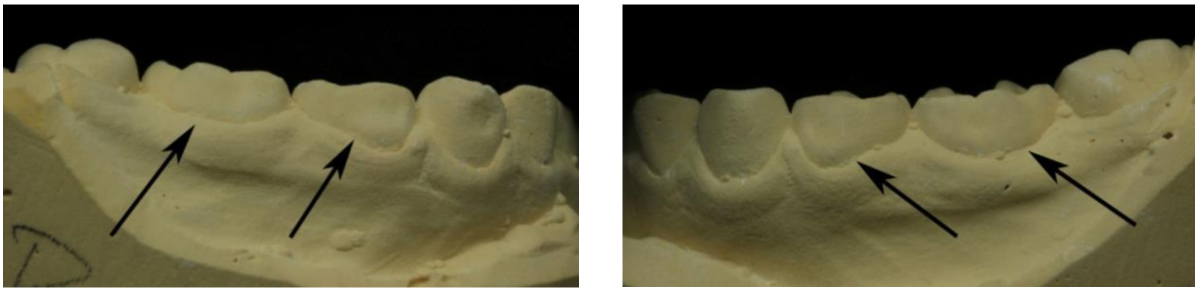
Figure 9.4. (continued) The pattern of progression of infraocclusion through different stages of dental development (ID 451).

Study models of an MZ twin pair (ID 489) were examined at different stages. Models of both members of the MZ twin pair were available for examination.

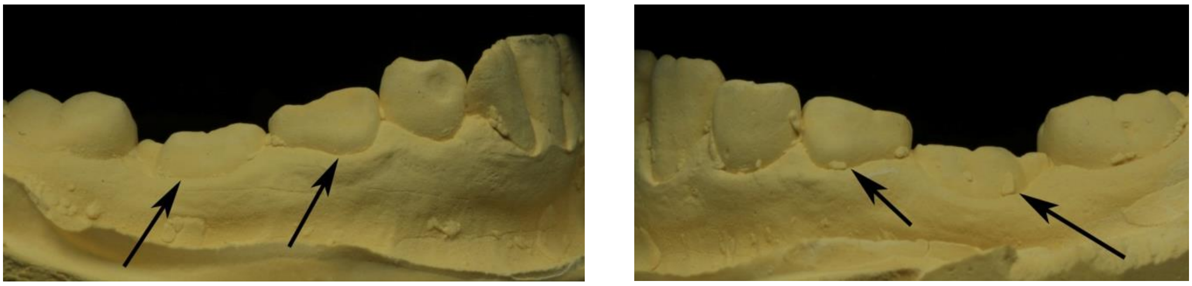
Similar to the first case, the progress of infraocclusion through different stages of development was examined. Similarities or differences of expression among members of the pair are also reported in this case, to clarify how epigenetic or environmental influences may affect phenotypic expression (Figure 9.5). Twin A and Twin B had signs of infraocclusion at Stage 1, affecting the primary mandibular right and left first and second molars. The level of infraocclusion progressed in Stage 2, varying between mild to moderate. Twin A had a mild level of infraocclusion affecting the primary mandibular right first molar and the left second molar, and a moderate level of infraocclusion affecting the primary mandibular right second molar and the left first molar at Stage 2. In contrast, Twin B showed a mild level of infraocclusion affecting the primary mandibular left first molar and the other teeth were moderately affected. At Stage 3, infraocclusion progressed to a more severe level in both twins. It was noted that the primary mandibular right and left second molars in Twin A and Twin B became more severely infraoccluded, in addition to the primary mandibular right first molar in Twin B (Figure 9.5). This case provides an opportunity to further explore changes that occur over time. It can be postulated from the images that the teeth associated with infraocclusion have initially reached the full occlusal level in Stage 1and then become infraoccluded in Stage 2 as a result of continuous eruption of the adjacent teeth. However, this cannot be addressed in any depth due to variation in the timing of tooth eruption and emergence and variation in the timing of obtaining the study models.

Twin A Mandibular right quadrant Mandibular left quadrant

Stage 1



Stage 2



Stage 3

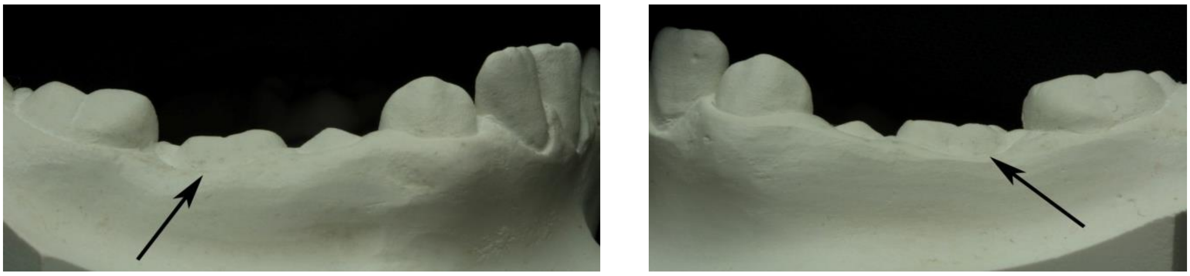
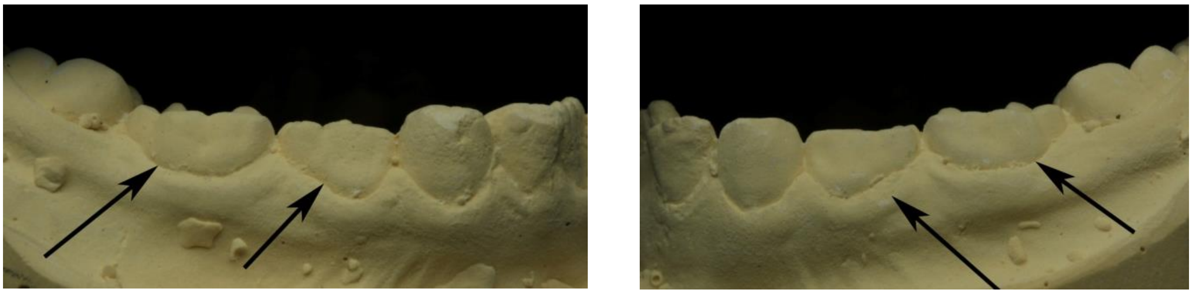


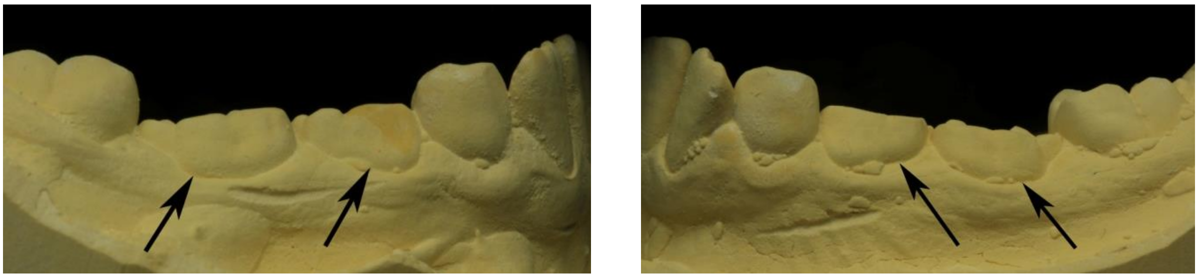
Figure 9.5. The pattern of progression of infraocclusion through different stages of dental development of Twin A and Twin B (ID 489).

Twin B Mandibular right quadrant Mandibular left quadrant

Stage 1



Stage 2



Stage 3

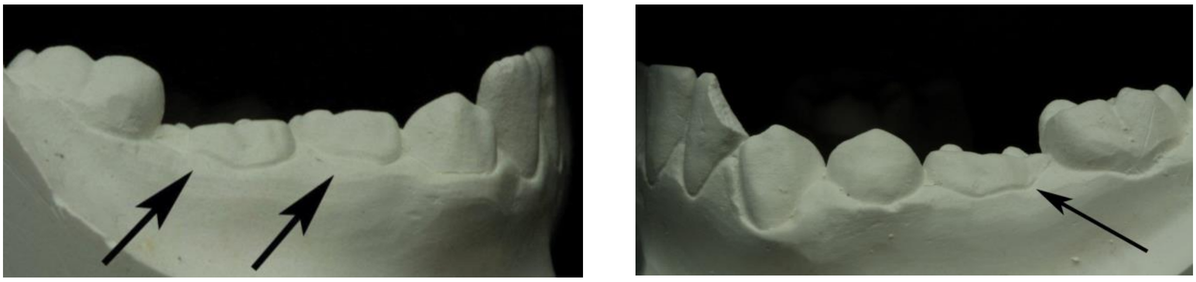
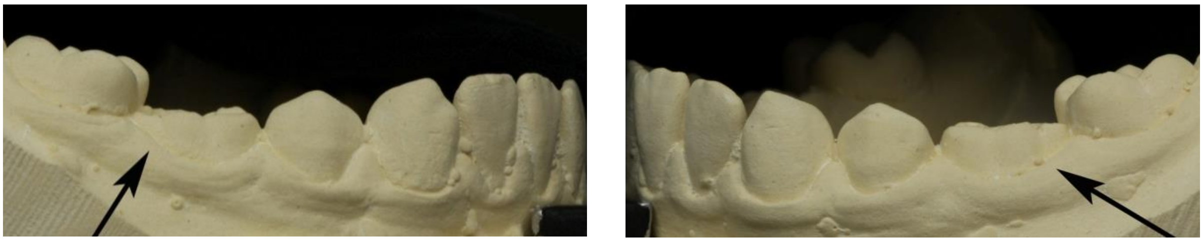


Figure 9.5. (continued) The pattern of progression of infraocclusion through different stages of dental development of Twin A and Twin B (ID 489).

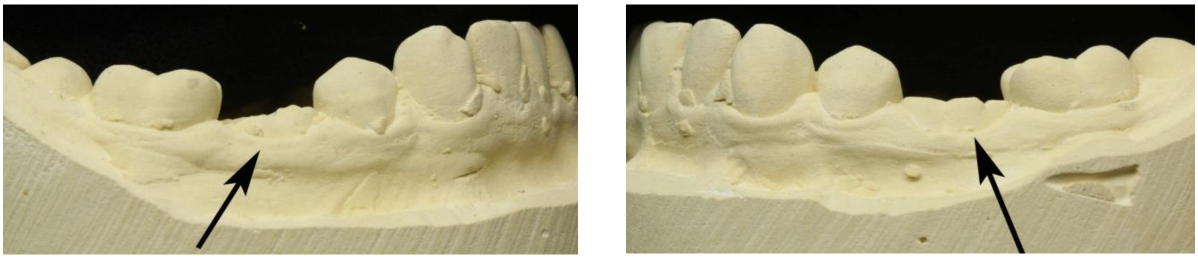
Another case showing progression of infraocclusion involved a DZ twin pair (ID 568A). Models were available at Stages 2 and 3 of development (Figure 9.6). Twin A had infraocclusion affecting the primary mandibular right and left second molar moderately and, at Stage 2, both molars became more severely infraoccluded. Twin B had moderate infraocclusion affecting the primary mandibular first and second right and left molars at Stage 2 and, at Stage 3, the primary mandibular second molars on the right and left showed persistence of the infraocclusion. Moreover, the mandibular left first premolar was rotated at this stage. Twin B also showed severe infraocclusion of the primary maxillary right second molar at Stage 2, which persisted with similar severity to Stage 3.

Twin A Mandibular right quadrant Mandibular left quadrant

Stage 2

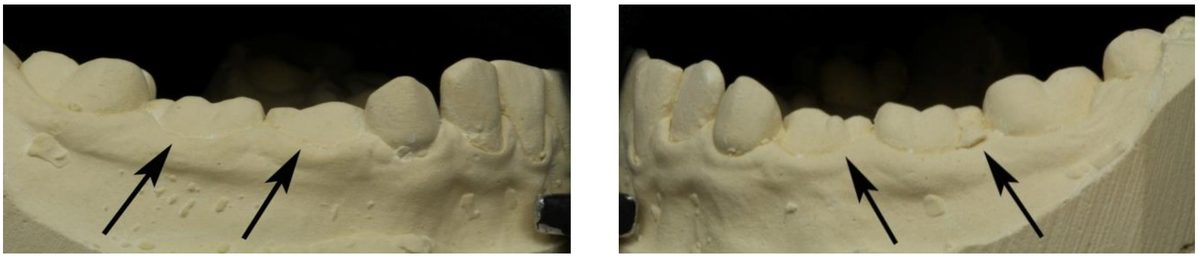


Stage 3



Twin B Mandibular right quadrant Mandibular left quadrant

Stage 2



Stage 3

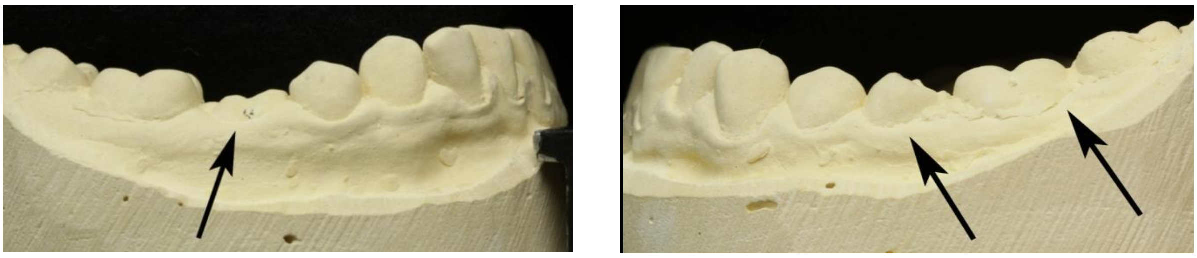


Figure 9.6. The pattern of progression of infraocclusion through different stages of dental development of Twin A and Twin B (ID 568).

.

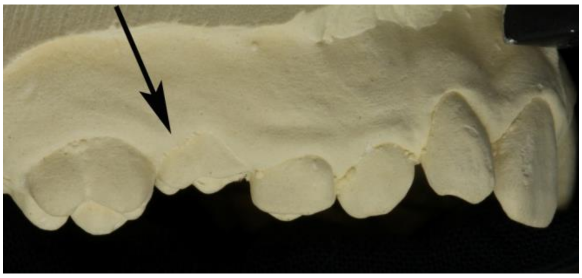
Twin A Maxillary right quadrant Maxillary left quadrant

Stage 2

Stage 3

Twin B Maxillary right quadrant Maxillary left quadrant

Stage 2



Stage 3

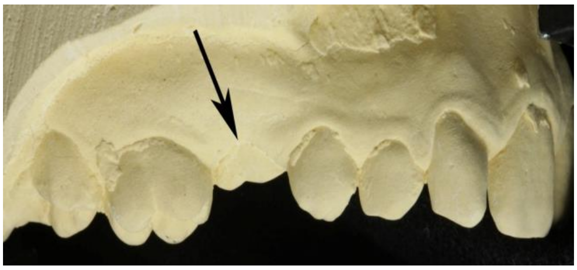


Figure 9.6. (continued) The pattern of progression of infraocclusion through different stages of dental development of Twin A and Twin B (ID 568).

9.3. Triplets

The triplets presented in this case (twin ID 223) showed infraocclusion to varying degrees in all mandibular quadrants. Twin A showed severe infraocclusion of the primary mandibular right and left first molars and a mild level of infraocclusion affecting the primary mandibular right and left second molars. In contrast, Twin B, showed severe infraocclusion of the primary mandibular right first molar and a moderate level of infraocclusion affecting the primary mandibular left first molar and right second molar, while the left second molar was mildly affected. Twin C showed a similar pattern of expression as Twin B (Figure 9.7).

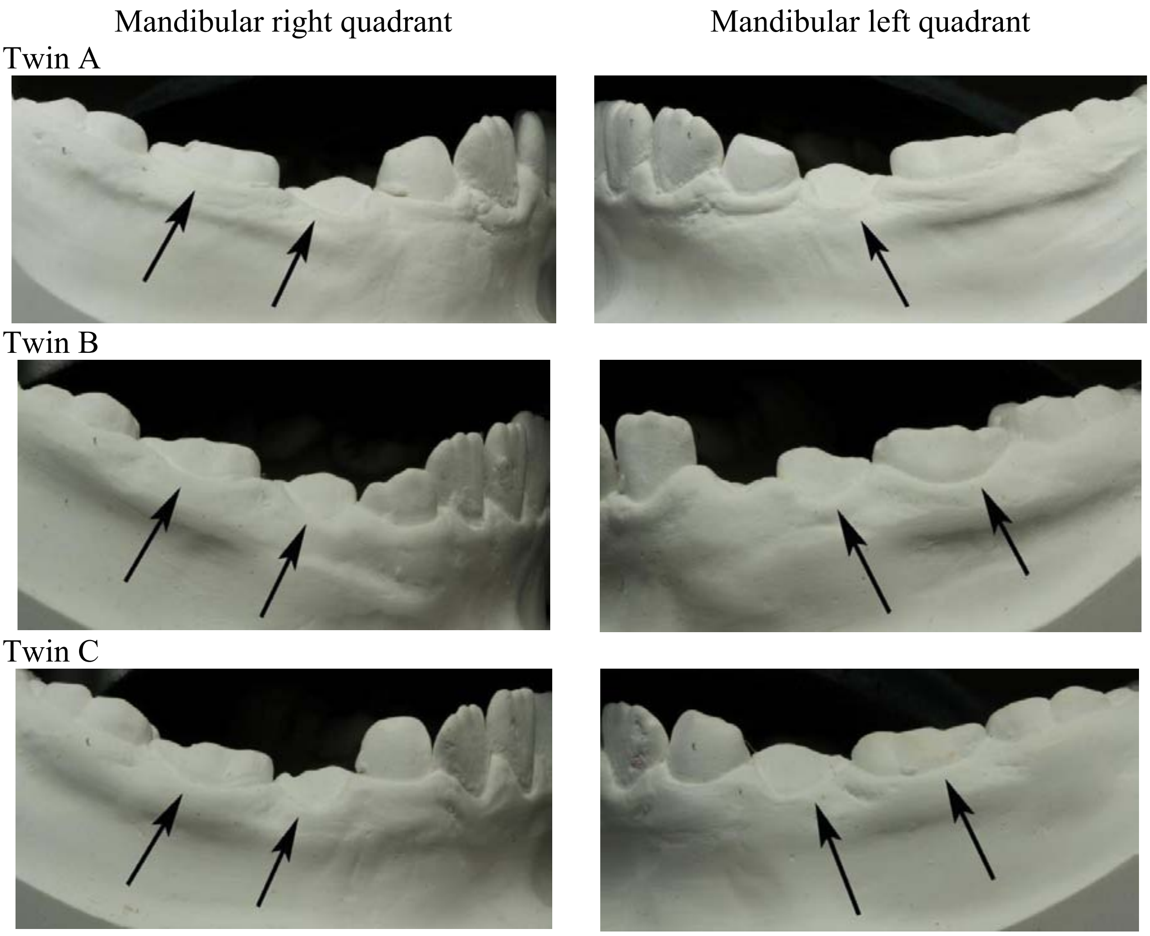
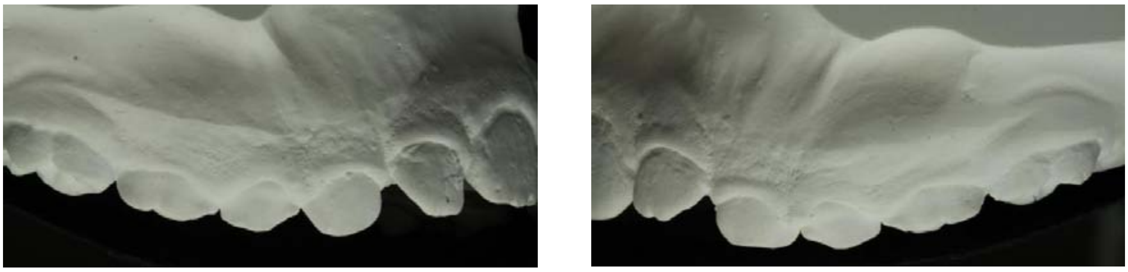


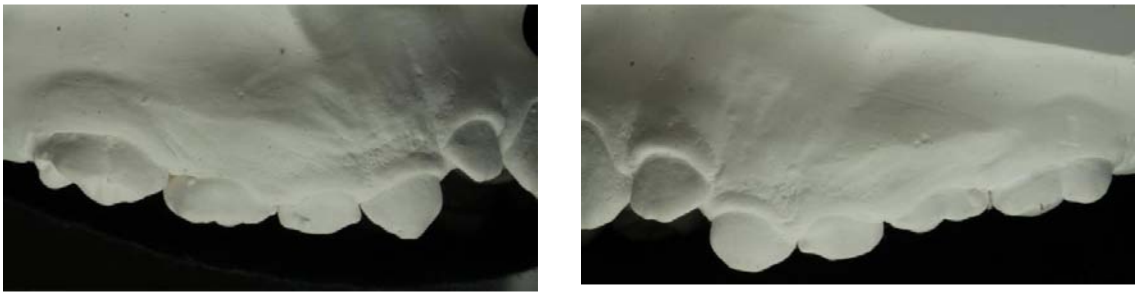
Figure 9.7.The pattern of expression and severity of infraocclusion among members of the triplets (ID 223).

Maxillary right quadrant Maxillary left quadrant

Twin A



Twin B



Twin C

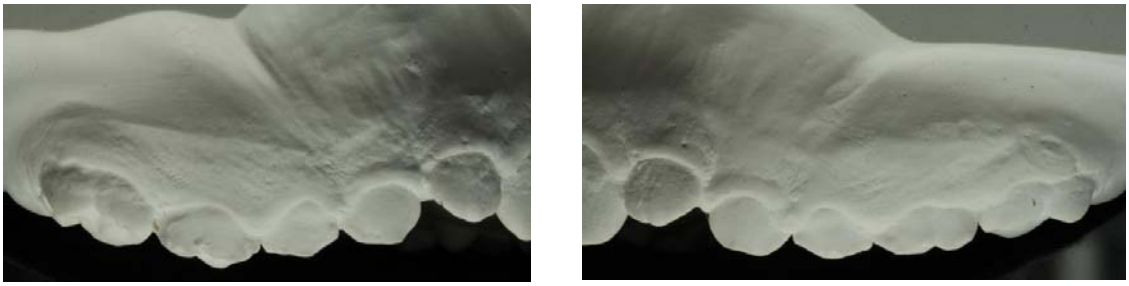


Figure 9.7. (continued) The pattern of expression and severity of infraocclusion among members of the triplets (ID 223).

9.4. Families of twins showing infraocclusion

As was proposed in the literature review and confirmed by the analyses performed in this thesis, there is evidence of a familial tendency in the occurrence of infraocclusion and associated dental anomalies. The prevalence of infraocclusion in siblings of affected individuals has been reported to be higher than that reported in the general population. A prevalence of 44 % in siblings of individuals displaying infraocclusion was reported by Via (1964) and a prevalence of 18% was reported by Kurol (1981). Furthermore, a familial tendency in the expression of infraocclusion has been highlighted in case reports. Cozza et al.

(2004) reported a case of severe infraocclusion affecting three siblings, while other studies have reported infraocclusion occurring in twin pairs (Stewart and Hansen, 1974; Helpin and Duncan, 1986; Dewhurst et al., 1997; Zengin et al., 2008). Similarly, it has been reported in the literature that infraocclusion occurs in association with other dental anomalies (Baccetti, 1998; Shalish et al., 2010). This finding was confirmed by the analyses of dental anomalies in association with infraocclusion that was performed in this thesis and reported in Chapter 8.

Given that there are associations of infraocclusion within families, associations of infraocclusion with other dental anomalies and associations of infraocclusion with dental development and tooth size, it is likely that common genetic factors contribute to variation in all of these features. However, it is still unknown how these genes act and at what stages of dental development. Investigating study models of twins, their parents and siblings provides some insight into how infraocclusion is expressed within a family, over one or two generations. This should further clarify possible roles of genetic factors. Moreover, by reviewing available records of family groups such as questionnaires and pre- and post-natal health records, the possible association of infraocclusion with environmental factors may be clarified.

Examining family group study models

The presence of infraocclusion and associated dental anomalies was investigated by visual examination in parents and siblings of twins who showed infraocclusion of any tooth. The following features were noted: infraocclusion of any tooth; retained primary teeth; agenesis of the premolars; and small size of teeth. Out of the 132 pairs of twins having infraocclusion, no records were available for 90 families. Of the remaining 42 families, 16 families (21 individuals) showed infraocclusion and/or missing premolars in one or more members of the family, while the remaining 26 families showed no signs of infraocclusion or associated dental anomalies. The findings are summarised in Table 9.2.

Table 9.2. Summary of findings of infraocclusion and associated anomalies in families of twin pairs with infraocclusion.

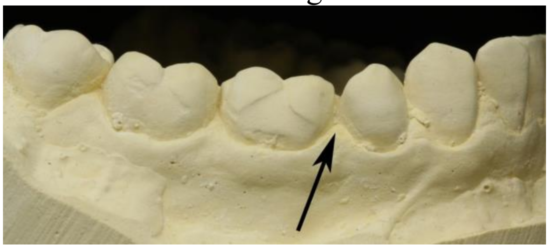
Twin ID	Twin Zygosity	Sex M/F	Father	Mother	Siblings
219	MZ	F	agenesis 35, 45, small 18, 25, 28	small 12	
234	MZ	F	agenesis 15, 25, 35, 45		
243	MZ	M		agenesis 15, 25, 35, 45	
254	MZ	M		infra 34	infra man RD and E, man LD and E
316	MZ	F			infra man RD, man LD
320	MZ	F			agenesis 15, 25, 35, 45
326	MZ	M			infra man RD, man LD
350	DZ	F			agenesis 35, 45
440	MZ	F			retained man RE, man LE
447	MZ	M			infra man RD and E, max RE, man LD and E, max LD
531	DZ	F			S(infra 35,45), B1(infra 45), B2(infra man RD)
538	DZ	F			infra man RD and E, man LD and E. Rotation of 34,44
562	DZ	M			infra man RD, man LD
596	MZ	M		agenesis 25	
609	MZ	M			agenesis15, 25, 35 45
648	MZ	M			infra man R and L D

Monozygotic (MZ), Dizygotic (DZ), Infraocclusion (infra), Mandibular (man), Maxilla (max), Primary first molar (D), Primary second molar (E) Sister (S), Brother (B). (n=16).

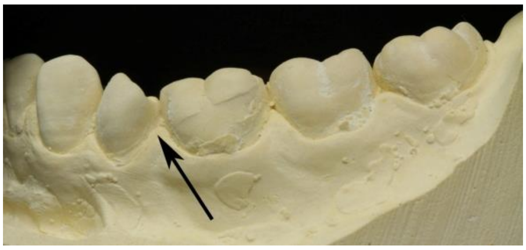
Among the 16 families investigated, 12 were families of MZ twin pairs and four were family members of DZ twin pairs. Examination of the study models revealed that some of the families of the twins showing infraocclusion also displayed one or more other dental anomalies. A case of mild infraocclusion was diagnosed in one member of an MZ twin pair (ID 219). On examining the study models of family members, it was found that the father had missing permanent mandibular second premolars on the right and left sides. It was also noted that the sizes of the maxillary third molars were reduced compared to the other molars. The mother in the same family had a small permanent maxillary left lateral incisor (Figure 9.8).

Father (ID 219)

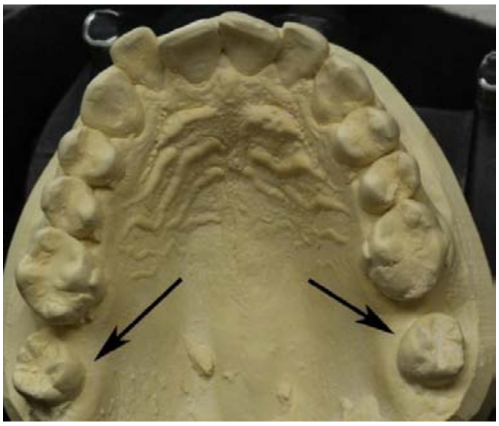
Mandibular right view



Mandibular left view



Occlusal view



Mother (ID 219)

Labial view

Figure 9.8. 2D images of study models showing some of the dental anomalies revealed in the families of twin pairs who showed infraocclusion (ID 219).

In another MZ twin pair (ID 440), both members displayed mild infraocclusion. Their sibling had retained primary mandibular right and left second molars (Figure 9.9).

Sibling (ID 440) Occlusal view

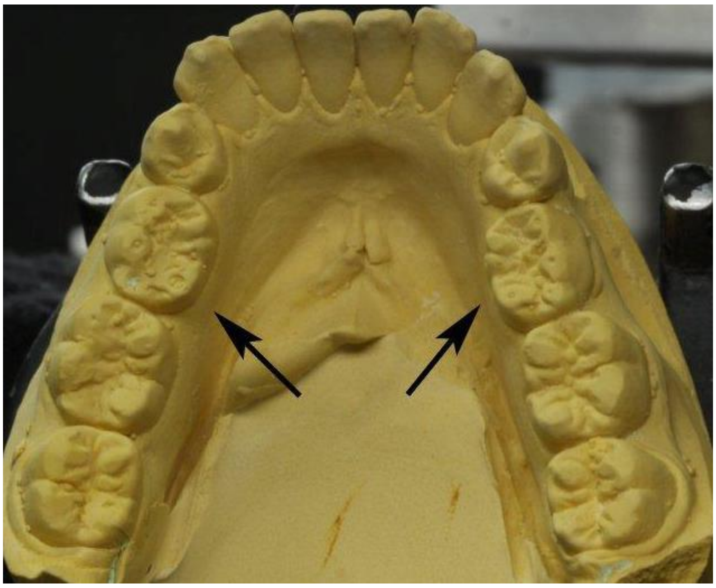


Figure 9.9. 2D image of a study model showing retained primary molars in a sibling of a twin pair who showed infraocclusion (ID 440).

A mild level of infraocclusion was revealed in one member of an MZ twin pair (ID 447). Examination of their siblings revealed that the twins' sister had infraocclusion in the maxillary and mandibular arches (Figure 9.10). Infraocclusion was displayed in all quadrants with varying severity.

Sibling (ID 447)

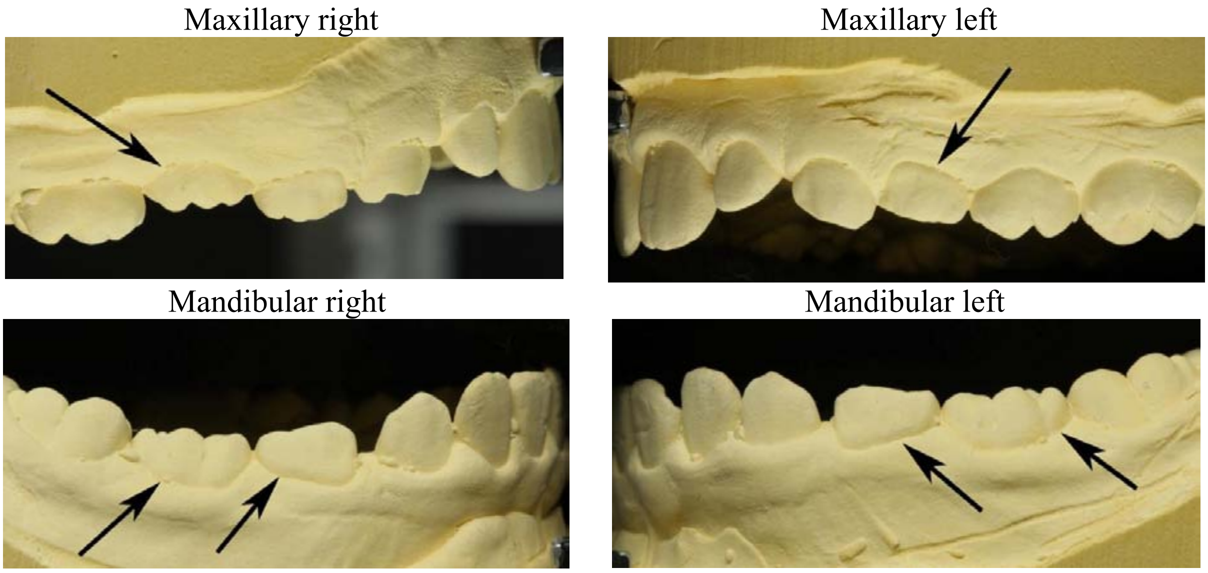
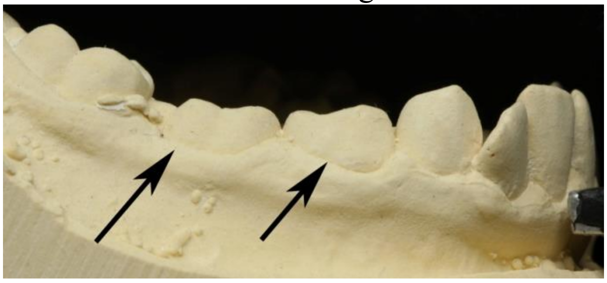


Figure 9.10. 2D images of study models showing infraocclusion in a sibling of one of the twins (ID 447).

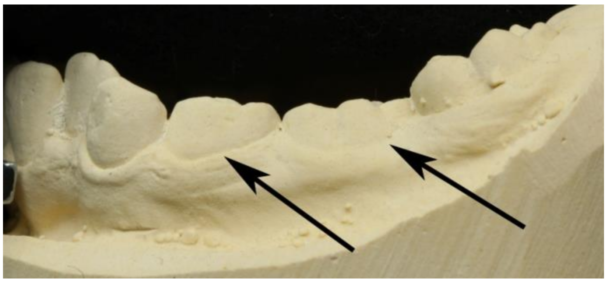
Models of the sibling of a DZ twin pair (ID 538) were available at two different stages: primary dentition and permanent dentition (21 years old). On examination of the first stage study models, infraocclusion was associated with the primary mandibular right and left first and second molars. Examination of the second stage study models revealed mild crowding of the permanent mandibular right and left first premolars, and infraocclusion of the permanent mandibular left second premolar (Figure 9.11).

Sibling (ID 538) stage 1

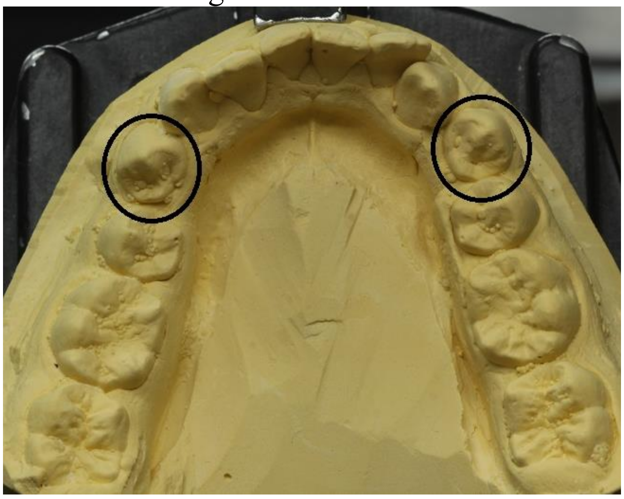
Mandibular right



Mandibular left



Stage 2 Occlusal view



Mandibular right

Mandibular left

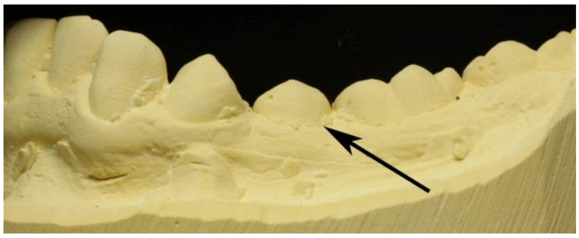
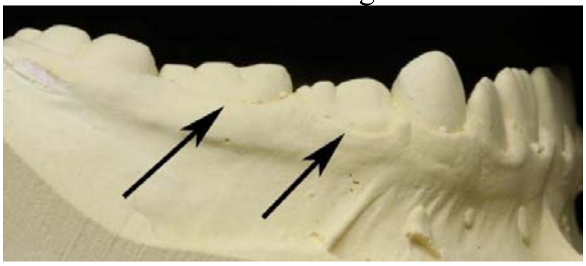


Figure 9.11. 2D images of study models showing infraocclusion and associated anomalies at later stage in a sibling of one of the twins showing infraocclusion (ID 538).

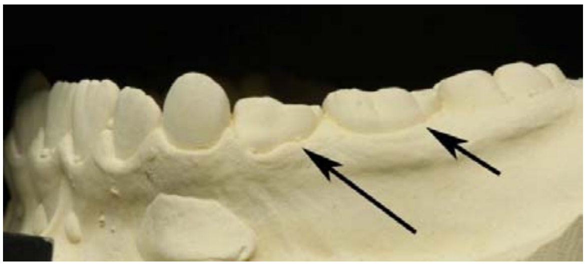
For another MZ twin pair (ID 254), one member displayed a mild level of infraocclusion, and investigation of the study models of other family members revealed that infraocclusion was present in two other members. The twins' sibling showed infraocclusion of the primary mandibular right and left first and second molars, and the mother showed mild infraocclusion associated with the permanent mandibular left first premolar when compared to the adjacent teeth and slight tipping of the tooth (Figure 9.12).

Sibling (ID 254)

Mandibular right



Mandibular left



Mother (ID 254) Mandibular left

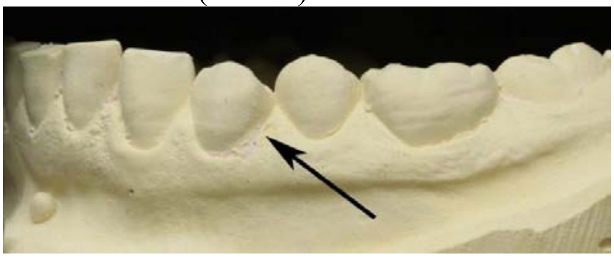
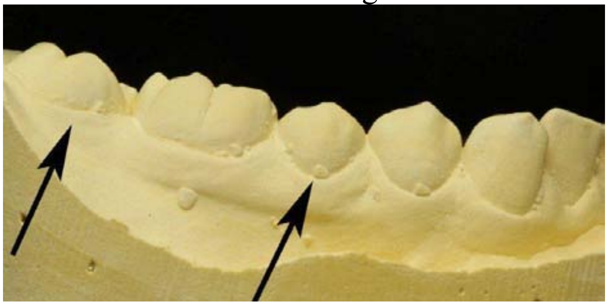


Figure 9.12. 2D images of study models showing infraocclusion and associated anomalies in family members of a twin pair of which one of the twins show infraocclusion (ID 254).

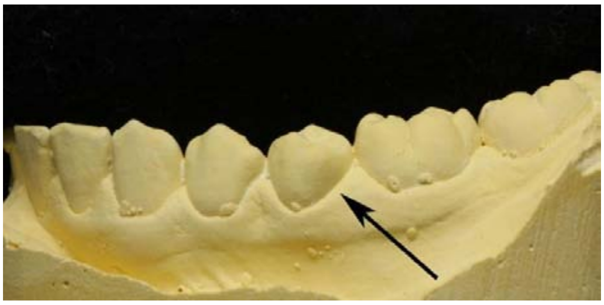
In another family (ID531), infraocclusion was revealed in three siblings of a DZ twin pair in whom only one member revealed a mild level of infraocclusion. All three siblings, one sister and two brothers, showed mild infraocclusion (Figure 9.13). This affected the mandibular right and left second premolars in one sibling. These teeth may not appear to be infraoccluded by looking at the 2D images only, but diagnosis was confirmed by examination of the models and comparison to the adjacent teeth. The left second premolar was also rotated slightly. Moreover, reviewing dental records also confirmed that the permanent mandibular second molar was rotated. In another sibling for whom study models were available at the primary dentition stage, mild infraocclusion was noted affecting the primary mandibular right and left first molars. The third sibling also showed infraocclusion affecting the permanent dentition, the infraoccluded tooth being the mandibular right second premolar.

Sibling 1 (ID 531)

Mandibular right

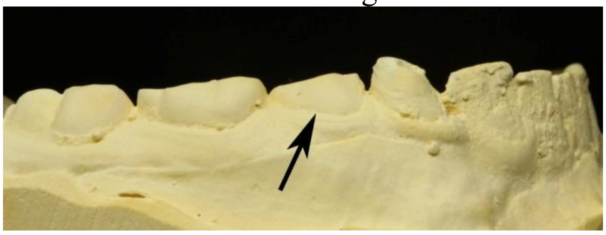


Mandibular left

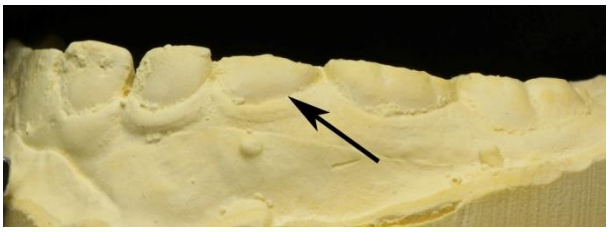


Sibling 2 (ID 531)

Mandibular right



Mandibular left



Sibling 3 (ID 531)

Mandibular right

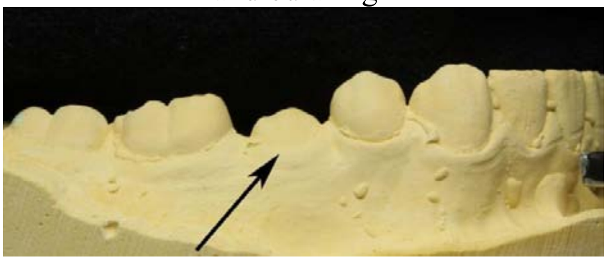


Figure 9.13. 2D images of study models showing infraocclusion in different members of the family of a DZ Twin pair (ID 531).

Another case was noted where different patterns of dental anomalies were expressed in family members of an MZ twin pair, where both members of the pair (ID 609) displayed bilateral infraocclusion ranging from mild to severe. It was noted that the sibling of this twin pair had a reduced crown size of the maxillary permanent left lateral incisor. It was also noted that the second premolar was absent in all quadrants (Figure 9.14).

Sibling (ID 609) occlusal view

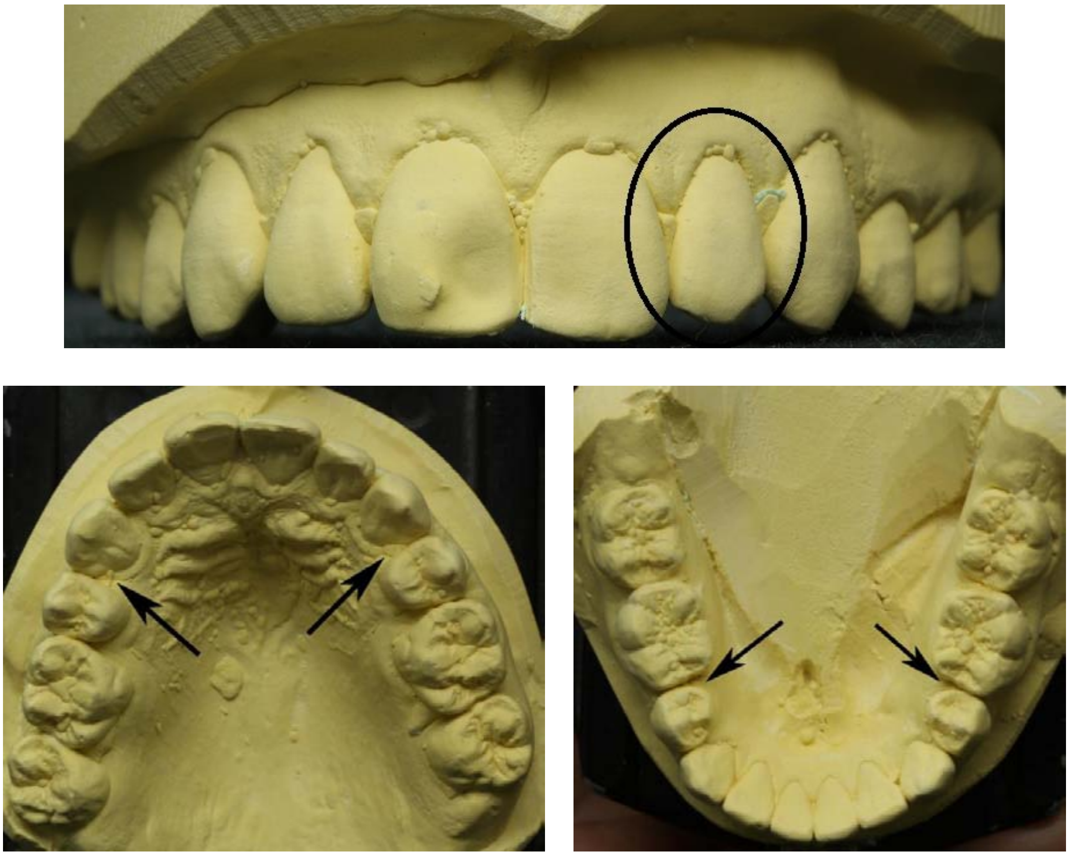


Figure 9.14. 2D images of a sibling of a twin pair showing associated dental anomalies.

Reviewing family group records

Reviewing the available records of the investigated family groups listed in Table 9.1 clarified some of the findings reported, e.g. whether the missing teeth were affected by agenesis or had been extracted, as well as information not apparent from study models, such as supernumerary teeth and records of the occlusion. Moreover, the records provided information about pre-natal complications during pregnancy, such as high blood pressure, and post-natal complications during or after birth, such as caesarean birth, reduced oxygen supply and the need for incubation of the twins. Gestational age and birth weight were also reported. After reviewing the available records, there seemed to be no one common factor among all family groups that could be related to the presence of infraocclusion and associated dental anomalies. Thus, there was variation of the environmental factors occurring within each of the family groups that could have contributed to the occurrence of infraocclusion. For instance, in some family groups, premature birth and low birth weight were reported (ID 234, 243, 326).

In contrast, normal birth weight was reported in other family groups (ID 531, 219, 254). In some other family records, reduced oxygen supply and other illnesses such as recurrent infection and asthma which required management, the intake of medications and antibiotics (ID 243, 254, 447, 596). All these could have acted as environmental triggers leading to the occurrence of infraocclusion with or without the association of other dental anomalies.

9.5. Discussion

Presentation of these cases consolidates the findings of the present study that were reported in previous chapters (Chapters 6, 7, 8). That is, infraocclusion is a multi-factorial condition that has a genetic basis to its occurrence and in some cases, is associated with the presence of other dental anomalies. In other cases, it occurs in the absence of other dental anomalies, with phenotypic variation evident in one or more members of the family of affected individuals in the form of other dental anomalies. A possible explanation for this phenotypic variation could be epigenetic and/or environmental factors acting in addition to genetic factors. This was further expressed in MZ twin pairs showing different expression of the condition, for example, where one member of a pair showed infraocclusion while the other member did not manifest the condition. Another example would be the mirror-image effect that was also presented. There have been some explanations for this phenomenon, i.e. that mirror-imaging may be related to the timing of the division in the twinning event and therefore the type of placentation (Townsend et al., 2003; Townsend et al., 2009), but further studies are warranted.

The presentation of the twin study models at three different stages showed that this condition may start at a very mild level that could possibly be missed by clinicians, and progress in later stages of dental development to more severe levels. Examining models at later stages of dental development also showed some cases where consequences were associated with severe forms of infraocclusion. These are important factors that require careful evaluation, on a case by case basis, considering that infraocclusion is a possible sign of underlying associated dental anomalies. This will contribute to decisions regarding management.

In some cases infraoccluded teeth may exfoliate naturally and the dentition continue to develop normally. However, in other cases, early intervention and treatment planning are warranted for a better long term prognosis. In more severe cases, especially when several members of the family are affected and when other dental anomalies are diagnosed, genetic counselling is needed and, in the future, it may be possible to identify the genes responsible following genetic testing.

Future studies including clinical examination of patients and their family members showing infraocclusion at different stages of dental development, together with histological examination of exfoliated teeth, may further reinforce our current state of knowledge in understanding the biological processes of dental development and the disturbances associated with this process. Clinical examination conducted over a series of visits will allow clinicians to determine whether a tooth emerged and then subsequently stopped erupting before reaching the occlusal plane, or whether a tooth had erupted to the full occlusion position then stopped while the adjacent teeth continued to erupt, resulting in what appear to be infraoccluded tooth. This, in turn, will influence decision making about the best treatment plan to be undertaken.

9.6. Conclusion

Findings presented in this chapter, in the form of case reports, support hypotheses put forward in this thesis, that is: family members of twin pairs displaying infraocclusion are more likely to show infraocclusion and/ or other dental anomalies; and twins' study models examined at three different stages can display infraocclusion in Stage 1, which progresses in Stage 2, and then persists in Stage 3 in the presence or absence of associated dental anomalies or subsequent side effects. In some other cases, infraocclusion may not be present in Stage 3, although associated dental anomalies and side effects of the feature may persist.

10. General discussion

The results of this study are in agreement with some of the findings reported in previous studies. For example, the primary mandibular first molar is the primary tooth most commonly affected by infraocclusion and there are no significant differences in frequencies of occurrence of infraocclusion related to side (right/left) or sex (Brearley and McKibben, 1973; Steigman et al., 1973b; Kurol, 1981). The estimated prevalence of infraocclusion was within the ranges reported previously (Via, 1964; Steigman et al., 1973b; Kurol, 1981; Koyoumdjisky-Kaye and Steigman, 1982a), with a higher prevalence noted in the twin sample compared to the singleton sample. The present study has investigated the influence of genetic factors on infraocclusion, building on previous studies (Via, 1964; Stewart and Hansen, 1974; Kurol, 1981; Helpin and Duncan, 1986; Dewhurst et al., 1997; Cozza et al., 2004; Zengin et al., 2008) and, for the first time using genetic modelling, has shown that there is a significant genetic contribution to variation in infraocclusion of the primary mandibular first molar. Furthermore, the current study has revealed a significant association of ectopic canines and the lateral incisor complex with infraocclusion, a finding consistent with previous studies (Baccetti, 2000; Garib et al., 2009; Peck, 2009; Shalish et al., 2010).

A new association has been revealed between infraocclusion and dental development, with those individuals with infraocclusion showing delayed dental development compared with unaffected individuals. Another new finding has been that infraocclusion in the primary dentition is associated with smaller primary tooth size.

In the present study, the term ‘infraocclusion’ was deliberately chosen as it is a descriptive term for a tooth that is positioned below the level of occlusion. Because of the nature of the samples used, it was not possible to determine whether those teeth that showed infraocclusion had erupted to the normal plane of occlusion and then stopped erupting, while the adjacent teeth had continued to erupt, or whether affected teeth had never reached the plane of occlusion. The biological mechanism(s) underlying infraocclusion is/are not well understood. Localised ankylosis between the root and alveolar bone could be one explanation for failure of eruption prior to, during or after complete emergence.

The finding that the primary mandibular first molar is more commonly infraoccluded than the primary mandibular second molar may be related to the chronology of dental development. On average the primary mandibular first molar starts developing in utero between 12 to 15.5 weeks, its crown formation at approximately 6 months postnatally. It then emerges into the oral cavity at approximately 16 months of age. Root formation of the primary mandibular first molar is completed at around 2.25 years of age. The primary mandibular second molar, starts developing in utero between 12.5 and 18 weeks, completes its crown formation at approximately 10 months postnatally, and then emerges into the oral cavity at approximately 27 months of age. Root formation of the primary mandibular second molar is completed at around 3 years of age (Nanci and Ten Cate, 2008). Thus, the period of time from initial formation until emergence is shorter for the primary mandibular first molar than it is for the primary mandibular second molar. The fact that the primary mandibular second molar develops over a longer period of time, may enable any disturbances in cycles of bone or cementum deposition and resorption to be ‘repaired’, thus reducing the chances of ankylosis and subsequent infraocclusion occurring. Variations in the dental development process such as local space loss, reduced alveolar bone height and changes to dental arch length (Becker and Karnei-R'em, 1992b; 1992a; Becker et al., 1992) that are caused by infraocclusion of the primary mandibular first molar may then contribute to infraocclusion of the second molar as it erupts into the oral cavity. This pattern should be similar to that of primary failure of eruption (PFE), where all the teeth that are distal to the affected tooth fail to erupt to the normal plane of occlusion (Proffit and Frazier-Bowers, 2009). These propositions are consistent with findings from the current investigation, which revealed that infraocclusion of the primary mandibular first molar is under relatively strong genetic control, with additive genetic variance or ‘narrow sense’ heritability contributing by 94% to phenotypic variation, while infraocclusion of the primary mandibular second molar is associated more with environmental factors. A combination of common and unique environmental factors contributed to variation in infraocclusion of the primary mandibular second molar, with common environmental factors accounting for 60% of observed variation. Common environmental factors, sometimes referred to as maternal factors, that are experienced by both members of a twin pair and, for infraocclusion, are likely to occur in the prenatal or early post natal period. Unique environmental factors contributed to 39% of the observed variation in infraocclusion and this component can be attributed to factors associated with eruption that are unique to each member of a pair. A part of the unique environmental contribution is likely to be due to errors of measurement.

Tooth eruption is a complex interactive process occurring between osteoblasts, osteoclasts and dental follicular cell lines associated with the tooth germ. This complex interaction is under the control of a host of signalling molecules, receptors, transcription and cell adhesion factors (Wise et al., 2002; Brook, 2009). These interactions maintain the coordinated resorption and deposition of bone on opposing sides of the erupting tooth during its intraosseous movement and emergence into the oral cavity (Wise et al., 2002; Brook, 2009; Wang, 2013). Genetic and environmental factors contribute to the multifactorial nature of tooth eruption, which may be disturbed at any stage of development. These disturbances may result in abnormalities in the eruption process, such as infraocclusion or other dental anomalies, including agenesis, microdontia and supernumerary teeth (Wise et al., 2002; Brook, 2009; Cobourne and Sharpe, 2013).

Epithelial rests of Malassez (ERM) are part of a network of epithelial cells derived from Hertwig's epithelial root sheath that separate the cementum covering the root of the tooth from alveolar bone covering the socket (Rincon et al., 2006). This arrangement of epithelial cells has been likened to a basketball net hanging from a basketball ring. They appear to have an important role in maintaining the periodontal ligament space (Luan et al., 2006). ERM synthesise osteopontin which acts as an aid for cementum repair and mineralised tissue formation on the root surface. ERM also synthesise bone sialoprotein which acts as an adhesion molecule and initiator of mineralization on the root surface (MacNeil et al., 1995). A high affinity receptor of nerve growth factor and parathyroid hormone related protein (PTHrP) may also be secreted by ERM (Beck et al., 1995). Maintaining proper functioning of these epithelial cells is critical to the normal process of tooth eruption and subsequent maintenance of the periodontal ligament. Cellular epigenetic influences in the form of localised transient disruptions of the 3D network of the ERM may disturb the normal eruption process. Perhaps, by allowing contact to be established between developing tooth root cementum and alveolar bone, localised areas of 'ankylosis' may develop that can then be repaired during the eruptive process or at a later stage. In other cases, the severity of 'ankylosis' could prevent the tooth from ever erupting. Some cases with extensive ankylosis can be apparent on radiographs. However, if localised areas of 'ankylosis' are on the labial or lingual surfaces of a tooth, or even small areas on mesial and distal surfaces, ankylosis may not be evident on radiographs or it may be overlooked.

Perhaps certain individuals are genetically predisposed to having some break down of the ERM during the eruptive process of primary molars, thereby leading to ankylosis and infraocclusion. Traumatised teeth that have received a heavy blow (an extrinsic traumatic event) may subsequently become ankylosed (Rubin et al., 1984). What is not clear is whether a minor traumatic event could have a similar effect. It is not known if events, such as altered blood flow and/or temporo-spatial disturbances between molecules and cells at a local level, within the periodontium, might lead to a localised ankylosis (Figure 10.1).

There may be other situations where the affected teeth are not ankylosed but fail to follow the normal eruption path that has already been cleared; that is, there is an uncoupling between bone resorption and tooth eruption. The periodontal ligament may be abnormal, so that not only does an affected tooth fail to erupt, but it does not respond to orthodontic forces.

This condition is known as primary eruption failure (PEF) (Proffit and Frazier-Bowers, 2009). Indeed, both ankylosis and PEF may be linked genetically since they can be found in different quadrants of the same mouth.

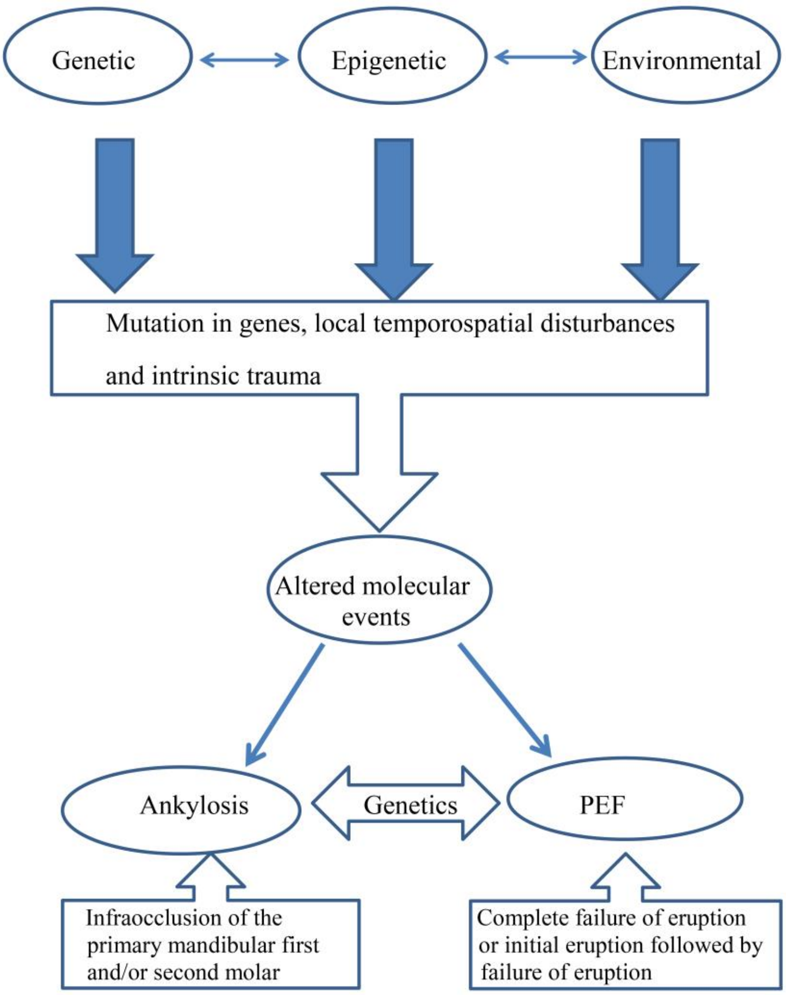


Figure 10.1. Flow chart showing the contribution of different factors associated with disturbances resulting in infraocclusion or primary eruption failure.

This study has shown that infraocclusion of the primary mandibular first molar is under significant genetic control. This was further supported by the presence of significant associations between infraocclusion and two selected dental anomalies (lateral incisor complex and ectopic canines). The fact that these anomalies are not related anatomically suggests that a common genetic factor may be responsible for their occurrence together, a concept known as 'pleiotropy'. A significant association was also revealed between infraocclusion and smaller tooth size and between infraocclusion and delayed dental development. These findings are consistent with previous studies which link delayed formation and eruption of teeth to reduced tooth size and missing teeth (Garn and Lewis, 1970; Brook, 1984; Becker and Chaushu, 2000; Arte et al., 2001; Brook et al., 2009a). These different clinical phenotypes are revealed at different stages of dental development (Figure 10.2). The first sign may be infraocclusion of the primary mandibular first molar and small size primary teeth, followed by agenesis of permanent teeth and ectopic permanent canines that are discovered clinically at later stages of development.

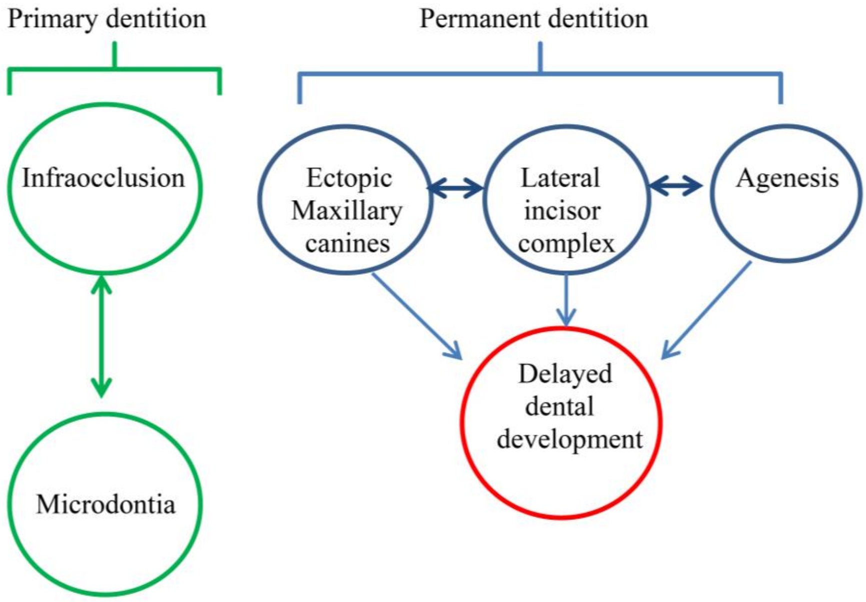


Figure 10.2. Presentation of infraocclusion and associated anomalies in the primary and permanent dentition.

The causative genes for some dental syndromic conditions have been identified but the underlying molecular mechanism(s) have not been fully explained (Brook, 2009; Cobourne and Sharpe, 2013). Evidence from mice and humans has revealed a role for several molecular signalling pathways in the regulation of tooth number (van den Boogard et al., 2012), tooth size (Cai et al., 2011), tooth morphology (Dassule et al., 2000), and tooth eruption (Decker et al., 2008). It has been found that a mutation in parathyroid hormone receptor 1 [PTH1R] is associated with Primary Eruption Failure (Decker et al., 2008). A mutation in the same hormone was also found to be associated with Blomstrad steochondrodysplasia [BOCD;

#215045], a lethal skeletal dysplasia associated with abnormal breast development and tooth impaction (Wysolmerski et al., 2001). Parathyroid hormone-like hormone [PTHLH] is essential for an appropriate spatio-temporal arrangement of bone cells and normal osteoclast function within the tooth germ and surrounding alveolar bone (Mekaapiruk et al., 2002). Further investigations are warranted in this area to reveal whether infraoccluded teeth are affected by mutations of the PTHLH gene, similar to primary failure of eruption.

The technique that was utilised in this study to measure and then categorise infraocclusion was established to enhance standardisation of the measurements obtained from both samples, and to be easily applied in a clinical case. However, there were some limitations to the methods used. For example, there was some subjectivity involved in locating the landmarks used to identify the occlusal plane and the occlusal table, although the results of double determination confirmed that the methods were reliable. Where there was evidence of wear or restoration obscuring a landmark, the case was excluded from further analysis. In cases of severe infraocclusion with tipping of adjacent teeth, the occlusal table can be altered locally and measurement of infraocclusion affected. Another limitation of the study, which was mentioned earlier, relates to the nature of the samples used as this prohibited the possibility of determining whether teeth were fully erupted and had then become infraoccluded or whether teeth had become infraoccluded before reaching the occlusal plane. Nevertheless, this is the first study to quantify the role of genetic factors on infraocclusion by studying twins. By using a model fitted approach, estimates of genetic and environmental contributions to observed variation were made that were associated with relatively small standard errors.

This study was not an experimental investigation using molecular genetic techniques but, rather, it focused on describing the nature and extent of infraocclusion in two samples as well as quantifying the relative contributions to variation due to genetic and environmental factors. Nevertheless, the findings can help us understand the biological mechanisms and associated features of infraocclusion. The findings will also assist clinicians in providing proper counselling, early diagnoses, prevention and treatment plans for affected individuals. This study highlights the need for further investigations and regular follow up to be conducted in all cases where infraocclusion is detected to eliminate the possibility of other associated dental anomalies. The finding of a significant genetic contribution to variation in infraocclusion has opened up new areas of research. Further genetic analyses may be undertaken in the future to identify candidate genes. This will require the collection of suitable family pedigrees and positional cloning of relevant loci. This is particularly critical where infraocclusion is found to occur in association with other dental and systemic anomalies and/or syndromes. Moreover, further studies are needed using molecular genetic techniques to identify the molecular signalling pathways that are disturbed during the eruption process that lead to the occurrence of infraocclusion. By identifying these pathways, it may be possible to prevent the condition occurring in the future.

11. Conclusions

The findings of this thesis support the hypothesis that the primary mandibular first molar is the most commonly infraoccluded tooth in singletons and twins. The patterns or trends of infraocclusion within each sample were similar; that is, no sex predilection was reported in association with infraocclusion and no evidence of association between infraocclusion and arch side (right/left) was revealed in either sample. However, the frequency of occurrence of infraocclusion was greater in the twin sample compared with the singleton sample, possibly reflecting a distinct feature in twins compared to singletons.

Percentage concordances for infraocclusion in MZ twin pairs (males and females) tended to exceed those in DZ twin pairs, supporting the hypotheses proposed earlier. This finding indicates a genetic influence on infraocclusion. However, epigenetic influences were also revealed by some MZ twin pairs who showed differences in the expression of infraocclusion, as well as presenting examples of mirror-imaging. New insights into genetic and environmental contributions to infraocclusion have been revealed by genetic modelling, with infraocclusion of the primary mandibular first molar being shown to be under strong genetic control. Unlike the first molar, variations in infraocclusion of the primary mandibular second molar could be attributed to a combination of common and unique environmental factors.

Investigations also supported the hypothesis that infraocclusion is not an isolated condition but can occur in association with other dental anomalies, reflecting a possible pleiotropic effect. Findings supported hypotheses proposed earlier, with an association being found between infraocclusion and delayed dental age and, moreover, dental crown size was reduced in cases of infraocclusion in both singletons and twins.

Findings also supported the hypothesis that family members of twin pairs displaying infraocclusion would be more likely to show infraocclusion and/ or other dental anomalies.

Examination of twins' dental models at three different stages confirmed that infraocclusion may progress to a more severe presentation in the presence or absence of associated dental anomalies or subsequent side effects. In some other cases, infraocclusion may not be present in the mixed dentition stage; however, associated dental anomalies and/or subsequent side effects of the feature may be present. These findings indicate that when infraocclusion is diagnosed, a combination of extensive clinical and radiographic examinations should be conducted to assess the dentition and reveal the possible existence of other dental anomalies. Moreover, proper counselling needs to be provided, including information about future treatment options. It should also be explained that where some family members are affected by infraocclusion, other dental anomalies may be present.

Future studies including multivariate modelling of the primary mandibular first and second molars, including relevant environmental factors and also other dental anomalies, may further elaborate the complex aetiology of infraocclusion in the primary dentition. Studies including clinical examination of patients showing infraocclusion at different stages of dental development, together with histological and molecular examination of exfoliated human teeth, may further reinforce our current state of knowledge in understanding the biological processes of dental development and disturbances associated with the process.

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13. Appendices

13.1. Appendix 1- List of achievements and professional development activities of Ruba Odeh during PhD candidature 2010-2013

Publication

Bockmann MR, Harris AV, Bennett CN, Odeh R, Hughes TE and Townsend GC (2011). Timing of colonization of caries-producing bacteria: an approach based on studying monozygotic twin pairs. International Journal of Dentistry 2011. Article ID 571573, http://dx.doi.org/10.1155/2011/571573.

Published abstracts for conference presentations during candidature

R. Odeh, G. Townsend, S. Mihailidis, T. Hughes, R. Lahdesmaki, A. Brook. Infraocclusion of primary molars and associated dental anomalies in twins and singletons: what is the underlying aetiology? Abstract submitted and oral presentation performed at the School of Dentistry, Research Day 2013, The University of Adelaide, August 2013.

R. Odeh, G. Townsend, S. Mihailidis, T. Hughes, R. Lahdesmaki, A. Brook. Genetic and environmental causes of variation in expression of infraoccluded primary teeth in twins and singletons. Abstract submitted and oral presentation performed at the Faculty of Health Sciences postgraduate research conference 2012, The University of Adelaide, August 2012.

R. Odeh, G. Townsend, S. Mihailidis, T. Hughes, R. Lahdesmaki, A. Brook. Genetic and environmental causes of variation in expression of infraoccluded primary teeth in twins and singletons. Abstract submitted and oral presentation performed at the School of Dentistry, Research Day 2012, The University of Adelaide, August 2013.

Successful grant application

Australian Dental Research Foundation (ADRF) grant 2011

Project: Genetic and environmental causes of variation in the expression of infraoccluded primary teeth

Investigators: R. Odeh, G. Townsend, S. Mihailidis, T. Hughes, R. Lahdesmaki

Other professional development activities

Review of manuscript (ODON-D-13-00114). Huge bilateral gemination of permanent maxillary central incisors: report of a case Odontology.

Completed Basic Statistics and Research Methods course, The University of Adelaide, Australia. June 2012.

Completed workshops on Managing an HDR Thesis with Word 2007 - Levels 1 & 2, The University of Adelaide, Australia. June 2011

Completed Endnote Training Sessions, Levels 1 & 2, The University of Adelaide, Australia. March 2011.

Completed a course on Being Supervised, The University of Adelaide, Australia. June 2010:

Completed a course on Readability in Research Documents, The University of Adelaide, Australia. May 2010.

Completed a course on Chemical Management, The University of Adelaide, Australia. April 2010.

Completed a course on Appropriate Citation and Avoiding Plagiarism, The University of Adelaide, Australia. March 2010

Completed a course on Reviewing Literature, The University of Adelaide, Australia. March 2010.

13.2. Appendix 2-**The following figures relate to material presented in Chapter 6, in particular they complement Figures 6.3 and 6.4.**

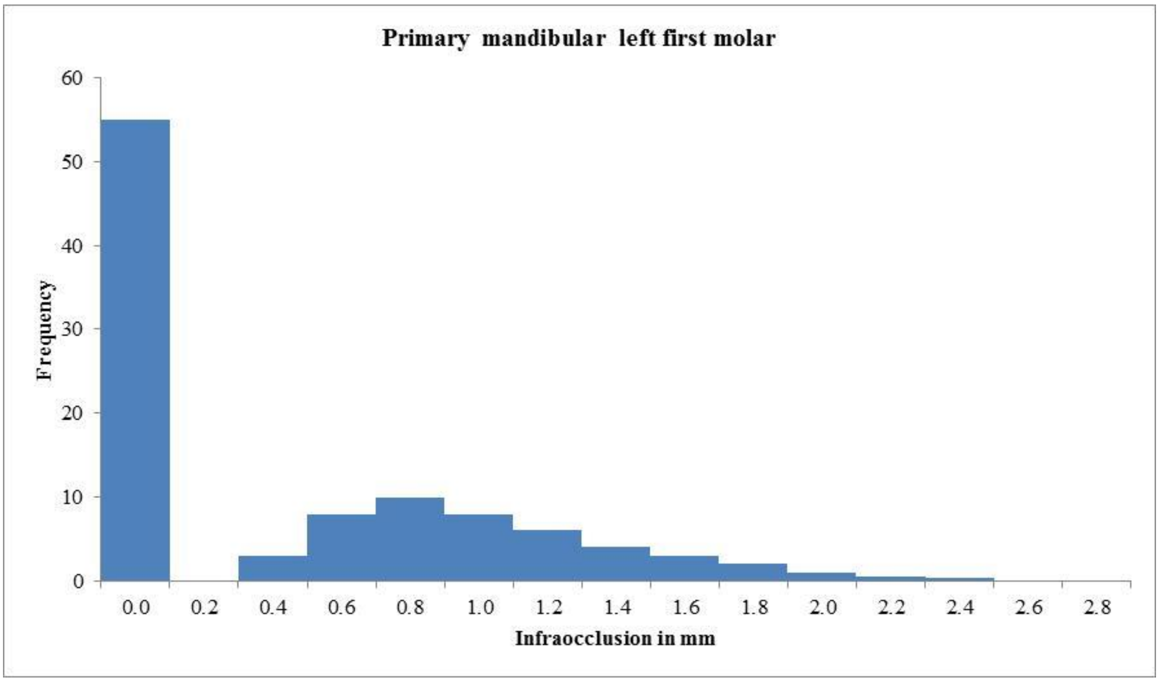

Figure 1. Bar chart presenting measurements obtained from the occlusal plane to the occlusal table of the primary mandibular left first molar in singletons.

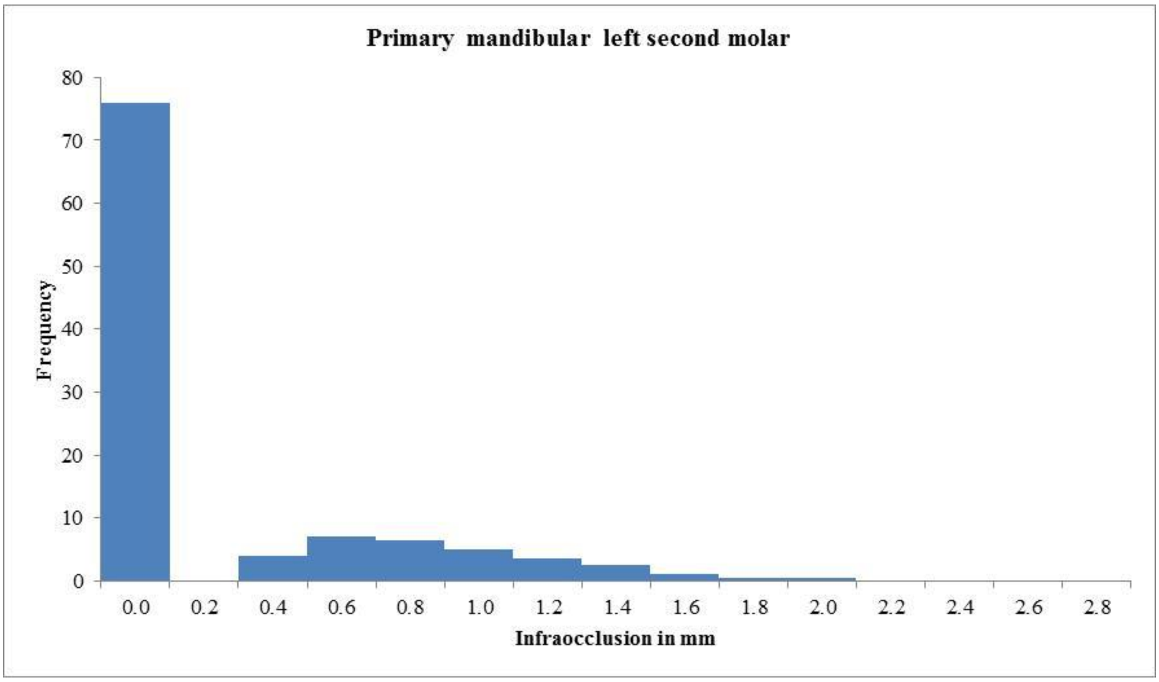

Figure 2. Bar chart presenting measurements obtained from the occlusal plane to the occlusal table of the primary mandibular left second molar in singletons.

The following tables relate to descriptive analysis of the singleton sample presented in Chapter 6.

Table 1. Comparison of the frequency of occurrence and degree of expression of infraocclusion between primary mandibular first (D) and second (E) molars (left side) - singleton sample. (Complements Table 6.5)

	MLD		MLE	
NI	937	(82.00%)	1270	(93.50%)
MI	190	(16.60%)	82	(82.00%)
MO	14	(1.22%)	6	(6.00%)
SE	1	(0.09%)	0	(0.00%)
Total	1142	(100%)	2716	(100%)

chi-square value = 79.3, dof = 3
P < 0.001

(When collapsed to a 2 x 3 table, with MO and SE combined, chi-square value = 78.9, dof = 2, P < 0.001)

Table 2. Comparison of the frequency of occurrence and degree of expression of infraocclusion of the mandibular left first molars between males and females - singleton sample. (Complements Table 6.8)

MLD

	M		F	
NI	518	(82.40%)	419	(81.50%)
MI	100	(15.90%)	90	(17.50%)
MO	9	(1.40%)	5	(0.10%)
SE	1	(0.16%)	0	(0.00%)
Total	628	(100%)	514	(100%)

chi-square value = 1.8, dof = 3
P > 0.05

(When collapsed to a 2 x 3 table, with MO and SE combined, chi-square value = 1.3, dof = 2, P > 0.05)

Table 3. Comparison of the frequency of occurrence and degree of expression of infraocclusion of the mandibular left second molars between males and females - singleton sample. (Complements Table 6.9)

MLE

	M		F	
NI	670	(93.00%)	600	(94.00%)
MI	47	(6.50%)	35	(5.50%)
MO	4	(0.60%)	2	(0.30%)
SE	0	(0.00%)	0	(0.00%)
Total	721	(100%)	637	(100%)

chi-square value = 1.1, dof = 2
P > 0.05

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, chi-square value = 0.9, dof = 1, P > 0.05)

Table 4. Comparison of the frequency of occurrence and degree of expression of infraoccluded primary mandibular first (D) and second (E) molars (right side) - singleton sample. (Additional results)

	MRD		MRE	
MI	153	(91.00%)	60	(87.00%)
MO	12	(7.14%)	9	(13.00%)
SE	3	(1.78%)	0	(0.00%)
Total	168	(100%)	69	(100%)

chi-square value = 3.2, dof = 2
P > 0.05

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 0.9, dof = 1, P > 0.05)

Table 5. Comparison of the frequency of occurrence and degree of infraoccluded primary mandibular first (D) and second (E) molars (left side) - singleton sample. (Additional results)

	MLD		MLE	
MI	190	(96.60%)	82	(93.20%)
MO	14	(7.36%)	6	(7.31%)
SE	1	(0.48%)	0	(0.00%)
Total	205	(100%)	88	(100%)

chi-square value = 0.4, dof = 2
P > 0.05

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 0.02, dof = 1, P > 0.05)

Table 6. Comparison of the frequency of occurrence and degree of infraocclusion between mandibular right and left first molars - singleton sample. (Additional results)

	MRD		MLD	
MI	153	(91.00%)	190	(96.60%)
MO	12	(7.14%)	14	(7.36%)
SE	3	(1.78%)	1	(0.48%)
Total	168	(100%)	205	(100%)

chi-square value =1.5, dof = 2
P > 0.05

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 0.3, dof = 1, P > 0.05)

Table 7. Comparison of the frequency of occurrence and degree of infraocclusion between mandibular right and left second molars - singleton sample. (Additional results)

	MRE		MLE	
MI	60	(87.00%)	82	(93.20%)
MO	9	(13.00%)	6	(6.81%)
SE	0	(0.00%)	0	(0.00%)
Total	69	(100%)	88	(100%)

chi-square value =1.7, dof = 1
P > 0.05

Table 8. Comparison of the frequency of occurrence and degree of infraoccluded mandibular right first molars between males and females - singleton sample. (Additional results)

MRD

	M		F	
MI	87	(93.50%)	66	(88.00%)
MO	4	(4.30%)	8	(10.60%)
SE	2	(2.15%)	1	(1.33%)
Total	93	(100%)	75	(100%)

chi-square value = 2.6, dof = 2
P > 0.05

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 1.6, dof = 1, P > 0.05)

Table 9. Comparison of the frequency of occurrence and degree of infraoccluded mandibular right second molars between males and females - singleton sample. (Additional results)

MRE

	M		F	
MI	32	(88.80%)	28	(84.80%)
MO	4	(11.10%)	5	(15.20%)
SE	0	(0.00%)	0	(0.00%)
Total	36	(100%)	33	(100%)

chi-square value = 0.2, dof = 1
P > 0.05

Table 10. Comparison of the frequency of occurrence and degree of infraoccluded mandibular left first molars between males and females - singleton sample. (Additional results)

MLD

	M		F	
MI	100	(90.00%)	90	(94.70%)
MO	9	(8.20%)	5	(5.26%)
SE	1	(1.00%)	0	(0.00%)
Total	110	(100%)	95	(100%)

chi-square value = 1.6, dof = 2
P > 0.05

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 1.1, dof = 1, P > 0.05)

Table 11. Comparison of the frequency of occurrence and degree of expression of infraoccluded mandibular left second molars between males and females - singleton sample. (Additional results)

MLE

	M		F	
MI	47	(92.20%)	35	(94.60%)
MO	4	(7.84%)	2	(5.40%)
SE	0	(0.00%)	0	(0.00%)
Total	51	(100%)	37	(100%)

chi-square value = 0.2, dof = 1
P > 0.05

Table 12. Comparison of the frequency of occurrence and degree of expression of infraoccluded mandibular first molar between right and left sides within individuals - male singleton sample. (Additional results)

	Males	MRD			
		MI	MO	SE	Total
MLD	MI	39	3	0	42
	MO	5	0	2	7
	SE	0	1	0	1
	Total	44	4	2	50

chi-square value = 24.6, dof = 4
P < 0.05, % concordance = 78%

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 5.8, dof = 1, P < 0.05)

Table 13. Comparison of the frequency of occurrence and degree of expression of infraoccluded mandibular second molar between right and left sides within individuals - male singleton sample. (Additional results)

	Males	MRE			
		MI	MO	SE	Total
MLE	MI	12	3	0	15
	MO	3	0	0	3
	SE	0	0	0	0
	Total	15	3	0	18

chi-square value = 0.7, dof = 1
P > 0.05, % concordance = 66.6%

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 0.7, dof = 1, P > 0.05)

Table 14. Comparison of the frequency of occurrence and degree of expression of infraoccluded mandibular first molar between right and left sides within individuals - female singleton sample. (Additional results)

	Females	MRD			
		MI	MO	SE	Total
MLD	MI	32	5	1	38
	MO	3	0	0	3
	SE	0	0	0	0
	Total	35	5	1	41

chi-square value = 0.6, dof = 2
P > 0.05, % concordance = 78.05%

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 0.6, dof = 1, P > 0.05)

Table 15. Comparison of the frequency of occurrence and degree of expression of infraoccluded mandibular second molars between right and left sides within individuals - female singleton sample. (Additional results)

	Females	MRE			
		MI	MO	SE	Total
MLE	MI	11	2	0	13
	MO	2	0	0	2
	SE	0	0	0	0
	Total	13	2	0	15

chi-square value = 0.4, dof = 1
P > 0.05, % concordance = 73.3%

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 0.4, dof = 1, P > 0.05)

The following tables relate to descriptive analysis of the twin sample presented in Chapter 6.

Table 16. Comparison of the frequency of occurrence and degree of expression of infraocclusion between primary mandibular first (D) and second (E) molars (left side) - twin sample. (Complements Table 6.15)

	MLD		MLE	
NI	177	(72.20%)	247	(82.30%)
MI	54	(22.00%)	43	(14.30%)
MO	9	(3.70%)	7	(2.30%)
SE	5	(2.00%)	3	(1.00%)
Total	245	(100%)	300	(100%)

chi-square value = 9.7, dof = 3
$P < 0.05$

(When collapsed to a 2 x 3 table, with MO and SE combined, chi-square value = 8.0, dof = 2, $P < 0.05$)

Table 17. Comparison of the frequency of occurrence and degree of expression of infraocclusion of the mandibular left first molars between males and females - twin sample. (Complements Table 6.18)

MLD

	M		F	
NI	100	(76.90%)	94	(75.20%)
MI	23	(17.70%)	24	(19.20%)
MO	5	(3.90%)	3	(2.40%)
SE	2	(1.50%)	4	93.20%)
Total	130	(100%)	125	(100%)

chi-square value = 1.27, dof = 3
$P > 0.05$

(When collapsed to a 2 x 3 table, with MO and SE combined, chi-square value = 0.2, dof = 2, $P > 0.05$)

Table 18. Comparison of the frequency of occurrence and degree of expression of infraocclusion of the mandibular left second molars between males and females - twin sample. (Complements Table 6.19)

MLE

	M		F	
NI	126	(82.90%)	117	(80.70%)
MI	19	(12.50%)	24	(16.60%)
MO	5	(3.30%)	2	(1.40%)
SE	2	(1.30%)	2	(1.40%)
Total	152	(100%)	145	(100%)

chi-square value = 2.03, dof = 3
P > 0.05

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 1.6, dof = 2, P > 0.05)

Table 19. Comparison of the frequency of occurrence and degree of infraoccluded primary mandibular first (D) and second (E) molars (right side) - twin sample. (Additional results)

	MRD		MRE	
MI	40	(70.17%)	30	(81.08%)
MO	8	(14.03%)	4	(10.81%)
SE	9	(15.78%)	3	(8.10%)
Total	57	(100%)	37	(100%)

chi-square value = 1.57, dof = 2
P > 0.05

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 0.006, dof = 1, P > 0.05)

Table 20. Comparison of the frequency of occurrence and degree of infraoccluded primary mandibular first (D) and second (E) molars (left side) - twin sample. (Additional results)

	MLD		MLE	
MI	54	(79.40%)	43	(81.10%)
MO	9	(13.20%)	7	(2.30%)
SE	5	(7.40%)	3	(1.00%)
Total	68	(100%)	53	(100%)

chi-square value = 0.14, dof = 2
P > 0.05

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 0.05, dof = 1, P > 0.05)

Table 21. Comparison of the frequency of occurrence and degree of infraocclusion between mandibular right and left first molars - twin sample. (Additional results)

	MRD		MLD	
MI	40	(70.17%)	54	(79.41%)
MO	8	(14.03%)	9	(13.23%)
SE	9	(15.78%)	5	(7.35%)
Total	57	(100%)	68	(100%)

chi-square value =2.33, dof = 2
P > 0.05

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 1.4, dof = 1, P > 0.05)

Table 22. Comparison of the frequency of occurrence and degree of infraocclusion between mandibular right and left second molars - twin sample. (Additional results)

	MRE		MLE	
MI	30	(81.08%)	43	(81.13%)
MO	4	(10.80%)	7	(13.20%)
SE	3	(8.10%)	3	(5.66%)
Total	37	(100%)	53	(100%)

chi-square value =0.3, dof = 2
P > 0.05

(When collapsed to a 2 x 2 table, with MO and SE combined, Fisher’s exact test, P > 0.05)

Table 23. Comparison of the frequency of occurrence and degree of infraoccluded mandibular right first molars between males and females - twin sample. (Additional results)

MRD

	M		F	
MI	17	(73.90%)	15	(62.50%)
MO	2	(8.60%)	6	(25.00%)
SE	4	(17.30%)	3	(12.50%)
Total	23	(100%)	24	(100%)

chi-square value = 2.2, dof = 2
P > 0.05

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 0.7, dof = 1, P > 0.05)

Table 24. Comparison of the frequency of occurrence and degree of infraoccluded mandibular right second molars between males and females - twin sample. (Additional results)

MRE

	M		F	
MI	18	(78.26%)	17	(89.47%)
MO	4	(17.39%)	2	(10.52%)
SE	1	(4.34%)	0	(0.00%)
Total	23	(100%)	19	(100%)

chi-square value = 1.32, dof = 2
P > 0.05

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 0.9, dof = 1, P > 0.05)

Table 25. Comparison of the frequency of occurrence and degree of infraoccluded mandibular left first molars between males and females - twin sample. (Additional results)

MLD

	M		F	
MI	23	(76.66%)	24	(77.42%)
MO	5	(16.66%)	3	(9.60%)
SE	2	(6.66%)	4	(12.90%)
Total	30	(100%)	31	(100%)

chi-square value = 1.17, dof = 2
P > 0.05

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 0.005, dof = 1, P > 0.05)

Table 26. Comparison of the frequency of occurrence and degree of infraoccluded mandibular left second molars between males and females - twin sample. (Additional results)

MLE

	M		F	
MI	19	(73.07%)	24	(85.71%)
MO	5	(19.23%)	2	(7.14%)
SE	2	(7.69%)	2	(7.14%)
Total	26	(100%)	28	(100%)

chi-square value = 1.8, dof = 2
P > 0.05

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 1.3, dof = 1, P > 0.05)

Table 27. Comparison of the frequency of occurrence and degree of infraoccluded mandibular first molar between right and left sides within individuals - male twin sample. (Additional results)

	Males	MRD			
		MI	MO	SE	Total
MLD	MI	12	3	1	16
	MO	2	1	0	3
	SE	0	1	2	3
	Total	14	5	3	22

chi-square value = 9.88, dof = 4
P < 0.05, % concordance = 68.2%

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 3.8, dof = 1, P < 0.05)

Table 28. Comparison of the frequency of occurrence and degree of infraoccluded mandibular second molar between right and left sides within individuals - male twin sample. (Additional results)

	Males	MRE			
		MI	MO	SE	Total
MLE	MI	6	1	0	7
	MO	2	2	0	4
	SE	1	1	0	2
	Total	9	4	0	12

chi-square value = 1.9, dof = 2
P > 0.05, % concordance = 66.6%

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 1.9, dof = 1, P > 0.05)

Table 29. Comparison of the frequency of occurrence and degree of infraoccluded mandibular first molar between right and left sides within individuals - female twin sample. (Additional results)

	Females	MRD			
		MI	MO	SE	Total
	MI	11	0	0	11
MLD	MO	2	2	0	4
	SE	1	1	3	5
	Total	14	3	3	20

chi-square value = 17.4, dof = 4
P < 0.001, % concordance = 80%

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 9.33, dof = 1, P < 0.05)

Table 30. Comparison of the frequency of occurrence and degree of infraoccluded mandibular second molars between right and left sides within individuals - female twin sample. (Additional results)

	Females	MRE			
		MI	MO	SE	Total
	MI	10	0	0	10
MLE	MO	1	0	0	1
	SE	0	1	0	1
	Total	11	1	0	12

chi-square value = 12, dof = 2
P < 0.05, % concordance = 83.3%

(When collapsed to a 2 x 2 table, with MO and SE combined, chi-square value = 5.4, dof = 1, P < 0.05)

The following tables relate to comparisons of infraocclusion among MZ and DZ twin pairs presented in Chapter 7.

Table 31. Comparison of the frequency of occurrence and degree of expression of infraocclusion in the primary mandibular left first molars among members of male MZ twin pairs. (Complements Table 7.9)

	MLD	Twin A				
		NI	MI	MO	SE	Total
Twin B	NI	28	4	0	0	32
	MI	4	5	2	0	11
	MO	0	1	1	0	2
	SE	0	0	0	0	0
	Total	32	10	3	0	45

Chi-square value = 19, dof = 4
P < 0.001, % concordance = 76%
Excluding NI % concordance = 35%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, Fisher's exact, P < 0.001).

Table 32. Comparison of the frequency of occurrence and degree of expression of infraocclusion in the primary mandibular left second molars among members of male MZ twin pairs. (Complements Table 7.10)

	MLE	Twin A				
		NI	MI	MO	SE	Total
Twin B	NI	34	4	1	0	39
	MI	2	2	0	0	4
	MO	0	2	0	0	2
	SE	0	0	0	0	0
	Total	36	8	1	0	45

Chi-square value = 13.6, dof =4
P < 0.05, % concordance = 80%
Excluding NI % concordance = 18%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, Fisher's exact, P < 0.05).

Table 33. Comparison of the frequency of occurrence and degree of expression of infraocclusion in the primary mandibular left first molars among members of male DZ twin pairs. (Complements Table 7.11)

	MLD	Twin A		
		NI	MI	Total
Twin B	NI	24	4	28
	MI	4	1	5
	Total	28	5	33

Fisher’s exact, P > 0.05
% concordance = 75.8%
Excluding NI % concordance = 11%

Table 34. Comparison of the frequency of occurrence and degree of expression of infraocclusion in the primary mandibular left second molars among members of male DZ twin pairs. (Complements Table 7.12)

	MLE	Twin A		
		NI	MI	Total
Twin B	NI	28	3	31
	MI	2	0	2
	Total	30	3	33

Fisher's exact, P > 0.05
% concordance = 84.8%
Excluding NI % concordance = 0%

Table 35. Comparison of the frequency of occurrence and degree of expression of infraocclusion in the primary mandibular left first molars among members of female MZ twin pairs. (Complements Table 7.13)

MLD		Twin A				
		NI	MI	MO	SE	Total
Twin B	NI	29	3	0	0	32
	MI	2	4	1	0	7
	MO	2	0	0	1	3
	SE	0	0	0	1	1
	Total	33	7	1	2	43

Chi-square value = 44, dof = 9
P < 0.001, % concordance = 79%
Excluding NI % concordance =36%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, Fisher's exact, P < 0.001).

Table 36. Comparison of the frequency of occurrence and degree of expression of infraocclusion in the primary mandibular left second molars among members of female MZ twin pairs. (Complements Table 7.14)

MLE		Twin A				
		NI	MI	MO	SE	Total
Twin B	NI	42	3	0	0	45
	MI	2	3	0	0	5
	MO	0	0	0	1	1
	SE	0	0	0	1	1
	Total	44	6	0	2	52

Chi-square value = 64.6, dof =6
P < 0.05, % concordance = 88.4%
Excluding NI % concordance = 40%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, Fisher's exact, P < 0.001).

Table 37. Comparison of the frequency of occurrence and degree of expression of infraocclusion in the primary mandibular left first molars among members of female DZ twin pairs. (Complements Table 7.15)

	MLD	Twin A				
		NI	MI	MO	SE	Total
Twin B	NI	21	5	1	0	27
	MI	4	0	0	1	5
	MO	0	1	0	0	1
	SE	0	0	0	0	0
	Total	25	6	1	1	33

Chi-square value = 11, dof =6
P > 0.05, % concordance=63%
Excluding NI % concordance = 0%

(When collapsed to a 2 x 2 table, with MI, MO and SE combined, Fisher's exact, P > 0.05).

Table 38. Comparison of the frequency of occurrence and degree of expression of infraocclusion in the primary mandibular left second molars among members of female DZ twin pairs. (Complements Table 7.16)

	MLE	Twin A		
		NI	MI	Total
	NI	27	4	31
Twin B	MI	1	3	4
	Total	28	7	35

Fisher's exact, P < 0.05
% concordance = 86%
Excluding NI % concordance = 37%

13.3. Appendix 3-Ethical approval

This thesis is part of a major project named **"Dentofacial variation in twins: genetics and environmental determinants"**, submitted to the Human Research Ethics Committee at the University of Adelaide, SA, Australia and approved under project number: **H-07-1984A**.