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EXPANDING THE DENTAL PHENOTYPE OF NON SYNDROMIC OROFACIAL CLEFTING

by

Brian James Howe

A thesis submitted in partial fulfillment
of the requirements for the Master of
Science degree in Oral Science
in the Graduate College of
The University of Iowa

December 2013

Thesis Supervisor: Assistant Professor Lina Moreno-Uribe

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CERTIFICATE OF APPROVAL

MASTER'S THESIS

This is to certify that the Master's thesis of

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has been approved by the Examining Committee
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The test of our progress is not whether we add more to the abundance of those who have much it is whether we provide enough for those who have little.

Franklin D. Roosevelt,
Inaugural Address
January 20, 1937

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LIST OF ABBREVIATIONS

BCL/P: Bilateral cleft lip with or without cleft palate

BCLP: Bilateral cleft lip with cleft palate

CI: Central incisor

CL/P: Cleft lip with or without cleft palate

CLP: Cleft lip with cleft palate

CP: Cleft palate along

DMFT: Decayed missing filled teeth –used for permanent teeth

dmft: decayed missing filled teeth – used for primary teeth

LI: Lateral incisor

IRB: Internal Review Board

IRCID: Iowa Registry of Congenital and Inherited Disorders

NS: Nonsyndromic

OO: Orbicularis Oris muscle

PM: Premolar

UCL/P: Unilateral cleft lip with or without cleft palate

UCLP: Unilateral cleft lip with cleft palate

UCL: Unilateral cleft lip

UCLA: Unilateral cleft lip and alveolus

CHAPTER I
INTRODUCTION

Non Syndromic Cleft Lip and/or Palate

Development

The development of the lip and palate requires the precise coordination of various cellular mechanisms including cell migration, growth, differentiation, and apoptosis which occur in a highly organized sequence of events leading to the proper formation and function of these orofacial structures. Neural crest cells migrate into the developing central face and by the 4th week of embryonic development they contribute to the formation of the frontonasal prominence, paired maxillary processes, and the paired mandibular processes. These structures surround the primitive oral cavity. The nasal placodes form by the 4th week of embryonic development and divide into the paired medial and lateral nasal processes. During the 6th and 7th weeks of embryonic development the medial nasal processes fuse to form the philthrum of what is to be the upper lip. Next the medial nasal processes and the maxillary processes fuse to from the upper lip. The lateral nasal processes become the ala of the nose (Mossey, Little, Munger, Dixon, & Shaw, 2009).

Development of the primary palate occurs during the 6th week of embryonic development when the medial nasal processes merge and fuse. The secondary palate develops during the 6th week of embryonic development where the paired palatal shelves form as an outgrowth of the maxillary processes. These processes first grow vertically down along the side of the developing tongue. During the 7th week of embryonic development the palatal shelves rise to a horizontal position, merge and fuse, from anterior to posterior, to form the midline. The secondary palate fuses with the primary

palate and the nasal septum. This process is complete by the 10th week of embryonic development (Mossey et al., 2009).

The lip and primary palate have unique developmental origins from the secondary palate; failure of these structures to develop properly can result in distinct developmental abnormalities. These abnormalities can be subdivided into: cleft lip with or without cleft palate (CL/P), and cleft palate alone (CP).

Genetic and Environmental Factors

CL/P and CP have been shown to be associated with syndromes, such as Van der Woude, comprising approximately 3-8% of clefting cases. Non Syndromic (NS) CL/P and CP make up the largest amount, 92-97% of clefting cases. With-in NS CL/P and CP there are two broad etiologic categories: genetic and environmental (Weinberg et al., 2006). The genetic contribution to NS CL/P is supported in twin studies first by those indicating the higher concordance rates among monozygotic versus dizygotic twins (Christensen & Fogh-Andersen, 1993) and then by additional studies that demonstrate an increased relative risk among offspring of both affected and unaffected twins (Grosen et al., 2010). Extensive genetics research has been undertaken to identify candidate genes influencing CL/P and CP risk. So far, several genes involved in craniofacial development have been consistently associated with orofacial clefting risk (Table 1). Muscle segment homeobox 1 (MSX1) has been identified from knockout animal models to show its involvement in NS CL/P. Jezewski found that approximately 2% of NS CL/P cases have mutations in MSX1 (Jezewski et al., 2003; Liu et al., 2005a; Satokata & Maas, 1994). Other genes or loci that have been found to play a role in CL/P are transforming growth factor alpha (TGFA) (Ardinger et al., 1989; Marazita et al., 2009), interferon regulatory factor 6 (IRF6) (Kondo et al., 2002; Marazita et al., 2009; Zucchero et al., 2004), paired box 9 (PAX9) (Marazita et al., 2009; Sasaki, O'Kane, Dixon, Dixon, & Ferguson, 2007), forkhead box E1 (FOXE1) (Beaty et al., 2013; Marazita et al., 2009; Moreno et al., 2009), 8q24 (Beaty et al., 2010; Beaty et al., 2013; Birnbaum et al., 2009; Grant et al.,

2009), paired box 7 (PAX7) (Beaty et al., 2010; Beaty et al., 2013; Bohmer et al., 2013; Pan et al., 2013), ATP binding cassette sub family A (ABCA4) (Beaty et al., 2010; Beaty et al., 2013), thyroid adenoma associated (THADA) (Beaty et al., 2013; Pan et al., 2013), ephrin receptor 3 (EPHA3) (Pan et al., 2013), DDB1 and CUL4 associated factor like 2 (DCAF4L2) (Beaty et al., 2013), ventral anterior homeobox 1 (VAX1) (Beaty et al., 2010; Mangold et al., 2010; Soria et al., 2004), sprout homolog 2 (SPRY2) (Goodnough, Brugmann, Hu, & Helms, 2007; Pan et al., 2013; Vieira et al., 2005), tropomysin 1 (TPM1) (Beaty et al., 2013; Pan et al., 2013), noggin (NOG) (Ashique, Fu, & Richman, 2002; He et al., 2010; Mangold et al., 2010), and v maf musculoaponeurotic fibrosarcoma oncogene homolog B (MAFB) (Beaty et al., 2010; Mangold et al., 2009). Summary of genes in table 1.

Environmental risk factors have been shown to increase risk for oral clefts. Maternal smoking during pregnancy has consistently been linked to an increased risk of CL/P and CP with a relative risk of 7.5% for CL/P and 5% for CP. This would mean that women who smoke during pregnancy have a 30% increased risk of giving birth to a child with CL/P and a 20% increased risk of giving birth to a child with CP. There are studies to suggest that second hand smoke is an environmental risk factor and presents gene/environmental interactions to increase the risk of giving birth to a child with CL/P (Honein et al., 2007; Jia et al., 2011; Little, Cardy, & Munger, 2004). Alcohol use during pregnancy has not been consistently shown to be associated with CL/P and CP with studies showing varying results (Bille et al., 2007; Romitti et al., 2007). Use of multivitamins before and during pregnancy has been shown to decrease the prevalence of CL/P and CP in births by 25% (Johnson & Little, 2008). The role of folic acid intake in CL/P is not certain and studies show conflicting results. Folate deficiency has been shown to cause clefts in animals,(Asling, Nelson, Doughty, Wright, & Evans, 1960) but in humans the evidence is mixed. Ulrich et.al. found that randomly assigning folic acid doses of 1.0 or 2.5mg did not result in a dose dependent difference yet another study by

the same author found that women who took folic acid supplements before the 7th week of pregnancy were found to have a significantly lower prevalence of malformations in their offspring (Ulrich et al., 1999a; Ulrich et al., 1999b). Timing of exposure to the environmental insult also seems to modulate the expression of the cleft phenotype. For instance, Ferguson examined alligator eggs and found that 98% had closure of the right nasal groove approximately one hour before the left nasal groove. By varying the time of an insult or teratogenic event it was possible to preferentially create a left or right sided cleft (Ferguson, 1981).

Epidemiology

Craniofacial anomalies make up a significant part of birth defects that occur worldwide. They require extensive care such as surgical, nutritional, dental, speech, and behavioral interventions (Murray, 2002). CL/P and CP frequency is not known in all parts of the world as not all countries collect these data and not all children are born in hospitals or in a medically supervised environments. The World Health Organization (WHO) estimates that the frequency of CL/P and CP to be 1 in 700 live births (Mossey P, 2003). CL/P and CP frequency varies based on the ethnic population. Amerindian populations have the highest frequency of approximately 1 in 500 births, with White and Asian populations in the middle at approximately 1 in 1000 births, and African populations with the lowest frequency at approximately 1 in 2500 births (Cooper, Ratay, & Marazita, 2006; Murray, 2002). CL/P and CP show gender differences as well, CL/P is most frequently seen in males at a 2 to 1 ratio (M:F) whereas CP is more frequently seen in females (Mossey P, 2002). A review by Vanderas shows that there is variability in the gender differences as well as the frequency of CL/P and CP due to sample size, ethnic population, and who was included in the study, whether live births, still births, or abortions. Given this variability, there is still generally the same ethnic and gender trend across these populations (Vanderas, 1987).

Abnormalities Found as Part of the Cleft Phenotypic Spectrum

Dental Abnormalities

Prevalence

Affected individuals with NS CL/P and CP have an increased rate of dental anomalies in the permanent dentition when compared to control populations. The types of dental anomalies noted in the literature include: exaggerated mammelons, excess mammelons, supernumerary teeth, thick curved central incisors, hypodontia, microdontia including peg laterals, T shaped lateral incisors, incisal fissures, and delayed eruption (Jordan, Kraus, & Neptune, 1966; Kraus, Jordan, & Pruzansky, 1966; Ranta, Stegars, & Rintala, 1983). Dental anomalies in deciduous teeth have also been found to be more common in subjects with clefts than in the normal age matched population. The dental anomalies range from hypodontia, supernumerary teeth, geminated, fused, and peg shaped teeth (Poyry & Ranta, 1985). Deciduous teeth also show a difference in the distribution of dental anomalies compared to permanent teeth. Anterior permanent teeth are affected four times more often than posterior permanent teeth which is in contrast to deciduous teeth where the anterior and posterior teeth are affected equally. More specifically, permanent incisor teeth are affected six times more frequently compared to permanent molars. Dental anomalies in permanent maxillary molars are five times more frequent than in permanent mandibular molars. In general, maxillary teeth have a higher frequency of dental anomalies in cleft affected subjects than mandibular teeth in both the permanent and the primary dentition, with maxillary teeth being three times more frequently affected than mandibular teeth (Kraus et al., 1966). Similar results where found by Rawashdeh and Abu Sirdaneh in cleft affected subjects with maxillary teeth being affected two times more than mandibular teeth and anterior teeth being affected three times more than posterior teeth (Rawashdeh & Abu Sirdaneh, 2009). Jordan et al. found that out of 87 noncleft subjects only 13 (14.9%) showed dental anomalies whereas

57 (54.3%) of the 105 subjects in the cleft population showed dental anomalies indicating a statistically significant difference between cases and controls. Within the cleft group there was also a greater number of dental anomalies per subject (1.29) compared to the non-cleft group (0.17). This was also highly significant (Jordan et al., 1966). Schroeder and Green found similar results in the cleft population indicating 1.02 dental anomalies per subject. This study also looked at siblings of the cleft affected subjects and found that they also have an increased frequency of dental anomalies (0.38) compared to the noncleft group (0.17) (Schroeder & Green, 1975). Kraus et al. looked at dental casts of 39 subjects with clefts and found a higher frequency of dental anomalies per subject (4.8 anomalies) (Kraus et al., 1966). Another study recently found results of 2.0 dental anomalies per cleft affected subject, slightly higher than Jordan et al. and Schroeder and Green but less than Kraus et al (Rawashdeh & Abu Sirdaneh, 2009). A more recent study comparing the frequency of dental anomalies between different types of clefts (bilateral CL/P (BCL/P), unilateral CL/P (UCL/P), unilateral CL (UCL), and CP) found that the CP group had the smallest frequency of dental anomalies in the maxillary anterior; however, there was no significant difference between the groups in the frequency of dental anomalies outside the cleft affected area (Wu, Chen, Lo, Cheng, & Ko, 2011). The evidence to date shows that subjects with orofacial clefting have more dental anomalies in permanent and deciduous teeth compared to controls. There is evidence to suggest that, compared to controls, siblings have a higher frequency of dental anomalies; however, a limited number of studies have evaluated the presence of dental anomalies in parents and siblings of cleft affected subjects, thus more studies are needed to better define these anomalies. Study results supporting prevalence of dental abnormalities are summarized in Table 2.

Hypodontia

The most commonly reported anomaly in CL/P is hypodontia. In the general population maxillary lateral incisor agenesis ranges from 0.6 to 5.2% and the maxillary

second premolar ranging from 1.1 to 1.6% (Bailit, 1975; Polder, Van't Hof, Van der Linden, & Kuijpers-Jagtman, 2004; Ranta, 1983). Eerens et al. examined hypodontia by looking at 50 NS CL/P affected subjects, 63 unaffected siblings and 250 control subjects. The control group was age and sex matched with the unaffected siblings. Of the 54 CL/P affected subjects, 15 (27.8%) showed hypodontia of one or more teeth outside the cleft area. Within the sibling group 7 (11.1%) showed hypodontia of one or more teeth. The control group showed 9 (3.6%) subjects with hypodontia of one or more teeth. The cleft affected group showed significantly more hypodontia when compared to the sibling and the control group. The sibling group alone also showed significantly more hypodontia when compared to the control group. The study found that second premolars were the most frequently missing teeth followed by the maxillary lateral incisor contralateral to the cleft (Eerens, Vlietinck, Heidbuchel et al., 2001). A previous study found similar results in regard to missing teeth when comparing the noncleft and cleft groups and the cleft group and siblings group; however, they did not find any statistically significant difference between the siblings group and the noncleft group (Schroeder & Green, 1975). Second premolars commonly fail to develop in the general population at a frequency ranging from 1.1% in the maxilla to 2.2% in the mandible. Comparing this rate to CP affected individuals, Ranta found that out of 416 children in the study, 11.1% of maxillary second premolars and 9.3% of mandibular second premolars were congenitally missing (Ranta, 1983). Jordan et al. examined dental casts of 105 cleft affected subjects and 87 control casts along with 800 noncleft human fetuses to serve as a second control group. They found that missing teeth in the area of the cleft was the most common dental anomaly, occurring in 25% of the cleft affected subjects (Jordan et al., 1966). Ranta et al. examined orthopantomograms of 251 cleft affected subjects and found that 31.5% had hypodontia, with the maxillary lateral incisor and maxillary and mandibular second premolar being the most commonly missing (Ranta et al., 1983). Agenesis alone was examined in 910 subjects with oral clefts by Hermus et al. and found that 35.1% had one

or more missing maxillary teeth and 11.5% had one or more missing mandibular teeth. Of these missing teeth the maxillary lateral incisor was the most common followed by the maxillary and mandibular second premolar respectively. They also examined the relationship of the extent of the cleft and agenesis and found that the prevalence of tooth agenesis was significantly associated with an increase in the cleft extent ranging from CL at 13.5% to CLP at 52.4% (Hermus, van Wijk, Tan, Kramer, & Ongkosuwito, 2013). However, Woolf et al. using 142 CL/P affected subjects along with 641 relatives of the affected subjects and 918 controls from the Salt Lake City, Utah area found that there was no significant difference in hypodontia of the maxillary lateral incisors between all the groups (Woolf, Woolf, & Broadbent, 1965). Research to date clearly shows that the frequency of agenesis of teeth in the cleft affected area (maxillary anterior) of cleft affected individuals is higher than in the general population. Outside the cleft affected area evidence also supports a higher frequency of agenesis, specifically the maxillary and mandibular second premolars, in cleft affected individuals. There are conflicting results in the frequency of agenesis in parents and siblings of cleft affected individuals. Further studies need to be conducted to define the frequency of agenesis in parents and siblings of cleft affected individuals when compared to a control group. Study results supporting hypodontia are summarized in Table 3.

Dental Asymmetry and Delayed Dental Development

Delayed dental development and asymmetry are not localized to the cleft defect, they can affect all teeth in both dental arches. Delayed or altered patterns of eruption in second premolars can affect individuals of the general population without hypodontia in 1.8 to 6.1% of the population and the delay in eruption ranges from 0.2 to 0.7 years. Comparing that to individuals affected with CP, the delay in eruption of second premolars is seen in 26.9-33.7% and the eruption delay ranges from 0.8 to 1.6 years. If one second premolar was delayed in eruption/development then the development of any remaining unerupted second premolars were also affected ranging from 2 to 6 years. The

severity of delayed eruption increases with the number of congenitally missing second premolars. The severity is also increased in relation to asymmetry. When one second premolar is missing the delay in the formation/eruption of the contralateral premolar is increased, and if both second premolars are congenitally missing in one jaw, then the opposing second premolars will be delayed even longer (Ranta, 1983). Deciduous teeth do not show any difference, compared to children without CL/P, in the timing of eruption (Poyry & Ranta, 1986). There are differences in timing in the eruption of permanent teeth between cleft types ranging from 2, 6.5, and 7 months for CL, unilateral CL/P, and bilateral CL/P respectively (Poyry, Nystrom, & Ranta, 1989). Another study conducted by Harris and Hullings looking at CL/P affected individuals found similar results to Ranta with a mean delay of all teeth to be 0.9 years (Harris & Hullings, 1990). Eerens et al. also examined asymmetric tooth formation and found that the cleft affected and sibling group showed significantly more asymmetric tooth development than the control group (Eerens et al., 2001).

Takahama and Aiyama studied impacted maxillary canines in parents of CL/P affected subjects as a possible microform of clefting and found that out of the 164 fathers, 3 had impacted maxillary canines. This finding was significant in comparison to the 1448 control fathers, with only 3 having impacted canines. This result was not found in the mothers (168) of CL/P affected subjects who did not show a higher frequency of impacted canines compared to control mothers (Takahama & Aiyama, 1982).

Delayed dental development/eruption in the permanent teeth of the cleft affected population is clearly increased when compared to controls and the general population. Dental asymmetry is also increased in the cleft affected population as well as their siblings when compared to control. However, more studies need to be conducted to further evaluate both delayed dental development/eruption and dental asymmetry in parents and siblings of cleft affected individuals. Currently there is not enough evidence to support any difference in delayed dental development/eruption or dental asymmetry in

deciduous teeth, further studies are needed. Evidence to date does suggest that delayed dental development/eruption and dental asymmetry may be a part of the phenotypic spectrum of CL/P but the reasoning for delayed dental development/eruption and dental asymmetry need to be further investigated as to how genetics and environment influence these dental anomalies. Study results supporting dental asymmetry and delayed dental development are summarized in Table 4.

Microdontia

Primary maxillary lateral incisors normally develop around the 16th week in utero with the crown completely developed by age 2.5. The permanent maxillary lateral incisors normally develop around 9-12 months of age with the development of the crown complete around 4-5 years of age. Incisor teeth develop from four distinct lobes, three facial lobes made up of a larger central lobe, and smaller mesial and distal lobes, and the fourth being the lingual lobe. The mesial and distal lobes are less prominent and slope from the junction of the incisal edge of the central lobe and mesial/distal lobes to the cervical portion of the clinical crown. When the mesial and distal lobes fail to develop, the cone shaped central lobe remains creating a cone or peg shaped lateral incisor instead. When the mesial or distal lobe fails to develop, only two facial lobes remain creating a smaller or malformed lateral incisor (Jordan et al., 1966; Major M. Ash, 1993). In the general population the overall prevalence of maxillary peg lateral incisors is 1.8% with left sided peg laterals being two times more common than right sided peg laterals. The occurrence rates for specific races do differ slightly from Mongoloid (3.1%), Black (1.5%), and White (1.3%). Gender differences are also apparent with women being 1.35 times more likely than men to have a maxillary peg shaped lateral incisor (Hua, He, Ngan, & Bouzid, 2013). Peg laterals were found in Jordan et al. to be the third most common dental anomaly out of the fifteen different anomalies examined. They found that in subjects with CL/P, peg laterals were seen in 11% compared to 7% of non-cleft subjects (Jordan et al., 1966). Wu et al. examined dental anomalies in 196 Chinese

children with CL, CL/P, and CP and found differences within cleft types and the frequency of peg laterals. They found that unilateral CL and alveolus had the highest frequency of peg laterals (61.3%) followed by bilateral CL/P (58%), unilateral CL/P (48.2), unilateral CL (45%), and CP (10%). The frequency of peg laterals in the CP group was found to be significantly lower when compared to the other cleft subtypes (Wu et al., 2011). Peg laterals are also seen in siblings of cleft affected subjects at an increased rate of up to 20% which is greater than the normal population of 1.8% reported by Hua et al (Hua et al., 2013; Schroeder & Green, 1975). Peg laterals are seen in the deciduous dentition of subjects affected with clefting at a prevalence of 0.5% compared to the noncleft population reported at 0.05-0.1% (Brabant, 1967; Poyry & Ranta, 1985). Tooth size reduction and tooth size asymmetry in the NS CL/P population are seen in the cleft affected area and also in the noncleft affected area. Werner and Harris found, out of 70 subjects with NS CL/P, that the average crown size reduction was 2.3% leading to a 5.2mm mean difference of tooth size across 28 teeth compared to control. If the CL/P was bilateral, the average crown size reduction was 4.2% leading to a 9.3mm mean difference of tooth size across 28 teeth compared to control (Werner & Harris, 1989). Walker et al. examined tooth size in UCLP, BCLP, and CP and found tooth sizes to be smaller in all cleft affected groups compared to control. In the UCLP group, teeth were 0.3 mm smaller in the maxilla and 0.2 mm smaller in the mandible with the maxillary lateral incisor being the smallest tooth, an average of 0.7 mm smaller than the control group. In the BCLP group teeth were, on average, smaller than control by 0.5 mm in the maxilla and 0.3 mm in the mandible. The maxillary lateral incisor was the smallest tooth in the group as well with an average of 1.2mm smaller than control. In the CP group, the teeth tended to be the smallest out of all the clefting groups, being 0.3 mm smaller in the mesiodistal dimension and 0.4 mm smaller in the buccolingual dimension compared with control (Walker, Mattick, Hobson, & Steen, 2009). Microdontia of permanent and primary maxillary lateral incisors, both contralateral and ipsilateral to the cleft, have

clearly been shown to be increased in frequency across all cleft types (CL, CL/P, CP) when compared to the frequency in the general population. Siblings of cleft affected subjects also show an increased frequency of microdontia in the maxillary lateral incisors; however, parents of cleft affected subjects have not been investigated and thus further research is needed in this area. Tooth size in cleft affected subjects is reduced across all teeth, excluding third molars, with the maxillary lateral incisor being the smallest. This reduction in tooth size is seen in all cleft types. No study to date have looked at tooth size reduction in parents and/or siblings of cleft affected subjects, more studies are needed to further define this dental anomaly. Evidence to date suggests that microdontia may be a part of the phenotypic spectrum of NS CL/P. Study results related to microdontia are summarized in Table 5.

Supernumerary Teeth

The prevalence of supernumerary teeth in the general population has been found to range from 1.2-3% with males being more affected than females (Anthonappa, King, & Rabie, 2013). Jordan et al. examined 15 dental anomaly traits in cleft affected subjects, noncleft affected subjects and cleft affected fetuses. They found that supernumerary teeth occurred at a frequency of 4.4% in the cleft affected subjects compared to 0% in the noncleft group. In the cleft affected fetuses, supernumerary teeth were tied for the third most common dental anomaly in the cleft affected fetuses with a frequency of 12.5% (Jordan et al., 1966). Supernumerary teeth can also be seen in the deciduous dentition in the general population at a frequency of 0.2 to 0.6%. In cleft affected subjects with deciduous dentition a frequency of 0.3% has been found, which is not significantly different than the normal population (Poyry & Ranta, 1985). When the cleft subtypes are broken down, UCL shows the highest frequency of supernumerary teeth at 15% followed by BCLP at 13.2%, unilateral cleft lip and alveolus (UCLA) with 9.7%, and UCLP with 4.8% all within the cleft area. Outside the cleft area UCLP was the only cleft subtype to exhibit supernumerary teeth with a frequency of 1.2%. The

frequency of supernumerary teeth within the cleft area decreased as the severity of cleft type increased (Wu et al., 2011). This general trend is also shown in Tortora et al. and Pegelow et al. Tortora et al. found that 7.3% of the 87 UCLP subjects had a supernumerary lateral incisor and 1.2% had a supernumerary central incisor. In the 29 BCLP subjects, 6.7% had supernumerary lateral incisors and 1.7% had supernumerary central incisors. They did not find any supernumerary teeth outside the clefting area (Tortora, Meazzini, Garattini, & Brusati, 2008). Pegelow et al. found slightly higher frequencies of supernumerary teeth in 129 subjects with UCLP, UCLA, and UCL. Out of the 67 UCLP subjects, 14.9% had supernumerary lateral incisors. In the UCLA group, 13.3% of the 15 subjects had supernumerary lateral incisors and in the UCL group, 21.3% of the 47 subjects had supernumerary lateral incisors (Pegelow, Alqadi, & Karsten, 2012). These studies suggest that supernumerary teeth can be seen in the permanent dentitions of cleft affected subjects at a higher frequency than the general population. Deciduous teeth, at this time, have not been shown to have an increased frequency of supernumerary teeth when compared to a control group, thus further investigation is needed. No studies to date have investigated the frequency of supernumerary teeth in parents and/or siblings of cleft affected subjects. Further research is needed to include this dental anomaly in the phenotypic spectrum of NS CL/P. Study results supporting supernumerary teeth are summarized in Table 6.

Mamelons

Permanent incisors generally have four distinct lobes, three facial and one lingual, the larger central lobe, smaller mesial and distal lobes, and a lingual lobe. Each of the three facial lobes end incisally as rounded protuberances called mamelons. Mamelons are seen on the incisal edge of maxillary and mandibular incisor teeth and are generally seen from the time of eruption and are worn off by the developing occlusion. Even after mamelons are worn by occlusal forces, there can be remnants of the lobes seen as labial/facial grooves (Major M. Ash, 1993). There are normal variations in mamelon

formation. Fitzgerald et al. found 12 variations from no mamelons to three mamelons with a smaller fourth mamelon. They also found that there is more mamelon variability in the maxillary incisor teeth, with the lateral incisor showing the most variability, when compared to mandibular incisors (Fitzgerald, Harris, Obermann, & McKnight, 1983). Jordan et al. in 1966 showed mammelons to be one of fifteen abnormalities seen out of 192 cleft and noncleft subjects. It was seen more often in the cleft affected subjects but was not significantly different from the noncleft control subjects (Jordan et al., 1966). Schroeder et al. examined dental casts of 56 cleft affected individuals, their siblings (66), and 94 control subjects and found that excessive mamelons where not seen in any of the groups; however, they found exaggerated or larger mamelons in the cleft affected subjects with none being found in the sibling or control group (Schroeder & Green, 1975). Rawashdeh and Abu Sirdaneh examined 100 cleft affected subjects and 60 controls from Jordan. They divided the cleft group into UCLP and BCLP. Excess mamelons where found to be the third most common dental abnormality with maxillary UCLP 8.8%, mandibular UCLP 8.1%, maxillary BCLP 8.45%, and mandibular BCLP 6.7% (Rawashdeh & Abu Sirdaneh, 2009). This study along with Schroeder and Green suggest that excess mamelons are more commonly seen in cleft affected subjects, but a large enough study has not been done to date to suggest that this trait is significantly different when compared to a control group. The reason for excess mamelons in the cleft affected population may be due to genetic or environmental influences. Altered occlusion in cleft affected subjects may cause the mamelons to persist due to lack of occlusal wear on the incisal edges of the mandibular and maxillary incisor teeth. The variation in mamelon morphology, in which mamelons represent the lobes that form the tooth, may give insight into how microdontia occurs. If a mamelon is missing or malformed then the lobe, which the mamelon represents, will be smaller and/or misshapen suggesting microdontia. It is unknown how genetics plays a role in the formation of excess or exaggerated mamelons in this population. Genetic studies would

be helpful in extrapolating the role genetics plays in the formation of excess mamelons in the cleft affected population. Study results supporting mamelons are summarized in Table 7.

Incisal Fissures

Incisal fissures are deep fissures or grooves on the incisal edge of the central or lateral incisors. Kraus et al. found incisal fissures to be one of 12 abnormalities found in the 39 cleft affected subjects they examined. No data on the incidence of incisal fissures was given by Kraus et al. (Kraus et al., 1966). Schroeder and Green also found incisal fissures as a dental abnormality in cleft affected subjects which accounted for 17.5% of the dental abnormalities in this group. Incisal fissures were also found in the sibling and noncleft groups with incisal fissures being the most frequent dental abnormality found in both groups, 56% and 43.8% of dental abnormalities respectively. Incisal fissures were significantly different only in the sibling group compared to the noncleft group (Schroeder & Green, 1975). Rawashdeh and Abu Sirdaneh found the frequency of incisal fissures in UCLP to be 0.99%, BCLP to be 2%, which was not significantly different than control (0%). In the lateral incisor, incisal fissures were found at a frequency of 1.4% in UCLP, and 2.8% for BLCP, again this was not significant when compared to control (0%) (Rawashdeh & Abu Sirdaneh, 2009). The origin of incisal fissures is unknown. It is possible that they are caused by exaggerated or deep mammelons that are not completely worn by usual occlusal forces. Although Kraus et al. and Schroeder and Green show that incisal fissures are present as a dental anomaly in the cleft affected population, a large enough study has not been done to date to suggest that this trait is significantly different when compared to a control group. Study results realted to incisal fissures are summarized in Table 8.

Dental Decay in the Cleft Population

Untreated dental decay has decreased since 1971 in the United States largely due to fluoridation efforts, yet it still affects a considerable number of children and adults.

The Department of Health and Human Services report on health in the United States characterized dental decay by age, sex, and race. The overall prevalence of untreated dental decay in the population ages 2-74 ranged from 15.6 to 23.7%. Breaking the prevalence down into age groups they found that overall untreated dental caries in the 2-5 year old age group was 19.3% from 1999-2002 (newer data not available), in the 6-19 year old age group:15.6% from 2007-2010, in the 20-64 year old age group: 23.7% from 2005-2008, in the 65-74 year old age group: 19.6% from 2005-2008, and in 75 year and older: 20.2% in 2005-2008. There were small differences in untreated dental caries between males and females ranging from 1.9 to 9.5% with the difference increasing with age. Larger differences exist in untreated dental caries when comparing Caucasian, Black, and Hispanic races with the Hispanics having the highest prevalence of 43.4% in the 75 year and older group and Caucasians having the lowest prevalence of 12.8% in the 6-19 year old group (National Center for Health Statistics., 2013). Children affected with CL/P generally have more dental caries compared to the overall population. Bokhout looked at 76 2.5 year olds with CL/P and 75 controls and found that 26.3% of subjects with CL/P had caries compared to 5.3% of controls. The mean decayed, filled teeth (dft) score was significantly different for the CL/P group (0.6 +/- 1.35) versus the control group (0.1 +/- 0.54) (Bokhout, Hofman, van Limbeek, Kramer, & Prahl-Andersen, 1996). A year later Bokhout et al. looked at primary teeth in 81 preschool subjects with CL/P and 77 control subjects and found that 30.9% of cleft affected subjects exhibited caries compared to 6.5% of the control group. The CL/P group also showed significantly poorer oral hygiene, and increased gingival inflammation compared to control. The incidence ratio of dental caries in the CL/P group was 9.25:1 (CL/P: controls) (Bokhout, Hofman, van Limbeek, Kramer, & Prahl-Andersen, 1997a). Turner et al. examined 89 Russian children with CL/P ages 5 to 9 years old for dft/DFT, oral hygiene, and caries rate. The mean dft was 7.31 and the mean DFT was 1.45. They classified the caries rate as follows: 0 caries means caries free, 1-3 carious teeth means low caries rate, 4-7 carious

teeth means moderate caries rate, and 8 or more carious teeth means high caries rate. Turner et al. found that 73/89 or 82% had a moderate to high caries rate. They also found that 66% had fair to poor oral hygiene (Turner, Zagirova, Frolova, Courts, & Williams, 1998). Oral health status was also looked at in 300 cleft affected children and their mothers (300) by de Castilho et al. who found that 62% of the cleft affected children displayed poor oral health, while 47% of the mothers showed poor oral health. Children who had mothers with poor oral health had a mean dmft of 6.0 which was not significantly different from children who had mothers with good oral health (mean dmft: 5.6) (de Castilho, das Neves, & de Carvalho Carrara, 2006). Hewson et al. looked at dmf/DMF in 90 Irish children with CL/P ages 18 months to 16 years old and 100 controls and found the mean dmf in cleft affected subjects to be 2.52 which was found to be significantly different than control (mean dmf: 1.99); however, the mean DMF for cleft affected and controls was not significantly different, 1.67 and 2.07 respectively (Hewson, McNamara, Foley, & Sandy, 2001). Kirchberg et al. examined 623 subjects ages 6-16 with CL/P and 47,646 controls. They used the decayed, missing, filled teeth (dmft) and Decayed, Missing, Filled Teeth (DMFT) scores for deciduous and permanent teeth respectively and found subjects in the 6-7 year old age group to have twice the caries when compared to the control group of the same age. In deciduous teeth, 70.6% of 6-8 year olds affected with CL/P had caries and needed treatment compared to 43.3% of 6-8 year olds in the control group with caries. In permanent teeth, presence of caries was significantly higher in the mixed dentition stage of cleft affected 6-12 year olds which ranged from 56 to 96% when compared to controls which ranged from 38 to 78%. The dmft and DMFT scores steadily increased in both control and CL/P affected groups from ages 6-16 (Kirchberg, Treide, & Hemprich, 2004). Chapple and Nunn looked at dmft and DMFT scores in 91 children with CL/P ages 4, 8, and 12 and found that 37% of 4 year olds had caries and 66% of 12 year olds had caries. The mean dmft score for the 4 year old group was 1.3 and the mean DMFT score for the 12 year old group was 1.8. They

also looked at enamel defects including opacities and hypoplasia. With respect to opacities, they found 56% of 4 year olds and 100% of 12 year olds affected with CL/P to have at least one opacity. Hypoplasia was not found in the primary dentition, but did affect the permanent dentition with 38% in the 8 year old group and 23% in the 12 year old group affected by CL/P (Chapple & Nunn, 2001). Al-Dajani looked at caries presence using DMFT score in 53 CL/P subjects ages 12-29 and one sibling for each of the subjects in the cleft affected group ages 12-29 as the control group and found that 85% of the cleft affected group exhibited caries compared to 45.3% of the sibling group, the difference was significant ($p > 0.05$). The mean DMFT score for the cleft affected subjects was significantly higher at 6.83 versus 3.81 for the sibling group ($p < 0.001$) (Al-Dajani, 2009). Overall, subjects with CL/P have a greater risk of having decay than the general population. The reasons for this increase are largely unknown. Cheng et al. suggests that simple preventive dental care for this population is paramount (Cheng, Moor, & Ho, 2007). Children with clefting may have poor oral hygiene habits due to poor self-motivation, lack of family support, or difficulty in cleansing rotated and/or displaced teeth. Orthodontic treatment may also increase caries risk in this populationdue to the longer duration of orthodontic treatment required tocorrect more complex malocclusions in these individuals. Such orthodontic appliances make it more difficult for children with orofacial clefts to cleanse their teeth. Scars from surgical procedures can often limit mobility of the upper lip, thus decreasing access to dental brushing. Oral bacterial loads may also be different in children with orofacial clefts due to open communication with the nasal cavity with any remaining defects in the palate acting as a reservoir for bacteria (Weiss, Weiss, Muller-Hartwich, Meier, & Jost-Brinkmann, 2005). As indicated above, poor self-esteem may also be a reason for poor oral hygiene. Interestingly, Nopoulos et al. suggested that the poor self esteem in children with clefts might be due to a decrease in size or underdevelopment of the frontal lobe of the brain, which controls social behavior , (Nopoulos et al., 2005). It is also possible that some of

the same genes responsible for clefting play a role in tooth development and thus susceptibility to dental decay. Several genes have been indentified in GWAS to show tentative relationships with dental decay and CL/P: NT5DC1 (6q22.1) (Shaffer et al., 2013; Vieira, McHenry, Daack-Hirsch, Murray, & Marazita, 2008b), TWSG1 which regulates BMP signaling (BMP knockout mice exhibit craniofacial defects) (Shaffer et al., 2013), RNF217 (6q22.31) has been associated with CL/P (Vieira et al., 2008b; Wang et al., 2013). Genes associated with CL/P and dental decay have been summarized in table 10. Study results supporting an increase in dental decay in the CL/P population are summarized in Table 9.

Orbicularis Oris Muscle Defect

The orbicularis oris muscle (OO) starts developing around the seventh week of embryonic development with OO muscle fibers present by the 12th week of embryonic development. By 16 weeks of embryonic development the OO muscle is complete (Marazita, 2007). Histologic examinations of the OO muscle in subjects with CL have shown that the muscle fibers stopped at the cleft margin and/or attempted to pass through the skin bridge. It was also noted that subjects with CL/P usually exhibited the OO muscle stopping at the cleft margin (Gundlach & Pfeifer, 1979). Martin et al. histologically examined the OO muscle of 30 apparent noncleft fetuses finding that two of the fetuses showed discontinuity of the OO muscle, one being unilateral and the second being bilateral. The term subepithelial cleft was coined for this defect in the OO muscle (Martin, Jones, & Benirschke, 1993). In a later publication, Martin et al. examined the OO muscle in 21 cleft affected subjects and their siblings and parents comparing them to 52 controls using ultrasonography and found that 40% of first degree relatives of CL/P affected subjects exhibited OO defects compared to 11% of control subjects. The difference in occurrence of OO defects between first degree relatives and controls was significant (Martin et al., 2000). Similar results were found by Neiswanger et al. in 2007 when they examined 525 noncleft affected family members of cleft affected

subject. They found that 10.3% of the family members exhibited an OO defect compared to 5.8% of control subjects exhibiting OO defects. The difference in subepithelial OO defects between control and noncleft affected family members was significant. Noncleft affected family members of cleft affected subjects were found to be two times as likely as controls to have an OO defect (subepithelial cleft). Neiswanger et al. also found sex differences as well, showing that OO defects occurred significantly more in male relatives when compared to male controls; however, CL/P incidence is higher in males which may account for the sex difference (Neiswanger et al., 2007). Mittal et al. examined 30 relatives of cleft affected subjects and found that 13.3% of relatives showed OO defects compared to zero defects found in 30 controls. This is similar to the results of Neiswanger et al. (Mittal et al., 2012). Marazita proposed the hypothesis that the poor fusion of the OO muscle leading to a subepithelial cleft might be the remnants of an attempted in utero repair of the cleft lip (Marazita, 2007). This hypothesis is supported by Lui et al who investigated BMP4 knockout mice and found that at 12 days post conception all the mice had bilateral CL, but at 14.5 days post conception only 22% still demonstrated bilateral CL. Lui et al. also hypothesizes that the CL was healed in utero (Liu et al., 2005b). Suzuki et al. further examined the subjects from Neiswanger et al. by searching for BMP4 mutations. They found that five of the 968 CL/P subjects showed BMP4 mutations versus zero of 529 controls showing BMP4 mutations. Interestingly, within the noncleft affected family group who also exhibited OO muscle defects (117) three were found to have a BMP4 mutation and the difference with controls (0/529) was significant. In these three cases of family members with BMP4 mutations all were parents of a child with CL/P. These children also exhibited the same BMP4 mutation (Neiswanger et al., 2007; Suzuki et al., 2009) supporting the hypothesis that OO defects are part of the phenotypic spectrum of NS CL/P. Study results supporting OO muscle defects are summarized in Table 11

Brain Structure

Development of the face and brain are closely related as the brain and face both develop from the prechordal region of the developing embryo. In the prechordal region the ectoderm becomes the face and the neuroectoderm becomes the brain (Van Der Plas, Conrad, Canady, Richman, & Nopoulos, 2010). Nopoulos et al. examined the brains of 50 boys and 24 girls with CL/P with age matched controls using magnetic resonance imaging (MRI) and found that CL/P subjects exhibited significantly smaller total gray matter, total white matter, total brain tissue, intracranial volume, cerebral volume, and cerebellar volume compared to controls, even after adjusting for height. The frontal lobes and cerebellum in particular were the most significantly reduced in the CL/P group. The temporal and parietal lobes showed no difference with controls, but the occipital lobe was significantly larger in CL/P subject when compared with controls. In the male CL/P subjects cerebral white matter was significantly decreased compared to male controls, thus the gray matter was relatively increased. There was no difference found in white matter or gray matter with female CL/P subjects compared to female controls (Nopoulos, Langbehn, Canady, Magnotta, & Richman, 2007). Van Der Plas et al. further examined brain structure in 33 boys with unilateral CL/P comparing them to 57 healthy boys using MRI. There were 14 right sided clefts and 19 left sided clefts. The boys with right sided CL/P were found to have significantly lower white matter volumes when compared to boys with left sided CL/P and controls. White matter volume in boys with left sided CL/P and controls were similar. However, the white matter volume in boys with right sided CL/P was uniform throughout the brain and not sided to the right or left. Intracranial volume in the boys with right sided CL/P was significantly different than control, but when boys with left sided CL/P and controls were compared is was not significant but trending ($p = 0.06$). These results suggest that there is a global difference between boys with right and left sided CL/P and brain structure (Van Der Plas et al., 2010). Weinberg et al. looked at brain morphology using three dimensional

morphometric analyses with landmarks. Magnetic resonance images were made on 31 adult males with CL/P, 14 males with CP, and 41 aged matched male controls. All three groups showed significant differences in brain shape. The CL/P and CP group showed enlargement of the anterior cerebrum and a relative reduction in the posterior cerebrum (Weinberg, Andreasen, & Nopoulos, 2009). Similar results were found in a previous study of 46 adult males with CL/P and 46 control males using magnetic resonance imaging (Nopoulos et al., 2002). All these findings suggest that anatomical changes in the brains of CL/P subjects may be a phenotype within the spectrum of NS CL/P. Study results supporting brain structural differences in CL/P are summarized in Table 12

Purpose

The purpose of the study is to expand the spectrum of dental phenotypes in children with orofacial clefts along with their parents and siblings. The motivation behind the study is to contribute knowledge to the etiology of orofacial clefting by quantifying all aspects of the dental phenotypic spectrum within orofacial clefting in the largest Multi-Center sample ever attempted. The dental phenotypes identified through this effort will allow the careful characterization of phenotypically homogenous individuals strenghthening the genotype-phenotype correlations in future genetic studies of this sample.

Table 1
Summary of Selected Genes Associated with CL/P

Gene	Locus	Phenotype	Associated Syndrome	Function	References *
ABCA4	1p22.1	CL/P	Retinopathies	Found to be associated with NS CL/P using GWAS	(Beaty et al., 2010; Beaty et al., 2013; Pan et al., 2013)
DCAF4L2	8q21.30	CL/P		Found to be associated with NS CL/P using GWAS	(Beaty et al., 2013)
EPHA4	3p11.1	CL/P		Found to be associated with NS CL/P using GWAS	(Beaty et al., 2013; Pan et al., 2013)
FOXE1	9q21	CL/P, CP	Bamforth-Lazarus syndrome	Associated with congenital hypothyroidism, thyroid agenesis, and CP. Also associated with CL/P.	(Marazita et al., 2009; Moreno et al., 2009)
IRF6	1q32	CL/P, CP, Lip pits	Van der Woude syndrome	Also found to be associated with NS CL/P. When inactivated in mice, results in CP.	(Kondo et al., 2002; Marazita et al., 2009; Zucchero et al., 2004)
MAFB	20q12	CL/P		Found to be associated with NS CL/P using GWAS	(Beaty et al., 2010; Mangold et al., 2009)
MSX1	4p16	CP, Hypodontia		When inactivated in mice, results in CL/P. Signals bone morphogenic protein (BMP).	(Jezewski et al., 2003; Liu et al., 2005a; Satokata & Maas, 1994)

Note *: Many references are available but selected original or most current appropriate references were used.

Table 1
Continued

Gene	Locus	Phenotype	Associated Syndrome	Function	References *
NOG	17q22	CL/P		Expressed in the frontonasal region along with BMP4 in birds. Helps regulate BMP in palatal region	(Ashique et al., 2002; He et al., 2010; Mangold et al., 2010)
PAX7	1p36	CL/P		Involved in craniofacial development	(Beaty et al., 2010; Beaty et al., 2013; Bohmer et al., 2013; Pan et al., 2013)
PAX9	14q13.3	CP, Hypodontia		When inactivated in mice, results in cleft of the secondary palate.	(Marazita et al., 2009; Sasaki et al., 2007)
SPRY2	13q31.1	CL/P		Plays role in outgrowth of facial prominances. Found to be associated with NS CL/P using GWAS	(Goodnough et al., 2007; Pan et al., 2013; Vieira et al., 2005)

Note *: Many references are available but selected original or most current appropriate references were used.

Table 1
Continued

Gene	Locus	Phenotype	Associated Syndrome	Function	References *
TGFA	2p13	CL/P		First reported linkage with CL/P	(Ardinger et al., 1989; Marazita et al., 2009)
TPM1	15q22.2	CL/P		Found to be associated with NS CL/P using GWAS	(Beaty et al., 2013; Pan et al., 2013)
VAX1	10q25	CL/P		Helps guide and organize neural stem cells	(Beaty et al., 2010; Mangold et al., 2010; Soria et al., 2004)
Gene Desert	8q24	CL/P		Found to be associated with NS CL/P using GWAS	(Beaty et al., 2010; Birnbaum et al., 2009; Grant et al., 2009)

Note *: Many references are available but selected original or most current appropriate references were used.

Table 2
Summary of Evidence Supporting
Prevalence of Dental Abnormalities

Study	Method of Evaluation	Study Population and Size	Results
(Jordan et al., 1966)	Dental Casts	105 CL/P 87 Controls 800 Fetus Controls	• Number of dental anomalies per subject: o CL/P: 1.29 * o Controls: 0.17 * o Cleft Fetuses: 3.0
Kraus et al., 1966	Dental Casts	39 CL/P	• Number of dental anomalies per subject: o CL/P: 4.8 • Maxillary teeth have higher frequency of dental anomalies in CL/P in permanent and primary dentitions
Rawashdeh & Abu Sirdaneh, 2009	Dental Casts	68 UCL/P 32BCL/P 60 Controls	• Number of dental anomalies per subject: o UCL/P: 2.2 o BCL/P: 1.7 o Mean for both CL/P groups: 2.0 o Controls: 0.33 • Maxillary teeth have 2x higher frequency of dental anomalies than mandibular teeth in CL/P • Anterior teeth more frequent than posterior
(Schroeder & Green, 1975)	Dental Casts and Radiographs	56 CL/P 66 Siblings 94 Controls	• Number of dental anomalies per subject: o CL/P: 1.02 * † o Siblings: 0.38 * o Controls: 0.17
Wu et al., 2011	Radiographs and Photographs	20 CL 31 Unilateral CL & alveolus (UCLA) 38 BCL/P 83 UCL/P 20 CP	• Frequency of dental anomalies in maxillary incisor region: o CP: 20% ‡ ▪ Lower than all other groups

Note: * Significant when compared to control. † Significant when compared to siblings/relatives.
‡ Significant when compared to all other groups

Table 3
Summary of Evidence Supporting Hypodontia

Study	Method of Evaluation	Study Population and Size	Results
(Eerens et al., 2001)	Radiographs	50 NS CL/P 63 Siblings 250 Controls	• 27.8% CL/P showed hypodontia * † • 11.1% Siblings showed hypodontia * • 3.6% Controls showed hypodontia • 2nd premolar (PM) most common • Maxillary Lateral Incisor (LI) 2nd most common

(Hermus et al., 2013)	Radiogrpahs	910 subjects with CL, CLP	• 35.1% had one or more missing maxillary teeth
			• 11.5% had one or more missing mandibular teeth
			• CL:
			  o 13.5% showing hypodontia
			• CLP:
			  o 52.4% showing hypodontia
			• Maxillary LI was most commonly missing
			• Maxillary 2nd PM was 2nd most common

(Jordan et al., 1966)	Dental Casts	105 CL/P 87 Controls 800 Fetus Controls	• 25% of CL/P subjects exhibited hypodontia

Letra et al., 2007	Dental Exam	500 CL/P
		500 Controls
			• Subjects with Agenesis:
			  o CL/P: 131 *
			  o Controls: 36

(Ranta, 1983)	Radiographs	416 CP	• 11.1% hypodontia of maxillary 2nd PM • 9.3% hypodontia of mandibular 2nd PM

Note: * Significant when compared to control. † Significant when compared to siblings/relatives

Table 3
Continued

Study	Method of Evaluation	Study Population and Size	Results
Ranta et al., 1983	Radiographs	251 CL/P	• 31.5% CL/P showed hypodontia • Maxillary LI was most common • Maxillary 2nd PM was 2nd most common
(Schroeder & Green, 1975)	Dental Casts and Radiographs	56 CL/P 66 Siblings 94 Controls	• 40.4% CL/P showed Hypodontia • 16% of siblings showed hypodontia • 6.25% of controls showed hypodontia
(Woolf et al., 1965)	Interviews, Review of Dental records	142 CL/P 641 Relatives 918 Controls	• 1.56% relatives showed maxillary LI hypodontia • 1.2% controls showed maxillary LI hypodontia • Suggest that LI hypodontia is not a microform of clefting

Note: * Significant when compared to control. † Significant when compared to siblings/relatives

Table 4
Summary of Evidence Supporting
Dental Asymmetry and Delayed Dental Development

Study	Method of Evaluation	Study Population and Size	Results
(Eerens et al., 2001)	Radiographs	50 NS CL/P 63 Siblings 250 Controls	• Difference in chronological age and mean dental age: o CL/P: 0.29 years o Siblings: 0.27 years o Controls: 0.47 years • Asymmetrical tooth development: o CL/P: 50% * o Siblings: 54% * o Controls: 22.8%
Harris & Hulling, 1990	Radiographs Canine to 3rd molar	54 CL/P 54 controls	• CL/P group showed asymmetry in maxillary 2nd PM teeth compared to control * • Difference in chronological age and mean dental age: o CL/P: 0.9 years • 89% of CL/P showed delayed dental age compared to chronological age
Poyry & Ranta, 1986	Radiographs	58 CL 303 CLP	• In UCLP 94% showed delayed development of deciduous teeth on the cleft side • Children under 1 years old had delayed development of 1-2wks
Poyry et al., 1989	Radiographs 2 year follow up	131 CL/P	• Delayed eruption o CL: 0.2 years o UCL/P: 0.65 years o BCL/P: 0.7 years • The difference in chronological age and mean dental age ranged from 0.16 to 0.52 years for all groups
(Ranta, 1983)	Radiographic	416 CP	• Delayed eruption of 2nd PM: 0.8 to 1.6 years • Delay seen in 26.9 to 33.7%

Note: * Significant when compared to control. † Significant when compared to siblings/relatives

Table 5
Summary of Evidence Supporting Microdontia

Study	Method of Evaluation	Study Population and Size	Results
Jordan et al.,1966	Dental Casts	105 CL/P 87 Controls 800 Fetus Controls	• Peg Lateral incisors (LI): o CL/P: 11% o Controls: 7% • Peg LI third most common dental anomaly out of 15 different anomalies examined
Letra et al., 2007	Dental Exam	500 CL/P 500 Controls	• Subjects with microdontia: o CL/P: 9 * o Control: 1
Poyry & Ranta, 1985	Radiographs & Medical records	4 CL 23 CLP 37 CP	• Prevalence of peg shaped deciduous teeth: 0.5%
Schroeder & Green, 1975	Dental Casts and Radiographs	56 CL/P 66 Siblings 94 Controls	• Prevalence of peg LI: o CL/P: 7% o Siblings: 20% o Controls: 25%
Walker et al., 2009	Dental Casts	100 UCLP 49 BCLP 63 CP 100 Controls	• Tooth size in comparison to control: o UCLP: ▪ maxilla - 0.3 mm ▪ mandible- 0.2 mm ▪ Maxillary LI (UCLP) being the smallest, 0.7 mm smaller than control o BCLP: ▪ maxilla – 0.5 mm ▪ mandible – 0.3 mm ▪ Maxillary LI (BCLP) being the smallest, 1.2 mm smaller than control o CP: ▪ maxilla – 0.3 mm ▪ mandible – 0.4 mm

Note: * Significant when compared to control. † Significant when compared to siblings/relatives

Table 5
Continued

Study	Method of Evaluation	Study Population and Size	Results
Werner & Harris, 1989	Orthodontic Clinical exam	70 CL/P 200 Controls	• Tooth size reduction in comparison to control: o CL/P: ▪ Average crown size reduction – 2.3% ▪ 5.2 mm difference in tooth size over of 28 teeth, compared to control o BCL/P: ▪ Average crown size reduction - 4.2% ▪ 9.3 mm difference in tooth size over 28 teeth, compared to control
Wu et al., 2011	Radiographs & Photographs	20 CL 31 Unilateral CL & alveolus (UCLA) 38 BCL/P 83 UCL/P 20 CP	• Frequency of peg LI o CL: 45% o UCLA: 61.3% o BCL/P: 58% o UCL/P: 48.2% o CP: 10% ‡

Note: ‡ Significant when compared to all other groups. † Significant when compared to siblings/relatives

Table 6
Summary of Evidence Supporting Supernumerary Teeth

Study	Method of Evaluation	Study Population and Size	Results
Jordan et al.,1966	Dental Casts	105 CL/P 87 Controls 800 Fetus Controls	• Frequency of supernumerary teeth: o CL/P: 4.4% o Controls: 0% o Fetuses with CL/P: 12.5%
Letra et al., 2007	Dental Exam	500 CL/P 500 Controls	• Subjects with Supernumerary teeth: o CL/P: 22 * o Controls: 1
Pegelow et al., 2011	Dental Casts and Photographs	47 CL 67 UCLP 15 UCLA	• Frequency of supernumerary LI: o CL: 21.3% o UCLP: 14.9% o UCLA: 13.3%
Poyry & Ranta, 1985	Radiographs and Medical records	4 CL 23 CLP 37 CP	• Frequency of supernumerary primary teeth o All cleft types: 0.3%
Tortora et al., 2008	Radiographs	87 UCL/P 29 BCL/P	• Frequency of supernumerary LI: o UCL/P: 7.3% o BCL/P: 6.7% • Frequency of supernumerary CI: o UCL/P: 1.2% o BCL/P: 1.7%
Wu et al., 2011	Radiographs and Photographs	20 CL 31 Unilateral CL & alveolus (UCLA) 38 BCL/P 83 UCL/P 20 CP	• Frequency of supernumerary teeth in cleft area: o CL: 15% o UCLA: 9.7% o UCL/P: 4.8% o BCL/P: 13.2% o CP: 0% • Frequency of supernumerary teeth outside cleft area: o UCL/P: 1.2% • Frequency increased as the severity of clefting decreased

Note: * Significant when compared to control. † Significant when compared to siblings/relatives

Table 7
Summary of Evidence Supporting Mamelons

Study	Method of Evaluation	Study Population and Size	Results
Jordan et al.,1966	Dental Casts	105 CL/P 87 Controls 800 Fetus Controls	• Frequency of mamelons: o CL/P: 2.2 - 5.9% o Controls: 13% o Cleft Fetuses: 0 - 4.2%
Rawashdeh & Abu Sirdaneh, 2009	Dental Casts	68 UCL/P 32 BCL/P 60 Controls	• Frequency of excess mamelons: o UCL/P: ▪ Maxillary: 8.8% ▪ Mandibular: 8.1% o BCL/P: ▪ Maxillary:8.45% ▪ Mandibular: 6.7% o Controls: 0%
Schroeder & Green, 1975	Dental Casts and Radiographs	56 CL/P 66 Siblings 94 Controls	• Excessive mamelons were not seen in any group • Exaggerated (larger) mamelons only found in the CL/P group

Note: * Significant when compared to control. † Significant when compared to siblings/relatives

Table 8
Summary of Evidence Supporting
Incisal Fissures

Study	Method of Evaluation	Study Population and Size	Results
Kraus et al., 1966	Dental Casts	39 CL/P	• One of 12 dental abnormalities found
Schroeder & Green, 1975	Dental Casts and Radiographs	56 CL/P 66 Siblings 94 Controls	• Frequency of Incisal Fissures: o CL/P: 17.5% o Siblings: 56% * o Controls: 43.8%
Rawashdeh & Abu Sirdaneh, 2009	Dental Casts	68 UCL/P 32 BCL/P 60 Controls	• Frequency of Incisal Fissures: o All cleft types: 1.3% ▪ LI: 1.9%

Note: * Significant when compared to control. † Significant when compared to siblings/relatives

Table 9
Summary of Evidence Supporting
Dental Decay in the Cleft Population

Study	Method of Evaluation	Study Population and Size	Results
Al-Dajani, 2009	Dental exam	53 CL/P 53 Siblings, unaffected, age matched	• Frequency of caries in permanent teeth: o CL/P: 85% † o Siblings: 45.3% • Mean DMFT score: o CL/P: 6.83 † o Siblings: 3.81
Bokhout et al., 1996	Dental exam	76 CL/P 75 Controls Aged 2.5 years	• Frequency of caries in primary teeth: o CL/P: 26.3% * o Control: 5.3% • Mean dft: o CL/P: 0.59 +/- 1.35 * o Control: 0.11 +/- 0.54
Bokhout et al., 1997	Dental exam	81 CL/P 77 Controls	• Frequency of caries in primary teeth: o CL/P: 30.9% * o Controls: 6.5% • CL/P had significantly: o Poorer oral hygiene * o More gingival inflammation * • Incidence rate ratio for caries in CL/P compared to controls o 9.25:1*
Chapple & Nunn, 2001	Dental exam	91 CL/P Ages 4,8,12	• Frequency of caries overall: o 4 year olds: 37% o 8 year olds: 64% o 12 year olds: 66% • Mean dmft score: o 4 year olds: 1.3 o 8 year olds: 1.8 o 12 year olds: 0.9 • Mean DMFT score: o 8 year olds: 0.4 o 12 year olds: 1.8

Note: * Significant when compared to control. † Significant when compared to siblings/relatives

Table 9
Continued

Study	Method of Evaluation	Study Population and Size	Results
Hewson et al., 2001	Dental exam	90 CL/P 100 controls	• Mean dmf score: o CL/P: 2.52 * o Control: 0.93 • Mean DMF score: o CL/P: 1.67 o Control: 2.07
Kirchberg et al., 2004	Dental exam	623 CL/P 47,646 controls	• Frequency of caries in primary teeth of 6-8 year olds: o CL/P: 70.6% * o Controls: 43.3% • Frequency of caries in permanent teeth of 6-12 year olds: o CL/P: 56-96% * o Controls: 38-78% • dmft score for 6-9 year olds: o CL/P: 3.38-5.16 * o Controls: 2.05-2.64 • DMFT score for 6-16 year olds: o CL/P: 0.21-5.61 * o Controls: 0.06-3.84 o CL/P was significant in all groups except ages 13,14,16
Turner et al., 1998	Dental exam	89 CL/P	• Mean dft score: o 7.31 • Mean DFT: o 1.45 • Oral Hygeine: o 66% had fair to poor oral hygeine

Note: * Significant when compared to control. † Significant when compared to siblings/relatives

Table 10
Summary of Selected Genes Associated with CL/P and Dental Decay

Gene	Locus	Function	Possible Role in Dental Decay	References
NT5DC1	6q22-q23	Associated with dental anomalies and CL/P	Associated with decay in mandibular anterior teeth	(Shaffer et al., 2013; Vieira, McHenry, Daack-Hirsch, Murray, & Marazita, 2008a; Wang et al., 2013)
RNF217	6q22.31	Associated with dental anomalies and CL/P	Associated with ligase activity, ubiquitin protein ligase activity, and small conjugated protein ligase activity	(Shaffer et al., 2013; Vieira et al., 2008a; Wang et al., 2013)
TWSG1	18p11	Regulates BMP signaling in mandible. BMP knockout mice display craniofacial defects	Associated with decay in mandibular anterior teeth	(MacKenzie et al., 2009; Melnick et al., 2006; Shaffer et al., 2013)

Table 11
Summary of Evidence Supporting
Orbicularis Oris Muscle Defect

Study	Method of Evaluation	Study Population and Size	Results
Liu et al., 2005b	Histologic	BMP 4 knockout mice	• 12 days post conception all mice had Bilateral CL • 14.5 days post conception, 22 % still had bilateral CL • Hypothesized that CL healed in utero
Gundlach & Pfeifer, 1979	Histologic	58 CL/P	• CL/P subjects had OO muscle fibers stop at the cleft margin • CL/P subject had OO muscle fibers attach to the ala of the nose
Martin et al., 1993	Histologic	30 non cleft fetuses	• 2/30 fetuses had OO muscle discontinuity o 1 unilateral o 1 bilateral
Martin et al., 2000	Ultrasound	21 CL/P Parents and siblings of CL/P subjects 52 controls	• Percent OO defect: o first degree relatives of CL/P subjects: 40% * o Controls: 11%
Mittal et al., 2012	Ultrasound	25 CL/P 30 unaffected relatives 30 controls	• Percent OO defect: o Unaffected relatives: 13.3% o Contols:0%
Neiswanger et al., 2007	Ultrasound	525 unaffected familymember of CL/P affected subject 527 controls	• Percent OO defect: o Unaffected family: 10% * o Controls: 5.8% • Male relatives 2x more likely to have OO defect than control
Suzuki et al., 2009	Genetics: BMP 4 mutation	968 CL/P 525 unaffected family members 529 controls	• Evaluated BMP 4 mutation in Neiswanger et al., 2007 subjects • Frequency of BMP 4 mutation: o CL/P: 5/968 o Unaffected family: 3/525 o Controls: 0/529 • Frequency of BMP 4 mutation and OO defect: o CL/P: 3 o Unaffected family: 3 o Mutations in the CL/P matched parents with BMP 4 mutation

Note: * Significant when compared to control. † Significant when compared to siblings/relatives

Table 12
Summary of Evidence Supporting
Brain Structural Differences in CL/P

Study	Method of Evaluation	Study Population and Size	Results
Nopoulous et al., 2002	Magnetic resonance imaging (MRI)	46 adult males NS CL/P 46 adult male controls	• NS CL/P: o Cerebral gray matter greater when compared to control * o Temporal and Occipital lobe showed reduction *
Nopoulous et al., 2007	MRI	74 CL/P 74 controls	• NS CL/P: o Smaller total gray matter * o Smaller total brain tissue * o Smaller cerebral volume * o Smaller cerebellar volume * o Smaller frontal lobe * o Larger occipital lobe * o Males had increased cerebral white matter * o Females showed no difference in cerebral white matter
van der Plas et al., 2010	MRI	37 boys NS CL/P 57 boys as controls	• Right sided cleft o Lower total white matter compared to left sided cleft * o White matter was uniform
Weinberg et al., 2009	MRI & 3D morphometric analysis	31 males CL/P 14 males CP 41 controls	• CL/P & CP o Enlargement of anterior cerebrum * o Reduction in posterior cerebrum

Note: * Significant when compared to control. † Significant when compared to siblings/relatives

CHAPTER II
MATERIALS AND METHODS

Subject Selection

This is a multi-center study that includes subjects recruited from The University of Iowa Children's Hospital, Iowa City, IA, through the Iowa Registry of Congenital and Inherited Disorders (IRCID); Children's Hospital of Pittsburgh, Cleft and Craniofacial Clinic, Pittsburgh PA and the University of Pittsburgh Clinical and Translational Science Institute, Pittsburgh, PA; and The University of Texas - Houston, Houston, TX. Internal review board (IRB) approval was attained at each site by the appropriate IRB process and committee. Controls were recruited through advertisement. Each subject was given a study identification number (study ID) and an individual identification number (individual ID) that was unique to each subject.

A total of 1181 subjects were recruited from all three sites, 9 subjects were removed due to syndromic status and 16 duplicates were removed (this was due to the calibration process), 25 subjects were excluded (22 from Iowa, 2 from Pittsburgh, and 1 from Texas) due to: having no teeth, being uncooperative thus unable to take photos, or unable to score photos due to poor quality. Leaving a total of 1131 subjects included in the study, 686 from Iowa, 35 from Pittsburgh, and 410 from Texas. There were 509 males and 622 females. There were 199 cleft affected subjects (case probands (PB)), 322 unaffected parents, 248 unaffected siblings, 98 affected and unaffected relatives, and 264 unaffected controls (149 control probands (PB), 44 control siblings, and 71 control parents). The rater, BJH, was blinded to sex, clefting status, if obvious clefting is not present, and status of subject such as: case proband, unaffected parent, sibling, or control proband, parent or sibling.

Inclusion criteria for case probands: subject with a nonsyndromic form of CL/P or CP. Inclusion criteria for unaffected parents, siblings and control parents and siblings: be

a first degree relative, parent or sibling, of the proband (either cleft affected or control/unaffected).

Exclusion criteria for case probands: subjects with syndromic forms of CL/P or CP. Exclusion criteria for unaffected parents, siblings, and control parents, siblings, and probands: subjects with no teeth present, with a history of clefting, genetic syndromes, facial trauma, or facial surgery.

Intraoral photos

Each site was provided a camera pack with a Cannon Rebel XSI or a Cannon 7D DSLR digital camera with a Cannon EF 100mm f/2.8 macro USM lens, a Cannon macro MR-14EX ring flash, 1 GB memory card, lithium battery pack, batteries for the ring flash, USB cable, CD's, and appropriate software for the camera to download pictures. Also provided in the pack were adult and child intraoral mirrors, plastic lip and cheek retractors, and latex gloves

Before the pictures were taken, all removable appliances, dentures, partials, retainers, are removed. Each photo was taken by two people, one to take the photo and one to place and hold the mirror and retractors. A total of six photographs were recommended: 1) study ID, 2) retractors in place, head on, 3) retractors in place, back right side of teeth 4) retractors in place, back left side of teeth, 5) retractors in place, upper arch using mirror, 6) retractors in place, lower arch using mirror. Additional photos were taken as needed to appropriately display the entire oral cavity. A picture of the study ID was taken for each subject and all the following pictures were assumed to belong to that study ID. All reusable equipment that was contaminated was cleaned with either an autoclave or cold sterilization. After the photos are taken they are downloaded with the equipment provided to appropriate software on a local computer designated for the research project. The photos for each subject were put into a folder that was named using the study ID and the unique identifier for each site, Iowa: GW, Pittsburgh: FC, Texas: TX. An example would be TX105.

Image Analysis

Each subject's photos are scored with an orofacial cleft dental phenotype part 2 intraoral photo form created in collaboration with The University of Iowa and The University of Pittsburgh (Figures 1 & 2). There is one page per quadrant starting in the upper right quadrant and proceeding to the upper left quadrant, lower left quadrant, and lower right quadrant. The information provided on the first page is: initials of evaluator, date the photos were rated, study ID, and individual ID. All subsequent pages contain the individual ID. The overall quality of the photographs was scored as either poor or good quality. This was determined by evaluating: blurring/motion, focus, retraction, mirror, and ability to visualize all teeth (third molars excluded). If the majority of photos were poor in these categories then that series of photos were marked as poor (Figure 1).

Each tooth is rated using the dental examination portion of the intraoral photo rating form (Figure 2). All appropriate teeth are marked as either primary or permanent. If the permanent tooth is erupting and remnants of the primary tooth are present, the tooth is marked as permanent and a note is made in the quadrant notes area of the form to indicate that the primary tooth is still present. Next, each tooth is marked as either "present" or "missing"; under the missing category it is marked at either "agenesis" or "other". If a tooth is missing and thought to be agenesis, specific rules are followed (Figure 3). For analysis purposes, if the primary second molar is still present and the permanent second premolar is not ectopically erupted and the subject is 14 years or older and/or the second permanent molars are erupted then agenesis will be assumed for the second premolar. If a tooth or teeth are marked as "other", specific rules are followed (Figure 4).

Decayed, filled teeth score for permanent (DFT) and deciduous (dft) teeth were determined using the present status portion of the intraoral photo rating form. Third molars were not included because of the difficulty in reliably determining presence or absence in the intraoral photos. Missing teeth for any reason were excluded due to the

inability to determine the reason for extraction. DFT and dft were dichotomized into good oral health and poor oral health. The parameters for poor oral health are: primary teeth decay ≥ 1, mixed dentition decay ≥ 2, and for permanent dentition decay ≥ 4.

DFT and dft were calculated by adding all teeth with decay or restorations of any type on the existing teeth. Each tooth can only have one indicator, decay or filled. If a tooth has decay and a restoration, only decay will be recorded. If a subject has mixed dentition, a DFT and dft score will be given for permanent and deciduous teeth respectively. To determine the percentage of DFT or dft for a subject, the number of decayed and filled teeth is divided by the number of teeth present then multiplied by 100 to attain the percentage.

Calibration

The photo rater, BJH, was calibrated against two experienced dentists, LMU and ARV. Fifteen randomly chosen subjects from the Texas subject population were used for calibration. Each subject was rated two times by each rater (BJH, LMU, ARV). Inter-rater and intra-rater reliability was calculated using *kappa* statistic with 95% confidence interval. Intra-rater reliability for BJH was 100% agreement with kappa = 0.95, LMU was 96.9% agreement with kappa = 0.88, ARV was unable to be calculated due to poor photo quality. Inter-rater reliability for BJH and LMU was 97.3% agreement with kappa = 0.93, BJH and ARV was 97.3% agreement with kappa = 0.91, LMU and ARV was 97.1% agreement with kappa = 0.92.

Statistical Methods

Descriptive analyses was completed for all aspects of the study population. Chi squared tests and Wilconxen rank sum tests were performed on the dental anomaly data, with p value indicating significance ≤ 0.017 after Bonferroni correction. For oral health status, regression analysis was performed. For analysis of oral health status, race, education, and age were taken into account and adjusted, with p value indicating significance ≤ 0.017 after Bonferroni correction.

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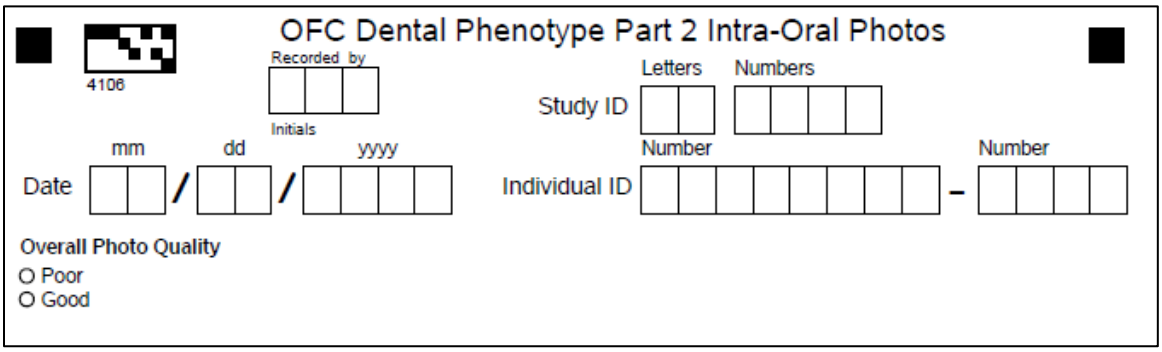
Figure 1
Intraoral Photo Rating Form
Subject Identification and Photo Quality


OFC Dental Phenotype Part 2 Intra-Oral Photos

4106

Recorded by

Initials

Study ID Letters Numbers

Date mm / dd / yyyy

Individual ID Number – Number

Overall Photo Quality

O Poor

O Good

Figure 2
Intraoral Photo Rating Form
Dental Examination

1. Dental Examination, Maxillary Teeth

Rate each tooth by marking the bubbles below. Each tooth should have an entry. Fill-in the bubble for primary or permanent tooth. Teeth can either be missing or present. If there is space or a supernumerary tooth, mark the box between the two adjacent teeth.

Quadrant 1	18 1	17 2	16 3	15 ○ 4 55 ○ A	14 ○ 5 54 ○ B	13 ○ 6 53 ○ C	12 ○ 7 52 ○ D	11 ○ 8 51 ○ E
Missing: Agenesis	○	○	○	○	○	○	○	○
Other	○	○	○	○	○	○	○	○
Present	○	○	○	○	○	○	○	○
Present Status:								
Full coverage (crown)	○	○	○	○	○	○	○	○
Partial coverage (onlay, cusp replacement, veneers)	○	○	○	○	○	○	○	○
Filling (amalgam, composite)	○	○	○	○	○	○	○	○
Gross decay	○	○	○	○	○	○	○	○
Attrition more than 2/3 of the clinical crown	○	○	○	○	○	○	○	○
If present:								
Fluorosis	○	○	○	○	○	○	○	○
Hypoplasia	○	○	○	○	○	○	○	○
Hypocalcification	○	○	○	○	○	○	○	○
Microdontia	○	○	○	○	○	○	○	○
Impacted	○	○	○	○	○	○	○	○
Rotation	○	○	○	○	○	○	○	○
Displaced	○	○	○	○	○	○	○	○
Mammalons	○	○	○	○	○	○	○	○
Incisal Fissures	○	○	○	○	○	○	○	○
Other (specify below)	○	○	○	○	○	○	○	○
Confidence: Low	○	○	○	○	○	○	○	○
High	○	○	○	○	○	○	○	○

Extra Teeth	○	○	○	○	○	○	○	○
Space Between Teeth	○	○	○	○	○	○	○	○

Quadrant 1 Notes

Note: This is a representative sample of one quadrant.

Figure 3
Rules for Determining Agenesis

Maxillary Lateral Incisor (LI):

- If subject is an adult with permanent canines erupted and lateral incisor (LI) not erupted: Mark Agenesis
    - Unless subject has multiple teeth with gross decay and/or multiple missing teeth likely due to extractions
- If subject is an adult with poor dentition (gross decay): mark Other, make note in quadrant notes section = Not Present.
- If permanent canine is erupted or ectopically eruption into LI space, and LI not present: mark Agenesis
- If obvious cleft and LI not present:
    - If 1st permanent molar(s) erupted: mark Agenesis with low confidence
    - If permanent canines erupted: mark Agenesis with high confidence

Maxillary Central Incisor (CI):

- If obvious cleft and central incisor (CI) not present:
    - If 1st permanent molar(s) is/are erupted: mark Agenesis with low confidence.
    - If permanent canines are erupted: mark Agenesis with high confidence.

Second Premolar:

- If second permanent molar is erupted, have minimal restorations, no gross decay, no history of tooth extraction given: mark Agenesis with low confidence
    - Unless subject has a history of orthodontics: mark Other, make note in quadrant notes section: mark Not Present
- If second permanent molar is erupted and subject has multiple teeth with gross decay and 2nd premolar not present: mark Not Present

Figure 4
Rules for Determining "Other"

- If permanent tooth is erupting and remnants of primary tooth is still present: mark the permanent tooth as present.
- If tooth is blurry or unable to clearly see the outline of the tooth: mark Other, make a note in quadrant notes section: Not Visible
- If teeth are not visible in photo: mark Other, make a note quadrant notes section: Not Visible
- If unable to determine the position of the tooth: mark Other, make a note in quadrant notes section: Not Visible, Unable to tell position
- If root tips are present and unable to tell position of the tooth: mark Other, make a note in quadrant notes section: Unable to tell position, root tips
- If primary teeth are present and permanent first molar and posterior have not erupted: mark Other, make a note in quadrant notes section: Not Visible
- If primary teeth present and permanent first molar erupted, but second permanent molar not erupted: mark Other, make a note quadrant notes section: Not Visible
- If permanent second molar is erupted and permanent canine not erupted: mark Other, make a note in quadrant notes section: Not Present, impacted
- If 3rd molars are not present, with no history of 3rd molar extractions: mark Other, make a note in quadrant notes section: Not Visible

CHAPTER III
RESULTS

The analysis was completed on all primary teeth (A-T) and from first molar to first molar in each arch (teeth number: 3-14, 19-30). The second and third molars were excluded from the study due to the inability to consistency visualize these teeth in the photographs. The analysis was restricted to first degree relatives, parents and siblings, of the proband (either cleft affected or control). Upon review of of 1131 subjects, 105 subjects were excluded due to not being first degree relatives or being cleft affected siblings and parents of the case probands, for a total of 1026 subjects, 199 cleft affected subjects (case probands (PB)), 322 unaffected parents, 248 unaffected siblings, and 257 unaffected controls (149 control probands (PB), 37 control siblings, and 71 control parents). The mean ages of the different groups ranged from: case probands 9, control probands 23, unaffected siblings 10, control siblings 9, unaffected parents 38, control parents 38. The breakdown of the study population and the breakdown of the cleft population are summarized in tables 13 and 14 respectively.

Anomalies

Anomalies were analyzed in two ways, first by examining how many subjects have at least one dental anomaly and second by attaining the mean number of dental anomalies per subject. In the first method, the primary dentition of the case probands have significantly more subjects with dental anomalies: 57% (114) compared to 12% (18) of control probands ($p = 5 \times 10^{-19}$). Looking specifically at each of the anomalies in the primary dentition, displaced, hypocalcification, rotation, and agenesis are significantly different than control probands. Whereas hypoplasia, incisal fissures, mamelons, and microdontia were not found to be significantly different in the primary dentition. Results are summarized in table 15. No significant differences were found between the case probands and control probands for the permanent dentition. Looking specifically at each

of the anomalies in the permanent dentition of case probands, the presence of mamelons, microdontia, and agenesis are significantly higher compared to control probands. However, rotation was seen significantly more in the control probands (89%) compared to the case probands (69%) ($p = 2 \times 10^{-5}$). There was no significant difference between case probands and control probands in hypocalcification of the permanent dentition. Displaced, hypoplasia, and incisal fissures were not significantly different than control probands. Results are summarized in table 16. In unaffected siblings, the number of subjects with at least one dental anomaly in total or by separate anomalies in primary and permanent dentitions was not significantly different than control siblings. Results are summarized in tables 17 and 18. In unaffected parents, the number of subjects with at least one dental anomaly in total or by separate anomalies in the permanent dentition was not significantly different compared to control parents. Results are summarized in table 19.

The second method of analyzing dental anomalies was done by attaining the mean number of dental anomalies per subject, this was done because many subjects have more than one dental anomalyand this was not taken into account in the first method. A limited number of dental anomalies were examined with the second method due to insufficient numbers. These include: displaced, hypocalcification, rotation, and agenesis. In the primary dentition of case probands the total number of dental anomalies per subject was significantly more (1.6 anomalies per subject) when compared to control probands (0.3 anomalies per subject) with $p < 0.0001$. The results are summarized in table 20. In the permanent dentition, case probands only showed significantly more agenesis per subject than control probands with 0.613 for case probands versus 0.1 in control probands ($p < 0.0001$). Control probands showed significantly more hypocalcification and rotation in the permanent dentition when compared to the case probands ($p < 0.003$ and $p < 0.0008$ respectively). This is similar to the findings from the first method of anomaly analysis where rotation was seen more commonly in the permanent dentition of control

probands. The dental anomaly displaced was not found to be significant, but warrents futher investigation (p=0.06). Results are summarized in table 21. No difference was found in the primary or permanent dentition between unaffected siblings and control siblings. Results summarized in tables 22 and 23. No significant difference was found in the permanent dentition between unaffected parents and control parents; however, rotation was trending (p=0.03) and warrents further investigation. Results are summarized in table 24.

A total of 15 supernumerary teeth were found in all subjects. The case proband (cleft affected) group had the most supernumerary teeth with 11 (2 mesiodens and 9 lateral incisors). All of the supernumerary teeth in this group were in the maxillary anterior (8 lateral incisors and 2 mesiodens) except one, which occurred in the mandible (lateral incisor). Unaffected family members, parents and siblings of case probands, had 3 supernumerary teeth: Unaffected parents with one (maxillary premolar), and unaffected siblings with two (one mandibular mesiodens and one mandibular premolar). There was only one control out of all subjects with a supernumerary tooth which was in the control proband group with a mandibular mesiodens. No statistical tests were completed due to the small number of supernumerary teeth. Supernumerary teeth are summarized in table 25.

Oral Health

DFT/dft was dichotomized into good and poor oral health while adjusting for age, race, and education. Before adjustment, case probands had significantly more subjects with poor oral health, 28%, when compared to control probands, 15% (p = 0.004); however, after adjusting for age, race and education there was no significant difference (p = 0.02). The resulting p value after adjustment is trending and suggests that case probands may have poorer oral health compared to control probands, futher investigation is needed. No significance was found when comparing the unaffected siblings and parents with the control siblings and parents. Results are summarizd in table 26.

Decay

No statistical test were performed on this due to the variability of DFT/dft between the Texas and Iowa populations, it was decided that for comparing DFT/dft the Iowa controls could not be used for Texas, thus only descriptive data is provided. Resulsts are summarizd in table 27.

Table 13
Breakdown of Study Population

Population	Case PB	Unaffected Parents	Unaffected Siblings	Control PB	Control Parents	Control Siblings
N	199	322	248	149	71	37
Age	9 (1-22)	23 (2-58)	10 (1-29)	9 (2-17)	38 (19-50)	38 (25-58)

Note: Age range given in ( ), mean age given above.

Table 14
Breakdown of Case Proband - Cleft Population

Cleft Affected	CL	CLP	CP
Pitt N= 6	M= 0 F= 1	M= 2 F= 0	M= 2 F= 1
IOWA N= 110	M= 17 F= 10	M= 39 F= 21	M= 12 F= 11
TEXAS N= 83	M= 4 F= 9	M= 46 F= 24	M= 0 F= 0
Total N= 199	**M= 21 F= 20 41**	**M= 87 F= 45 132**	**M= 14 F= 12 26**

Table 15
Subjects With at Least One Anomaly in Primary Dentition – Probands

Anomaly	Case Proband	Control Proband	p value
N	**199**	**149**	
Displaced	52 (26%)	3 (2%)	5E-11*
Hypocalcification	30 (15%)	8 (5%)	0.005*
Hypoplasia	7 (4%)	2 (1%)	0.31
Incisal Fissures	1 (0.5%)	0	1.00
Mamelons	0	0	NA
Microdontia	4 (2%)	0	0.14
Rotation	97 (49%)	15 (10%)	2E-15*
Agenesis	25 (13%)	0	6E-07*
Total Subjects	114 (57%)	18 (12%)	5E-19*

*Significance: $p < 0.017$ (Bonferroni correction)

Table 16
Subjects With at Least One Anomaly in Permanent Dentition – Probands

Anomaly	Case Proband	Control Proband	p value
N	**199**	**149**	
Displaced	94 (47%)	57 (38%)	0.10
Hypocalcification	89 (45%)	85 (57%)	0.03
Hypoplasia	11 (6%)	4 (3%)	0.29
Incisal Fissures	8 (4%)	4 (3%)	0.57
Mamelons	114 (57%)	31 (21%)	5E-12*
Microdontia	16 (8%)	2 (1%)	0.006*
Rotation	138 (69%)	132 (89%)	2E-05*
Agenesis	72 (36%)	7 (5%)	2e-13*
Total Subjects	171 (86%)	139 (93%)	0.04

*Significance: $p < 0.017$ (Bonferroni correction)

Table 17
Subjects With at Least One Anomaly in Primary Dentition – Siblings

Anomaly	Unaffected Sibling	Control Sibling	p value
N	**248**	**37**	
Displaced	16 (6%)	1 (3%)	0.71
Hypocalcification	21 (8%)	3 (8%)	1.00
Hypoplasia	4 (2%)	1 (3%)	0.50
Incisal Fissures	0	0	NA
Mamelons	1 (0.5%)	0	1.00
Microdontia	2 (1%)	0	1.00
Rotation	61 (25%)	12 (32%)	0.32
Agenesis	1 (0.5%)	0	1.00
Total Subjects	74 (30%)	15 (40%)	0.19

*Significance: $p < 0.017$ (Bonferroni correction)

Table 18
Subjects With at Least One Anomaly in Permanent Dentition – Siblings

Anomaly	Unaffected Sibling	Control Sibling	p value
N	**248**	**37**	
Displaced	78 (31%)	11 (30%)	1.00
Hypocalcification	123 (50%)	17 (46%)	0.73
Hypoplasia	7 (3%)	0	0.60
Incisal Fissures	24(10%)	3 (8%)	1.00
Mamelons	101 (41%)	16 (43%)	0.86
Microdontia	6 (2%)	0	1.00
Rotation	198 (80%)	29 (78%)	0.83
Agenesis	5 (2%)	0	1.00
Total Subjects	219 (88%)	29 (78%)	0.11

*Significance: $p < 0.017$ (Bonferroni correction)

Table 19
Subjects With at Least One Anomaly in Permanent Dentition – Parents

Anomaly	Unaffected Parents	Control Parents	p value
N	**322**	**71**	
Displaced	168 (52%)	38 (53%)	0.9
Hypocalcification	178 (55%)	46 (65%)	0.15
Hypoplasia	22 (7%)	3 (4%)	0.59
Incisal Fissures	10 (3%)	3 (4%)	0.71
Mamelons	25 (8%)	2 (3%)	0.19
Microdontia	4 (1%)	0	1.00
Rotation	313 (97%)	68 (96%)	0.46
Agenesis	24 (7%)	2 (3%)	0.19
Total Subjects	317 (98%)	69 (97%)	0.61

*Significance: $p < 0.017$ (Bonferroni correction)

Table 20
Mean Number of Anomalies in Prmary Dentition – Probands

	Case Probands		Control Probands		
Anomaly	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**	**p value**
Displaced	0.4	0.8	0	0.2	<0.0001*
Hypocalcification	0.4	1.1	00.1	0.4	0.0036*
Rotation	1.6	2.1	00.2	0.8	<0.0001*
Agenesis	0.170	0.523	0	0	<0.0001*
Total	1.6	3.2	0.3	1.1	<0.0001*

*Significance: p < 0.017 (Bonferroni correction)

Table 21
Mean Number of Anomalies in Permanent Dentition – Probands

	Case Probands		Control Probands		
Anomaly	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**	**p value**
Displaced	1.1	1.7	0.8	1.4	0.06
Hypocalcification	2.4	4.2	3.8	5.1	0.003*
Rotation	3.4	3.4	4.3	2.7	0.0008*
Agenesis	0.613	0.993	0.1	0.3	<0.0001*
Total	8.8	7.5	8.9	6.2	0.55

*Significance: p < 0.017 (Bonferroni correction)

Table 22
Mean Number of Anomalies in Primary Dentition – Siblings

	Unaffected Siblings		Control Siblings		
Anomaly	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**	**p value**
Displaced	0.1	0.30	0.1	0.30	0.39
Hypocalcification	0.3	1.3	0.2	0.6	0.93
Rotation	0.6	1.4	0.6	1.0	0.46
Agenesis	0	0.1	0	0	0.71
Total	1.0	2.2	0.8	1.1	0.42

*Significance: $p < 0.017$ (Bonferroni correction)

Table 23
Mean Number of Anomalies in Permanent Dentition – Siblings

	Unaffected Siblings		Control Siblings		
Anomaly	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**	**p value**
Displaced	0.7	1.4	0.6	1.3	0.77
Hypocalcification	2.8	4.6	2.2	4.1	0.51
Rotation	4.1	3.2	3.7	2.9	0.60
Agenesis	0	0.2	0	0	0.39
Total	8.6	6.5	7.4	5.9	0.26

*Significance: $p < 0.017$ (Bonferroni correction)

Table 24
Mean Number of Anomalies in Permanent Dentition – Parents

	Unaffected Parents		Control Parents		
Anomaly	**Mean**	**Standard Deviation**	**Mean**	**Standard Deviation**	**p value**
Displaced	1.2	1.6	1.1	1.3	0.71
Hypocalcification	3.7	5.1	3.4	4.4	0.61
Rotation	5.8	2.9	5.0	2.6	0.03
Agenesis	0.1	0.6	0	0.2	0.15
Total	9.9	5.9	8.5	4.9	0.10

*Significance: $p < 0.017$ (Bonferroni correction)

Table 25
Supernumerary Teeth

	Case PB	Control PB	Unaffected Parents	Control Parents	Unaffected Siblings	Control Siblings
N	**198**	**149**	**322**	**71**	**248**	**37**
Mesiodens	2	0	0	0	1	0
Lateral Incisor	9	0	0	0	0	0
Premolar	0	1	1	0	1	0
Total Supernumerary Teeth	11	1	1	0	2	0

Note: PB= proband

Table 26
Oral Health Status

	Proband		Siblings		Parents	
	Case	Control	Unaffected	Control	Unaffected	Control
N	198	149	248	37	322	71
Poor Oral Helath	55	22	58	5	134	37
Poor Oral Health Percent	28%	15%	23%	14%	42%	37%
Unadjusted p value	0.004 *		0.40		0.51	
Adjusted p value †	0.02		0.40		0.46	

† p value adjusted for age, race, and education
*Significance: $p < 0.017$ (Bonferroni correction)

Table 27
Percent of Decayed Filled Teeth
in the Primary and Permanent Dentition

	Probands		Siblings		Parents	
	Case	Control	Unaffected	Control	Unaffected	Control
N	198	149	248	37	322	71
Primary						
Maxillary	28	5	23	9	0	0
Mandible	12	4	14	9	0	0
Total	40	9	37	18	0	0
Permanent						
Maxillary	6	16	5	2	37	28
Mandible	2	6	3	2	13	13
Total	8	22	8	4	50	41

Note: All numbers under Primary and Permanent are given as percentages

CHAPTER IV
DISCUSSION

The use of intraoral photographs to determine dental anomalies is a method to evaluate dental phenotypes and gross decay in the clefting population that to our knowledge, has never been used before. Most previous studies have used dental records, radiographs, dental casts, or a clinical exam to determine dental anomalies. Drawbacks to the current method include limited ability to visualize all aspects of the teeth due to poor photo quality, and not having radiographs limits the ability to confirm agenesis and impaction of teeth. However, rules were developed to justify our calls, based on the probability, that a tooth was impacted or did not develop (agenesis) (Figure 3 and 4). Other drawbacks of our study were that there was only one control population (Iowa). For decay, this was a concern because dental decay can be influenced by many factors such as: age, diet, education, socioeconomic status (SES), and race. In the Texas population, specifically case probands, the DFT was approximately 4 times more than the Iowa case probands suggesting that these two populations are different in terms of decay and filled history. In the Iowa population, 94% where white non Hispanic and approximately 2% were white Hispanic. This is in contrast to the Texas population were 66% are classified at white Hispanic and 20% are white non Hispanic. This is why, in the analysis, we dichotomized the DFT/dft status into good and poor oral health, adjusting for age, education (used as a proxy for SES), and race. For dental anomalies this is not a concern as they are developmental in origin and are not dependent on age or other factors that influence decay.

The mean number of dental anomalies in our study in the primary and permanent dentition for case probands were 1.6 and 8.8 respectively and in the control probands 0.3 and 8.9 respectivley. The mean number of dental anomlies in the primary dentition of case probands more closely resembles what other studies have found (1.02 – 2.2) (Jordan

et al., 1966; Rawashdeh & Abu Sirdaneh, 2009; Schroeder & Green, 1975) compared to the permanent dentition. The mean number of dental anomalies in our study in the permanent dentition may be higher due to rotation, displacement, and hypocalcification being more common in the adult population. They may be caused by other factors such as eruption of third molars or other occlusal factors causing rotation and/or displacement and trauma or illness unrelated to the cleft, yet associated with hypocalcification. With respect to hypocalcification, because no clinical exam was done, it is possible that a portion of the hypocalcification could be white spot lesions related to decay which would cause an increase in the number of dental anomalies in this category. The mean number of dental anomalies in the primary dentition of control probands (0.3) was similar to control groups from previous studies (0.14 – 0.33). The mean number of dental anomalies in case and control probands may be similar to the other studies because the previous studies primarily examined younger individuals with similar age ranges. In the permanent dentition of control siblings and parents the mean number of dental anomalies was 7.4 and 8.5 respectively. In Schroeder and Green who looked at siblings, they found that the average number of dental anomalies was 0.38 which is much smaller than what our study found in siblings (7.4). The previous studies did not separate the average number of dental anomalies into primary and permanent dentitions but combined them (Jordan et al., 1966; Rawashdeh & Abu Sirdaneh, 2009; Schroeder & Green, 1975). This suggests that age and thus the risk of getting dental anomalies like rotation, displacement, and hypocalcification over time may affect what and how we categorize dental anomalies in permanent teeth.

In the primary and permanent dentitions of case probands (cleft affected) both analyses of dental anomalies showed significnalty more dental anomalies in the primary dentition with no significance in the permanent dentition when compared to control probands, which is different than other studies (Jordan et al., 1966; Rawashdeh & Abu Sirdaneh, 2009; Schroeder & Green, 1975). The studies in table 2, that have controls,

show that cleft affected subjects have more dental anomalies in permanent teeth than controls. In the currernt study, primary teeth of case probands showed dental anomalies: displaced, hypocalcification, rotation, and agenesis to be significantly different when compared to controls. When looking at specific dental anomalies from previous studies in tables 3-8 and comparing them to the current results, no previous studies examined primary teeth individually from permanent teeth, they were combined. Permanent teeth of case probands showed the dental anomalies: microdontia and agenesis to be significant when compared to control probands which is similar to other studies (Eerens, Vlietinck, Heidbrüchel et al., 2001; Jordan et al., 1966; Letra, Menezes, Granjeiro, & Vieira, 2007; Rawashdeh & Abu Sirdaneh, 2009; Schroeder & Green, 1975). The current study suggests that the primary dentition may have a different distribution of dental anomalies when compared to permanent dentition, with the exception of agenesis.

In the analysis of subjects with at least one dental anomaly, rotation in the permanent dentition was more common in the control probands when compared to case probands, with hypocalcification trending (0.03). Similarily, in the analysis of the mean number of dental anomalies per subject, roatation and hypocalcification in the permanent dentition of control probands was more common than case probands. Hypocalcification has not previsouly been extensively examined and rotation has not been looked at individually. Rotated and displaced teeth have not often been examined. Letra et al. found different results, showing that malposition was significantly more common in the cleft affected population when compared to controls; however, they combined rotation and displacement into one category, malposition (Letra et al., 2007). Rotation and displacement in the primary dentition being significant in our study is likely due to the cleft and displacement of the primary palate or from the corrective surgical procedure in the area of the developing tooth buds. The primary tooth buds and possibly some permanent tooth germ may be disrupted, moved, or obliterated during the corrective procedure resulting in rotation, displacement or other dental anomalies.

Hypoplasia was not found to be significant in either the primary or permanent dentitions of case probands,unaffected siblings or parents. Hypoplasia has not been extensively examined previsouly. Our study found that 6% of case probands exhibited hypoplasia in the permanent dentition, this is much smaller than what Chapple and Nunn found at 38% of cleft affected 8 year olds and 23% of cleft affected 12 year olds (Chapple & Nunn, 2001). One reason that the percentages may be lower in the current study may be due to the lack of visibility in the photographs of all hypoplastic areas on the teeth. Hypoplasia can result from insults during tooth development which can be genetic in origin or possibly from the corrective surgical procedures to repair an orofacial cleft. Futher investigation is needed with a larger sample size to help determine if hypoplasia is indeed a dental phenotype of orofacial clefting.

Agenesis was seen significantly more in the primary (13%) and permanent (36%) dentitions of case probands when compared to control probands (0% and 5% respectively). Comparing these findings to the general population, the control probands have similar frequencies of agenesis to that in the general population (0.6 – 5.2%) but the case probands have a much higher incidence of agenesis than the general population (Bailit, 1975; Polder et al., 2004; Ranta et al., 1983). The 36% of case probands with agenesis in the permanent teeth of the current study is similar to Eerens et al. who found that 27.8% of CL/P affected subjects showed hypodontia/agenesis, Jordan et al. with 25%, Hermus et al. with 35.1% in the maxilla, Ranta et al. with 31.5%, and Schroeder and Green with 40.4% (Eerens et al., 2001; Hermus et al., 2013; Jordan et al., 1966; Ranta et al., 1983; Schroeder & Green, 1975). Previous studies have only looked at permanent teeth and no reports were found specifically for agenesis of primary teeth. No control siblings or parents exhibited agenesis while 0.5% and 2% of unaffected siblings in primary and permanent dentitions respectively exhibited agenesis and 2% of unaffected parents exhibited agenesis in the permanent teeth. These results are similar to the frequency of agenesis in the general population, but different from what Eerens et al.

(11.1%) and Schroeder and Green (16%) found in siblings who showed agenesis (Eerens et al., 2001; Schroeder & Green, 1975). Findings of this study are consistent with previous studies on agenesis in permanent teeth of cleft affected subjects. The current findings also support that agenesis occurs more commonly in the primary teeth of cleft affected subjects giving further support for agenesis as a dental phenotype in orofacial clefting

Mamelons were found to be significantly more common in the permanent teeth of case probands compared to controls. This is not consistent with other studies that did not find mamelons to be significant in the permanent dentition when compared to controls (Jordan et al., 1966; Rawashdeh & Abu Sirdaneh, 2009; Schroeder & Green, 1975). Although, previous studies looked specifically at excess and/or exaggerated mamelons, no studies that were found examined mamelons alone. In the current study mamelons were not subdivided into excess and exaggerated mamelons, thus showing that mamelons, overall, occur more commonly in the cleft population when compared to control. This is likely due to malocclusion leading to the lack of normal occlusal forces which would wear down and remove or minimize mamelons. However, it is also possible that the mamelons, which represent the lobes of the developing incisor teeth, may be seen more in cleft affected subjects because they are part of a modified developmental process. Genetic studies would need to be done to help isolate the genes that may modify the gowth of lobes and thus mamelons. Incisal fissures were not found to be significant in the cleft affected, sibling, or the parent group. Incisal fissures were seen in 4% of permanent teeth in the case probands, which is slightly higher than Rawashdeh and Abu Sirdaneh at 1.3% and lower than Schroeder and Green at 17.5% (Rawashdeh & Abu Sirdaneh, 2009; Schroeder & Green, 1975). Incisal fissures were found to be significant by Schroeder and Green in the siblings of cleft affected subjects and not in the cleft affected (proband) group (Schroeder & Green, 1975). However, it is of note in our study that in the sibling group 24 unaffected silbings had incisal fissures

compared to only 3 subjects in the control siblings group. This was not significant, but it warrents further investigation with a larger study population. It is possible that some incisal fissures are truly exaggerated mamelons that were too deep to be completely worn by occlusion and thus is not a seperate anomaly and belong in the mamelon category. It may be that incisal fissures are more rare and our sample size was too small to pick up a difference.

Microdontia in the primary dentition of case probands of our current study was 2%, which is higher than the general population at 0.05-0.1% (Brabant, 1967; Poyry & Ranta, 1985). In the permanent dentition of the case probands in the current study, microdontia occurred at a frequency of 8%, which is higher than the general population at a frequency of 1.8% for permanent lateral incisors but similar to 11% of Jordan et al. and 7% of Schroeder and Green but much smaller than the results of Wu et al (45-61.3%) (Hua et al., 2013; Jordan et al., 1966; Schroeder & Green, 1975; Wu et al., 2011). Microdontia occurred at a frequency of 1% to 2% in primary and permanent dentitions respectively of unaffected siblings, and 1% of permanent teeth of unaffected parents which is similar to the general population in permanent teeth but higher than the general population in the primary teeth. Microdontia was not seen in the control parents for the permanent dentition or for the control siblings for both dentitions. See table 5 for results from previous studies and tables 15-19 for current results. Current results lend further support to microdontia as a dental phenotype in orofacial clefting in the permanent dentition. Futher studies are needed to see if microdontia in the primary dentition is indeed a dental phenotype of orofacial clefting.

Supernumerary teeth of case probands in the current study occurred at a frequency of 5% which is higher than the frequency reported for the general population of 1.2-3% (Anthonappa et al., 2013) but similar to Jordan et al. which reported a frequency of 4.4% in the CL/P population. Breaking down the supernumerary teeth into lateral incisors and cental incisors, the current study found that the lateral incisor had a frequency of 4.5%

and the central incsisor at 1.0% which is slightly lower, but similar to what Tortora et al. found and much lower than what Pegelow et al. and Wu et al. found (see table 6). No supernumerary teeth were found in control siblings or parents which is similar to what Jordan et al. found in controls (Jordan et al., 1966; Pegelow et al., 2012; Tortora et al., 2008; Wu et al., 2011). The current study suggests that supernumerary teeth may be a dental phenotype in oralfacial clefting but a larger sample size is needed to draw definitive conclusions.

The poor oral health status of the case probands compared to the control probands of the current study is consistant with previous studies (Bokhout, Hofman, van Limbeek, Kramer, & Prahl-Andersen, 1997b; de Castilho et al., 2006; Turner et al., 1998) which found that subjects with CL/P have significantly poorer oral hygiene and increased incidence of decay. In the current study, we found that 28% of case probands had poor oral health, which is lower than the 66% found in Turner et al. and 62% found in de Castilho et al. This may be due to the variable definition of poor oral health which can osilate from any need of dental treatment to scoring gingival bleeding, using a plaque indexes, or caries risk assesment. A drawback of our method of evaluation is that the oral hygiene status can not be evaluated using clinical examination methods such as gingival bleeding and a plaque index. Also no information was collected to evauate caries risk which can be included in the overall assessment for oral health status. In terms of decayed, filled teeth (dft/DFT) for the primary dentition, 40% of case probands had at least one decayed, filled tooth compared to 9% of control probands. This is slightly higher than the two studies by Bokhout et al. which found 26.3% and 30.9% for primary teeth but similar to and slightly lower than those from Chapple and Nunn (37-66%) and lower than Kirchberg (70.6%). The frequency of decay in the permanent teeth of case probands was much lower in our study when compared to Al-Dajani at 85% and Kirchberg at 56-96% (Al-Dajani, 2009; Bokhout et al., 1996; Bokhout, Hofman, van Limbeek, Kramer, & Prahl-Andersen, 1997c; Chapple & Nunn, 2001; Kirchberg et al.,

2004). Previous studies can be found in table 9 and descriptive results from the current study are summarized in table 27. Poor oral health is increased in the clefting population, even given the variability in evaluating oral health from previous studies, they all consistently found poorer oral health in this population. The current study suggests that the clefting population has poorer oral health with a p value of 0.02 (after adjusting for age, race, and education). A larger sample size is needed to further solidify poor oral health as a phenotype of orofacial clefting.

In conclusion, our results provide further evidence that individuals with clefts show distrubances and/or modified development of the primary and permanent dentitions and that several dental anomalies within this group are significantly more common than controls (displaced, hypocalcification, mamelons, microdontia, rotation, and agenesis). These dental anomalies that were found in the case probands may be seen in unaffected siblings and/or parents with a larger sample size. Previous studies have combined the number of dental anomalies in the primary and permanent dentitions, very few studies have separated primary and permanent dentitions. However, in the current study we were able to separate the dentitions and it suggests that the dental anomalies affecting the primary dentition (displaced, hypocalcification, and rotation) may be different than those affecting the permanent dentitions (mamelons and microdontia) of cleft affected subjects warrenting further inquiry. The evidence provided also helps to support mamelons as a dental phenotype in the permanent dentition of cleft affected subjects as well as lend further support to the dental anomaly agenesis in the primary and permanent dentitions and microdontia in the permanent dentition as phenotypes of orofacial clefting. Dental pheonotypes in the primary and permanent dentitions of unaffected siblings and parents were not found to be significant when compared to controls, however, with a larger sample size differences may be revealed. Few studies have examined siblings and/or parents of cleft affected subjects and found significant differences. In the current study, unaffected siblings in both the primary and permanent dentitions, the total number of

subjects with at least one dental anomaly compared to control siblings ($p = 0.19$ and 0.11 respectively) was not significant at this time, yet it deserves further investigation. In unaffected parents in the permanent dentition, the mean number of dental anomalies in total also shows promise ($p = 0.10$) as well as rotation ($p = 0.03$). With the previous studies, it is possible that the small sample size did not lend it self to a broad representation of all subjects from cleft affected, siblings, or controls. This may also be of concern for the current study were the number of controls was limited only to the Iowa population. With an overall larger sample size, differences may be found between unaffected siblings and/or parents and controls. A better understanding of dental anomalies in cleft affected subjects is provided with this research, but more research is needed to expand the knowledge of dental phenotypes and solidify their extent in orofacial clefting.

REFERENCES

Al-Dajani, M. (2009). Comparison of dental caries prevalence in patients with cleft lip and/or palate and their sibling controls. *The Cleft Palate-Craniofacial Journal : Official Publication of the American Cleft Palate-Craniofacial Association, 46*(5), 529-531. doi:10.1597/08-003.1; 10.1597/08-003.1

Anthonappa, R. P., King, N. M., & Rabie, A. B. (2013). Prevalence of supernumerary teeth based on panoramic radiographs revisited. *Pediatric Dentistry, 35*(3), 257-261.

Ardinger, H. H., Buetow, K. H., Bell, G. I., Bardach, J., VanDemark, D. R., & Murray, J. C. (1989). Association of genetic variation of the transforming growth factor-alpha gene with cleft lip and palate. *American Journal of Human Genetics, 45*(3), 348-353.

Ashique, A. M., Fu, K., & Richman, J. M. (2002). Endogenous bone morphogenetic proteins regulate outgrowth and epithelial survival during avian lip fusion. *Development (Cambridge, England), 129*(19), 4647-4660.

Asling, C. W., Nelson, M. M., Doughty, H. D., Wright, H. V., & Evans, H. M. (1960). The development of cleft palate resulting from maternal pteroylglutamic (folic) acid deficiency during the latter half of gestation in rats. *Surgery, Gynecology & Obstetrics, 111*, 19-28.

Bailit, H. L. (1975). Dental variation among populations. an anthropologic view. *Dental Clinics of North America, 19*(1), 125-139.

Beaty, T. H., Murray, J. C., Marazita, M. L., Munger, R. G., Ruczinski, I., Hetmanski, J. B., . . . Scott, A. F. (2010). A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4. *Nature Genetics, 42*(6), 525-529. doi:10.1038/ng.580; 10.1038/ng.580

Beaty, T. H., Taub, M. A., Scott, A. F., Murray, J. C., Marazita, M. L., Schwender, H., . . . Ruczinski, I. (2013). Confirming genes influencing risk to cleft lip with/without cleft palate in a case-parent trio study. *Human Genetics, 132*(7), 771-781. doi:10.1007/s00439-013-1283-6; 10.1007/s00439-013-1283-6

Bille, C., Olsen, J., Vach, W., Knudsen, V. K., Olsen, S. F., Rasmussen, K., . . . Christensen, K. (2007). Oral clefts and life style factors--a case-cohort study based on prospective danish data. *European Journal of Epidemiology, 22*(3), 173-181. doi:10.1007/s10654-006-9099-5

Birnbaum, S., Ludwig, K. U., Reutter, H., Herms, S., Steffens, M., Rubini, M., . . . Mangold, E. (2009). Key susceptibility locus for nonsyndromic cleft lip with or without cleft palate on chromosome 8q24. *Nature Genetics, 41*(4), 473-477. doi:10.1038/ng.333; 10.1038/ng.333

Bohmer, A. C., Mangold, E., Tessmann, P., Mossey, P. A., Steegers-Theunissen, R. P., Lindemans, J., . . . Ludwig, K. U. (2013). Analysis of susceptibility loci for nonsyndromic orofacial clefting in a european trio sample. *American Journal of Medical Genetics.Part A, 161*(10), 2545-2549. doi:10.1002/ajmg.a.36141; 10.1002/ajmg.a.36141

Bokhout, B., Hofman, F. X., van Limbeek, J., Kramer, G. J., & Prahl-Andersen, B. (1996). Increased caries prevalence in 2.5-year-old children with cleft lip and/or palate. *European Journal of Oral Sciences, 104*(5-6), 518-522.

Bokhout, B., Hofman, F. X., van Limbeek, J., Kramer, G. J., & Prahl-Andersen, B. (1997a). Incidence of dental caries in the primary dentition in children with a cleft lip and/or palate. *Caries Research, 31*(1), 8-12.

Bokhout, B., Hofman, F. X., van Limbeek, J., Kramer, G. J., & Prahl-Andersen, B. (1997b). Incidence of dental caries in the primary dentition in children with a cleft lip and/or palate. *Caries Research, 31*(1), 8-12.

Bokhout, B., Hofman, F. X., van Limbeek, J., Kramer, G. J., & Prahl-Andersen, B. (1997c). Incidence of dental caries in the primary dentition in children with a cleft lip and/or palate. *Caries Research, 31*(1), 8-12.

Brabant, H. (1967). Comparison of the characteristics and anomalies of the deciduous and the permanent dentition. *Journal of Dental Research, 46*(5), 897-902.

Chapple, J. R., & Nunn, J. H. (2001). The oral health of children with clefts of the lip, palate, or both. *The Cleft Palate-Craniofacial Journal : Official Publication of the American Cleft Palate-Craniofacial Association, 38*(5), 525-528. doi:2

Cheng, L. L., Moor, S. L., & Ho, C. T. (2007). Predisposing factors to dental caries in children with cleft lip and palate: A review and strategies for early prevention. *The Cleft Palate-Craniofacial Journal : Official Publication of the American Cleft Palate-Craniofacial Association, 44*(1), 67-72. doi:10.1597/05-112

Christensen, K., & Fogh-Andersen, P. (1993). Cleft lip (+/- cleft palate) in danish twins, 1970-1990. *American Journal of Medical Genetics, 47*(6), 910-916. doi:10.1002/ajmg.1320470620

Cooper, M. E., Ratay, J. S., & Marazita, M. L. (2006). Asian oral-facial cleft birth prevalence. *The Cleft Palate-Craniofacial Journal : Official Publication of the American Cleft Palate-Craniofacial Association, 43*(5), 580-589. doi:10.1597/05-167

de Castilho, A. R., das Neves, L. T., & de Carvalho Carrara, C. F. (2006). Evaluation of oral health knowledge and oral health status in mothers and their children with cleft lip and palate. *The Cleft Palate-Craniofacial Journal : Official Publication of the American Cleft Palate-Craniofacial Association, 43*(6), 726-730. doi:10.1597/04-205

Eerens, K., Vlietinck, R., Heidbrüchel, K., Van Olmen, A., Derom, C., Willems, G., & Carels, C. (2001). Hypodontia and tooth formation in groups of children with cleft, siblings without cleft, and nonrelated controls. *Cleft Palate-Craniofacial Journal, 38*(4), 374-378.

Eerens, K., Vlietinck, R., Heidbuchel, K., Van Olmen, A., Derom, C., Willems, G., & Carels, C. (2001). Hypodontia and tooth formation in groups of children with cleft, siblings without cleft, and nonrelated controls. *The Cleft Palate-Craniofacial Journal : Official Publication of the American Cleft Palate-Craniofacial Association, 38*(4), 374-378. doi:2

Ferguson, M. W. (1981). Review: The value of the american alligator (alligator mississippiensis) as a model for research in craniofacial development. *Journal of Craniofacial Genetics and Developmental Biology, 1*(1), 123-144.

Fitzgerald, L. R., Harris, E. F., Obermann, K., & McKnight, J. T. (1983). Incisor mamelon morphology: Diagnostic indicators of abnormal development. *Journal of the American Dental Association (1939), 107*(1), 63-66.

Goodnough, L. H., Brugmann, S. A., Hu, D., & Helms, J. A. (2007). Stage-dependent craniofacial defects resulting from Sprouty2 overexpression. *Developmental Dynamics : An Official Publication of the American Association of Anatomists, 236*(7), 1918-1928. doi:10.1002/dvdy.21195

Grant, S. F., Wang, K., Zhang, H., Glaberson, W., Annaiah, K., Kim, C. E., . . . Hakonarson, H. (2009). A genome-wide association study identifies a locus for nonsyndromic cleft lip with or without cleft palate on 8q24. *The Journal of Pediatrics, 155*(6), 909-913. doi:10.1016/j.jpeds.2009.06.020; 10.1016/j.jpeds.2009.06.020

Grosen, D., Bille, C., Pedersen, J. K., Skytthe, A., Murray, J. C., & Christensen, K. (2010). Recurrence risk for offspring of twins discordant for oral cleft: A population-based cohort study of the danish 1936-2004 cleft twin cohort. *American Journal of Medical Genetics, Part A, 152 A*(10), 2468-2474.

Gundlach, K. K., & Pfeifer, G. (1979). The arrangement of muscle fibres in cleft lips. *Journal of Maxillofacial Surgery, 7*(2), 109-116.

Harris, E. F., & Hullings, J. G. (1990). Delayed dental development in children with isolated cleft lip and palate. *Archives of Oral Biology, 35*(6), 469-473.

He, F., Xiong, W., Wang, Y., Matsui, M., Yu, X., Chai, Y., . . . Chen, Y. (2010). Modulation of BMP signaling by noggin is required for the maintenance of palatal epithelial integrity during palatogenesis. *Developmental Biology, 347*(1), 109-121. doi:10.1016/j.ydbio.2010.08.014; 10.1016/j.ydbio.2010.08.014

Hermus, R. R., van Wijk, A. J., Tan, S. P., Kramer, G. J., & Ongkosuwito, E. M. (2013). Patterns of tooth agenesis in patients with orofacial clefts. *European Journal of Oral Sciences, 121*(4), 328-332. doi:10.1111/eos.12054; 10.1111/eos.12054

Hewson, A. R., McNamara, C. M., Foley, T. F., & Sandy, J. R. (2001). Dental experience of cleft affected children in the west of ireland. *International Dental Journal, 51*(2), 73-76.

Honein, M. A., Rasmussen, S. A., Reefhuis, J., Romitti, P. A., Lammer, E. J., Sun, L., & Correa, A. (2007). Maternal smoking and environmental tobacco smoke exposure and the risk of orofacial clefts. *Epidemiology (Cambridge, Mass.), 18*(2), 226-233. doi:10.1097/01.ede.0000254430.61294.c0

Hua, F., He, H., Ngan, P., & Bouzid, W. (2013). Prevalence of peg-shaped maxillary permanent lateral incisors: A meta-analysis. *American Journal of Orthodontics and Dentofacial Orthopedics : Official Publication of the American Association of Orthodontists, its Constituent Societies, and the American Board of Orthodontics, 144*(1), 97-109. doi:10.1016/j.ajodo.2013.02.025; 10.1016/j.ajodo.2013.02.025

Jezewski, P. A., Vieira, A. R., Nishimura, C., Ludwig, B., Johnson, M., O'Brien, S. E., . . . Murray, J. C. (2003). Complete sequencing shows a role for MSX1 in non-syndromic cleft lip and palate. *Journal of Medical Genetics, 40*(6), 399-407.

Jia, Z. L., Shi, B., Chen, C. H., Shi, J. Y., Wu, J., & Xu, X. (2011). Maternal malnutrition, environmental exposure during pregnancy and the risk of non-syndromic orofacial clefts. *Oral Diseases, 17*(6), 584-589. doi:10.1111/j.1601-0825.2011.01810.x; 10.1111/j.1601-0825.2011.01810.x

Johnson, C. Y., & Little, J. (2008). Folate intake, markers of folate status and oral clefts: Is the evidence converging? *International Journal of Epidemiology, 37*(5), 1041-1058. doi:10.1093/ije/dyn098; 10.1093/ije/dyn098

Jordan, R. E., Kraus, B. S., & Neptune, C. M. (1966). Dental abnormalities associated with cleft lip and/or palate. *The Cleft Palate Journal, 3*, 22-55.

Kirchberg, A., Treide, A., & Hemprich, A. (2004). Investigation of caries prevalence in children with cleft lip, alveolus, and palate. *Journal of Cranio-Maxillo-Facial Surgery : Official Publication of the European Association for Cranio-Maxillo-Facial Surgery, 32*(4), 216-219. doi:10.1016/j.jcms.2004.02.003

Kondo, S., Schutte, B. C., Richardson, R. J., Bjork, B. C., Knight, A. S., Watanabe, Y., . . . Murray, J. C. (2002). Mutations in IRF6 cause van der woude and popliteal pterygium syndromes. *Nature Genetics, 32*(2), 285-289. doi:10.1038/ng985

Kraus, B. S., Jordan, R. E., & Pruzansky, S. (1966). Dental abnormalities in the deciduous and permanent dentitions of individuals with cleft lip and palate. *Journal of Dental Research, 45*(6), 1736-1746.

Letra, A., Menezes, R., Granjeiro, J. M., & Vieira, A. R. (2007). Defining subphenotypes for oral clefts based on dental development. *Journal of Dental Research, 86*(10), 986-991.

Little, J., Cardy, A., & Munger, R. G. (2004). Tobacco smoking and oral clefts: A meta-analysis. *Bulletin of the World Health Organization, 82*(3), 213-218.

Liu, W., Sun, X., Braut, A., Mishina, Y., Behringer, R. R., Mina, M., & Martin, J. F. (2005a). Distinct functions for bmp signaling in lip and palate fusion in mice. *Development (Cambridge, England), 132*(6), 1453-1461. doi:10.1242/dev.01676

Liu, W., Sun, X., Braut, A., Mishina, Y., Behringer, R. R., Mina, M., & Martin, J. F. (2005b). Distinct functions for bmp signaling in lip and palate fusion in mice. *Development (Cambridge, England), 132*(6), 1453-1461. doi:10.1242/dev.01676

MacKenzie, B., Wolff, R., Lowe, N., Billington, C. J.,Jr, Peterson, A., Schmidt, B., . . . Petryk, A. (2009). Twisted gastrulation limits apoptosis in the distal region of the mandibular arch in mice. *Developmental Biology, 328*(1), 13-23. doi:10.1016/j.ydbio.2008.12.041; 10.1016/j.ydbio.2008.12.041

Major M. Ash, J. (1993). *Wheeler;'s dental anatomy, physiology and occlusion* (7th ed.). Philadelphia, PA: W.B. Saunders Company.

Mangold, E., Ludwig, K. U., Birnbaum, S., Baluardo, C., Ferrian, M., Herms, S., . . . Nothen, M. M. (2010). Genome-wide association study identifies two susceptibility loci for nonsyndromic cleft lip with or without cleft palate. *Nature Genetics, 42*(1), 24-26. doi:10.1038/ng.506; 10.1038/ng.506

Mangold, E., Reutter, H., Birnbaum, S., Walier, M., Mattheisen, M., Henschke, H., . . . Diaz Lacava, A. (2009). Genome-wide linkage scan of nonsyndromic orofacial clefting in 91 families of central european origin. *American Journal of Medical Genetics.Part A, 149A*(12), 2680-2694. doi:10.1002/ajmg.a.33136; 10.1002/ajmg.a.33136

Marazita, M. L. (2007). Subclinical features in non-syndromic cleft lip with or without cleft palate (CL/P): Review of the evidence that subepithelial orbicularis oris muscle defects are part of an expanded phenotype for CL/P. *Orthodontics & Craniofacial Research, 10*(2), 82-87. doi:10.1111/j.1601-6343.2007.00386.x

Marazita, M. L., Lidral, A. C., Murray, J. C., Field, L. L., Maher, B. S., Goldstein McHenry, T., . . . Arcos-Burgos, M. (2009). Genome scan, fine-mapping, and candidate gene analysis of non-syndromic cleft lip with or without cleft palate reveals phenotype-specific differences in linkage and association results. *Human Heredity, 68*(3), 151-170.

Martin, R. A., Hunter, V., Neufeld-Kaiser, W., Flodman, P., Spence, M. A., Furnas, D., & Martin, K. A. (2000). Ultrasonographic detection of orbicularis oris defects in first degree relatives of isolated cleft lip patients. *American Journal of Medical Genetics, 90*(2), 155-161.

Martin, R. A., Jones, K. L., & Benirschke, K. (1993). Extension of the cleft lip phenotype: The subepithelial cleft. *American Journal of Medical Genetics, 47*(5), 744-747. doi:10.1002/ajmg.1320470529

Melnick, M., Petryk, A., Abichaker, G., Witcher, D., Person, A. D., & Jaskoll, T. (2006). Embryonic salivary gland dysmorphogenesis in twisted gastrulation deficient mice. *Archives of Oral Biology, 51*(5), 433-438. doi:10.1016/j.archoralbio.2005.09.010

Mittal, M., Maheshwari, N., Ahlawat, K., Sharma, V., Sultan, A., & Chopra, R. (2012). Detection of orbicularis oris muscle defects in first degree relatives of cleft lip children using ultrasound. *Journal of the Indian Society of Pedodontics and Preventive Dentistry, 30*(3), 237-241. doi:10.4103/0970-4388.105017; 10.4103/0970-4388.105017

Moreno, L. M., Mansilla, M. A., Bullard, S. A., Cooper, M. E., Busch, T. D., Machida, J., . . . Lidral, A. C. (2009). FOXE1 association with both isolated cleft lip with or without cleft palate, and isolated cleft palate. *Human Molecular Genetics, 18*(24), 4879-4896. doi:10.1093/hmg/ddp444; 10.1093/hmg/ddp444

Mossey P, C. E. (2002). Epidemiology of oral clefts: An international perspective. In D. Wyszinski (Ed.), *Cleft lip and palate: From origins to treatment* (pp. 127-158) New York:Oxford University Press.

Mossey P, C. E. (2003). *Global registry and database on craniofacial anomalies*. Geneva: World Health Organization.

Mossey, P. A., Little, J., Munger, R. G., Dixon, M. J., & Shaw, W. C. (2009). Cleft lip and palate. *The Lancet, 374*(9703), 1773-1785.

Murray, J. C. (2002). Gene/environment causes of cleft lip and/or palate. *Clinical Genetics, 61*(4), 248-256.

National Center for Health Statistics. (2013). *Health, united states, 2012: With special feature on emergency care.* (). Hyattsville, MD.: U.S. Department of Health and Human Services.

Neiswanger, K., Weinberg, S. M., Rogers, C. R., Brandon, C. A., Cooper, M. E., Bardi, K. M., . . . Marazita, M. L. (2007). Orbicularis oris muscle defects as an expanded phenotypic feature in nonsyndromic cleft lip with or without cleft palate. *American Journal of Medical Genetics.Part A, 143A*(11), 1143-1149. doi:10.1002/ajmg.a.31760

Nopoulos, P., Berg, S., Canady, J., Richman, L., Van Demark, D., & Andreasen, N. C. (2002). Structural brain abnormalities in adult males with clefts of the lip and/or palate. *Genetics in Medicine : Official Journal of the American College of Medical Genetics, 4*(1), 1-9. doi:10.109700125817-200201000-00001

Nopoulos, P., Choe, I., Berg, S., Van Demark, D., Canady, J., & Richman, L. (2005). Ventral frontal cortex morphology in adult males with isolated orofacial clefts: Relationship to abnormalities in social function. *The Cleft Palate-Craniofacial Journal : Official Publication of the American Cleft Palate-Craniofacial Association, 42*(2), 138-144. doi:10.1597/03-112.1

Nopoulos, P., Langbehn, D. R., Canady, J., Magnotta, V., & Richman, L. (2007). Abnormal brain structure in children with isolated clefts of the lip or palate. *Archives of Pediatrics & Adolescent Medicine, 161*(8), 753-758. doi:10.1001/archpedi.161.8.753

Pan, Y., Han, Y., Zhang, H., Zhou, L., Li, D., Cai, Q., . . . Wang, L. (2013). Association and cumulative effects of GWAS-identified genetic variants for nonsyndromic orofacial clefts in a chinese population. *Environmental and Molecular Mutagenesis, 54*(4), 261-267. doi:10.1002/em.21773; 10.1002/em.21773

Pegelow, M., Alqadi, N., & Karsten, A. L. (2012). The prevalence of various dental characteristics in the primary and mixed dentition in patients born with non-syndromic unilateral cleft lip with or without cleft palate. *European Journal of Orthodontics, 34*(5), 561-570. doi:10.1093/ejo/cjr074

Polder, B. J., Van't Hof, M. A., Van der Linden, F. P., & Kuijpers-Jagtman, A. M. (2004). A meta-analysis of the prevalence of dental agenesis of permanent teeth. *Community Dentistry and Oral Epidemiology, 32*(3), 217-226. doi:10.1111/j.1600-0528.2004.00158.x

Poyry, M., Nystrom, M., & Ranta, R. (1989). Tooth development in children with cleft lip and palate: A longitudinal study from birth to adolescence. *European Journal of Orthodontics, 11*(2), 125-130.

Poyry, M., & Ranta, R. (1985). Anomalies in the deciduous dentition outside the cleft region in children with oral clefts. *Proceedings of the Finnish Dental Society.Suomen Hammaslaakariseuran Toimituksia, 81*(2), 91-97.

Poyry, M., & Ranta, R. (1986). Formation of anterior maxillary teeth in 0-3-year-old children with cleft lip and palate and prenatal risk factors for delayed development. *Journal of Craniofacial Genetics and Developmental Biology, 6*(1), 15-26.

Ranta, R. (1983). Hypodontia and delayed development of the second premolars in cleft palate children. *European Journal of Orthodontics, 5*(2), 145-148.

Ranta, R., Stegars, T., & Rintala, A. E. (1983). Correlations of hypodontia in children with isolated cleft palate. *Cleft Palate Journal, 20*(2), 163-165.

Rawashdeh, M. A., & Abu Sirdaneh, E. O. (2009). Crown morphologic abnormalities in the permanent dentition of patients with cleft lip and palate. *The Journal of Craniofacial Surgery, 20*(2), 465-470. doi:10.1097/SCS.0b013e31819b97bf; 10.1097/SCS.0b013e31819b97bf

Romitti, P. A., Sun, L., Honein, M. A., Reefhuis, J., Correa, A., & Rasmussen, S. A. (2007). Maternal periconceptional alcohol consumption and risk of orofacial clefts. *American Journal of Epidemiology, 166*(7), 775-785. doi:10.1093/aje/kwm146

Sasaki, Y., O'Kane, S., Dixon, J., Dixon, M. J., & Ferguson, M. W. (2007). Temporal and spatial expression of Pax9 and sonic hedgehog during development of normal mouse palates and cleft palates in TGF-beta3 null embryos. *Archives of Oral Biology, 52*(3), 260-267. doi:10.1016/j.archoralbio.2006.09.012

Satokata, I., & Maas, R. (1994). Msx1 deficient mice exhibit cleft palate and abnormalities of craniofacial and tooth development. *Nature Genetics, 6*(4), 348-356. doi:10.1038/ng0494-348

Schroeder, D. C., & Green, L. J. (1975). Frequency of dental trait anomalies in cleft, sibling, and noncleft groups. *Journal of Dental Research, 54*(4), 802-807.

Shaffer, J. R., Feingold, E., Wang, X., Lee, M., Tcuenco, K., Weeks, D. E., . . . Marazita, M. L. (2013). GWAS of dental caries patterns in the permanent dentition. *Journal of Dental Research, 92*(1), 38-44. doi:10.1177/0022034512463579; 10.1177/0022034512463579

Soria, J. M., Taglialatela, P., Gil-Perotin, S., Galli, R., Gritti, A., Verdugo, J. M., & Bertuzzi, S. (2004). Defective postnatal neurogenesis and disorganization of the rostral migratory stream in absence of the Vax1 homeobox gene. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 24*(49), 11171-11181. doi:10.1523/JNEUROSCI.3248-04.2004

Suzuki, S., Marazita, M. L., Cooper, M. E., Miwa, N., Hing, A., Jugessur, A., . . . Murray, J. C. (2009). Mutations in BMP4 are associated with subepithelial, microform, and overt cleft lip. *American Journal of Human Genetics, 84*(3), 406-411. doi:10.1016/j.ajhg.2009.02.002; 10.1016/j.ajhg.2009.02.002

Takahama, Y., & Aiyama, Y. (1982). Maxillary canine impaction as a possible microform of cleft lip and palate. *European Journal of Orthodontics, 4*(4), 275-277.

Tortora, C., Meazzini, M. C., Garattini, G., & Brusati, R. (2008). Prevalence of abnormalities in dental structure, position, and eruption pattern in a population of unilateral and bilateral cleft lip and palate patients. *The Cleft Palate-Craniofacial Journal : Official Publication of the American Cleft Palate-Craniofacial Association, 45*(2), 154-162. doi:10.1597/06-218.1; 10.1597/06-218.1

Turner, C., Zagirova, A. F., Frolova, L. E., Courts, F. J., & Williams, W. N. (1998). Oral health status of russian children with unilateral cleft lip and palate. *The Cleft Palate-Craniofacial Journal : Official Publication of the American Cleft Palate-Craniofacial Association, 35*(6), 489-494. doi:2

Ulrich, M., Kristoffersen, K., Rolschau, J., Grinsted, P., Schaumburg, E., & Foged, N. (1999a). The influence of folic acid supplement on the outcome of pregnancies in the county of funen in denmark. part II. congenital anomalies. A randomised study. *European Journal of Obstetrics, Gynecology, and Reproductive Biology, 87*(2), 111-3; discussion 103-4.

Ulrich, M., Kristoffersen, K., Rolschau, J., Grinsted, P., Schaumburg, E., & Foged, N. (1999b). The influence of folic acid supplement on the outcome of pregnancies in the county of funen in denmark. part III. congenital anomalies. an observational study. *European Journal of Obstetrics, Gynecology, and Reproductive Biology, 87*(2), 115-8; discussion 103-4.

Van Der Plas, E., Conrad, A., Canady, J., Richman, L., & Nopoulos, P. (2010). Effects of unilateral clefts on brain structure. *Archives of Pediatrics and Adolescent Medicine, 164*(8), 763-768.

Vanderas, A. P. (1987). Incidence of cleft lip, cleft palate, and cleft lip and palate among races: A review. *The Cleft Palate Journal, 24*(3), 216-225.

Vieira, A. R., Avila, J. R., Daack-Hirsch, S., Dragan, E., Felix, T. M., Rahimov, F., . . . Murray, J. C. (2005). Medical sequencing of candidate genes for nonsyndromic cleft lip and palate. *PLoS Genetics, 1*(6), e64. doi:10.1371/journal.pgen.0010064

Vieira, A. R., McHenry, T. G., Daack-Hirsch, S., Murray, J. C., & Marazita, M. L. (2008a). Candidate gene/loci studies in cleft lip/palate and dental anomalies finds novel susceptibility genes for clefts. *Genetics in Medicine : Official Journal of the American College of Medical Genetics, 10*(9), 668-674. doi:10.1097GIM.0b013e3181833793; 10.1097GIM.0b013e3181833793

Vieira, A. R., McHenry, T. G., Daack-Hirsch, S., Murray, J. C., & Marazita, M. L. (2008b). A genome wide linkage scan for cleft lip and palate and dental anomalies. *American Journal of Medical Genetics.Part A, 146A*(11), 1406-1413. doi:10.1002/ajmg.a.32295; 10.1002/ajmg.a.32295

Walker, S. C., Mattick, C. R., Hobson, R. S., & Steen, I. N. (2009). Abnormal tooth size and morphology in subjects with cleft lip and/or palate in the north of england. *European Journal of Orthodontics, 31*(1), 68-75. doi:10.1093/ejo/cjn073; 10.1093/ejo/cjn073

Wang, Q., Jia, P., Cuenco, K. T., Zeng, Z., Feingold, E., Marazita, M. L., . . . Zhao, Z. (2013). Association signals unveiled by a comprehensive gene set enrichment analysis of dental caries genome-wide association studies. *PloS One, 8*(8), e72653. doi:10.1371/journal.pone.0072653; 10.1371/journal.pone.0072653

Weinberg, S. M., Andreasen, N. C., & Nopoulos, P. (2009). Three-dimensional morphometric analysis of brain shape in nonsyndromic orofacial clefting. *Journal of Anatomy, 214*(6), 926-936. doi:10.1111/j.1469-7580.2009.01084.x; 10.1111/j.1469-7580.2009.01084.x

Weinberg, S. M., Neiswanger, K., Martin, R. A., Mooney, M. P., Kane, A. A., Wenger, S. L., . . . Marazita, M. L. (2006). The pittsburgh oral-facial cleft study: Expanding the cleft phenotype. background and justification. *The Cleft Palate-Craniofacial Journal : Official Publication of the American Cleft Palate-Craniofacial Association, 43*(1), 7-20. doi:10.1597/04-122R1.1

Weiss, M., Weiss, J., Muller-Hartwich, R., Meier, B., & Jost-Brinkmann, P. G. (2005). Chlorhexidine in cleft lip and palate patients with multibracket appliances. results of a prospective study on the effectiveness of two different chlorhexidine preparations in cleft lip and palate patients with multibracket appliances. *Journal of Orofacial Orthopedics = Fortschritte Der Kieferorthopadie : Organ/Official Journal Deutsche Gesellschaft Fur Kieferorthopadie, 66*(5), 349-362. doi:10.1007/s00056-005-0508-2

Werner, S. P., & Harris, E. F. (1989). Odontometrics of the permanent teeth in cleft lip and palate: Systemic size reduction and amplified asymmetry. *Cleft Palate Journal, 26*(1), 36-41.

Woolf, C. M., Woolf, R. M., & Broadbent, T. R. (1965). Lateral incisor anomalies. microforms of cleft lip and palate? *Plastic and Reconstructive Surgery, 35*, 543-547.

Wu, T. T., Chen, P. K., Lo, L. J., Cheng, M. C., & Ko, E. W. (2011). The characteristics and distribution of dental anomalies in patients with cleft. *Chang Gung Medical Journal, 34*(3), 306-314.

Zucchero, T. M., Cooper, M. E., Maher, B. S., Daack-Hirsch, S., Nepomuceno, B., Ribeiro, L., . . . Murray, J. C. (2004). Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate. *The New England Journal of Medicine, 351*(8), 769-780. doi:10.1056/NEJMoa032909

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