FACIAL FORM AS A SUBCLINICAL PHENOTYPE OF NONSYNDROMIC OROFACIAL CLEFTING: AN ANTHROPOMETRIC ANALYSIS

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FACIAL FORM AS A SUBCLINICAL PHENOTYPE OF NONSYNDROMIC OROFACIAL CLEFTING: AN ANTHROPOMETRIC ANALYSIS

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Orofacial clefting (OFC) is the most common craniofacial anomaly, seen in every 1 in 500 to 2500 births worldwide. It has been identified that 60 to 70% of OFC are non-syndromic (NS) and are not associated with any single genetic marker. However, high recurrence rates of NSOFC have been identified in families. The recurrence risk is predicted on rather empirical data owing to poor gene mapping and poor correlation between genotype and phenotype of this anomaly. Considering the fact that OFC presents with significant etiologic heterogeneity and phenotypic diversity, subclinical manifestations need to be identified to complete the OFC phenotypic spectrum. This will improve correlation between genotype and phenotype and thus improve recurrence risk estimation. A large body of evidence suggests that subtle changes in craniofacial morphology may be a subclinical marker for cleft susceptibility. A vast majority of this evidence is based on cephalometric data with far fewer studies examining soft tissue features of the face. The purpose of the present study is to compare craniofacial characteristics of unaffected biological parents of NS OFC offspring with controls derived from the same population using direct anthropometry.

The study sample consisted of 67 male and 76 female unaffected parents of both NS Cleft lip and Cleft lip/palate children. Control sample comprised of 37 normal males and 59 normal females of the same race and ethnicity. Craniofacial measurements of both study and control

population were collected using direct anthropometry as was described by Farkas (1994) and Kolar & Salter (1997) and were subjected to stepwise discriminant functional analysis (DFA). DFA is similar to logistic regression; used to classify population into groups based on covariate variables. In this study discriminant models with high statistical significance (P‹0.001) were derived in males and females that could clearly distinguish unaffected parents from controls based on direct anthropometrically measured craniofacial characteristics. The study showed that salient discriminating features are localized to specific regions of the face in a partly gender-specific manner. The study showed that a model derived using a small subset of direct anthropometrically measured craniofacial features can be used to discriminate unaffected parents from the controls.

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PREFACE

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1.0 INTRODUCTION

Orofacial Clefting (OFC) is the most common craniofacial anomaly and is diverse in its phenotypic presentation and etiology. Biological mechanisms that lead to the development of OFC have been studied extensively but the cascade of events that start in the genome and translated into clefting is not clearly understood. It is believed that clefting results from genetic susceptibility, environmental factors, biomechanical breakdown or a combination of any/all of these factors reaching a certain threshold. No single genetic marker has been causally implicated in the etiology of OFC for the vast majority of cases likely reflecting the trait's complex etiologic heterogeneity. Given this inherent complexity, one would expect a wide range of phenotypic expression associated with the trait, including subclinical presentations at the low end of the susceptibility range.

A complete gamut of these subclinical manifestations (or endophenotypes) needs to be identified in order to completely describe the orofacial cleft (OFC) phenotypic spectrum. It is thought that presence of these subclinical phenotypes is indicative of underlying cleft susceptibility genes. Hence, identifying subclinical phenotypes typically involve examining traits in individuals who are clinically unaffected, but are at elevated genetic risk for OFC; namely the biological parents and sibs of affected individuals. To date numerous studies have documented differences in a variety of physical traits in unaffected "at risk" relatives compared with population controls; these include increased dental anomalies, aberrant dermatoglyphics, lip print

patterns, changes in brain morphology and function, subepithelial lip muscle defects, and altered speech patterns among others. Expanding the OFC phenotype to capture the full range of trait expression should improve the correlation between genotype and phenotype. These efforts, in the long term, have a potential to increase power of gene mapping and also to improve recurrence risk estimation.

There is a large body of evidence suggesting that subtle changes in craniofacial morphology may be a subclinical marker for cleft susceptibility. Numerous studies have documented changes in facial form in the biological parents and sibs of individuals with OFC compared with population-based controls. Specific findings, however, vary greatly among different studies, possibly reflecting etiologic and/or methodological heterogeneity. The vast majority of these studies are based on cephalometric data, focusing almost exclusively on skeletal form. Far fewer studies have examined the soft tissue features of the face, despite the fact that many mild cleft manifestations are limited to subtle dysmorphology of the soft tissue nose and upper lip. Thus, additional detailed studies of the facial soft tissue phenotype in OFC relatives are warranted.

The purpose of the present study is to compare craniofacial characteristics of unaffected biological parents of NS OFC offspring and controls derived from the same population using direct anthropometry.

2.0 BACKGROUND AND LITERATURE REVIEW

2.1 INTRODUCTION TO OROFACIAL CLEFTING

Cleft lip with or without palate (CL/P) is the most common congenital craniofacial anomalies (Christensen, 1999) and is reported in 1 in 700 newborns worldwide (Murray, 2002). However, incidence of CL/P varies among different ethnic groups (1 in 500 to 2500 newborns) (Murray, 2002). Mooney (2008) noted that incidence of CL/P is 2:1 in males compared with females. Lisi et al (2005) also pointed out that there is a tendency for males to be afflicted with CL/P more than females, even though the phenomenon hasn't been explained. They also pointed out that obstructive congenital defects (e.g.: heart defects etc.) and orofacial clefting are much more common on the left side of the body than on the right. The left sided and male gender predominance of these congenital anomalies appears to be universally distributed and is not limited to a particular ethnicity or race (Lisi et al., 2005). Unilateral pattern of CL/P with left sided predominance has been reported elsewhere as well (Shapira et al., 1999; Wyszynski, 1996).

It is well known that OFC deformities are a significant psychological and economic burden not only to the families but is also a public health issue, considering the fact that OFC is associated with significant morbidity and mortality (Christensen et al., 2004). Tolarova and Cervenka (1998) calculated that every day on an average there are 20 births with orofacial

clefting in the US and average lifetime cost per cleft per child is $100,000. Christensen et al (2004) studied mortality rates in a cohort of Danish population born with Cleft lip and palate between 1943 and1987; followed to 1998. They found that mortality increased not only the first year after birth but throughout the life span they collected data on.

2.1.1 Embryology and Classification of Orofacial clefts

An understanding of craniofacial embryology is helpful to better comprehend orofacial clefting. The first branchial arch and frontonasal process (Helms et al., 1997) form most of the facial skeleton and soft-tissues. Failure of the adjacent medial and/or lateral facial prominences (frontonasal process gives rise to medial and lateral nasal prominences) to meet with the maxillary prominence or breakdown thereafter will result in facial clefting along the lines of fusion. Orofacial clefts, hence can appear anywhere along the lines of fusion of these processes. Depending on the embryological tissue of origin and the spatial/temporal sequence of events during orofacial development, the type and severity of clefting would vary.

OFCs can be classified very simplistically into syndromic and non-syndromic forms, based on association with any other developmental abnormalities (Jugessur and Murray, 2005). Syndromic CL/P is known to be associated with at least 400 other conditions (Cohen, 2002). Murray (2002) quoted that incidence of isolated CL/P alone (also called non-syndromic CL/P) is 70%. Mitchell et al. (2002) pointed out that 50 to 70% of all OFCs are non-syndromic clefts. Evidently syndromic and non-syndromic forms differ in etiology and it is very important to delineate them accordingly, since clinical management will be different in each of these conditions. Based on careful analysis of cleft epidemiological data it has also been shown that CLP and CP are two very different entities (Mossey et al., 1998b).

Several classification systems of orofacial clefting have been proposed based on anatomy (Vento et al., 1991; Friedman et al., 1999), embryology (Kernahan et al., 1971) and severity (Friedman et al., 1999). Perhaps classification system based on embryologic origin of face could be based on the tissue of origin (primary/secondary palate, maxillary process, etc.) and more encompassing but can be cumbersome. Clefts of primary palate result in Cleft lip and/or alveolus; Clefts affecting both primary and secondary palate result in CLP. This classification may be descriptive but cannot be readily useful clinically, since there is no mention of the extent or severity of involvement.

Mooney (2008) made a case for factoring in etiopathogenesis in the classification of orofacial clefts. Considering the fact that historic classification systems were based on descriptive morphological and anatomical features, including etiopathogenesis in the discussion will help in genetic counseling and interdisciplinary communication. He grouped all the existing OFC classifications into three categories: morphological based classification systems, pathogenically based classification systems and etiologically based classification systems. He further pointed out that no existing classification system could incorporate all of these elements into one system.

2.1.2 Etiopathogenesis of Oro-Facial Clefting

It is well known that OFC is a complex disease trait with multifactorial etiology. Single genes, gene-gene interactions, gene- environmental interactions, environmental factors and mechanical factors have all been implicated in the etiology (Wyszynski et al., 1997). Even though it is evident that facial clefts run in families, very few genes have been directly implicated in the etiology of CL/P and CP, at least for the vast majority of cases (Jugessur et al., 2009; Mossey et

al., 1998a). Familial effects in NS CL/P and CP have long been identified and it has also been recognized that the transmission patterns are non-Mendelian (Marazita et al., 2002). Genetic heterogeneity has been noticed in the etiology of orofacial clefts. Jugessur and Murray (2005) suggested that single genes IRF6, MSX1 and FGFR1 are shown to be associated with isolated cleft abnormalities. Mossey et al. (1998a) implicated TGF-α allelic variants in the etiology of non-syndromic CP and CL/P while Zhu et al. (2010) suggested TGF- α might not be responsible as a possible culprit. Zhu et al. (2010) evaluated the possibility of MTHFR, TGFB3 polymorphisms association with an increased risk of isolated CP and CL/P in Chinese population. They concluded that MTHFR, TGFB3 polymorphisms are associated with increased risk in the population they studied. Dixon et al. (2011) pointed out that at least 20 genes have been implicated in the etiology of NS CL/P. They suggested that the genes that figured prominently in etiopathogenesis include IRF6, 8q24, VAX1, MSX1, FOXE1, MYH9, MAFB, ABCA4, 17q22, BMP4, and FGFR2.

Contemporary belief is that interaction of genes with one other and/or environmental agents are responsible for majority of OFCs (Marazita and Mooney, 2004). It has also been shown that most OFCs with genetic etiology don't follow a simple Mendelian form of inheritance (Mitchell et al., 2002), possibly because of incomplete penetrance, variable expressivity and allelic heterogeneity of perpetrator genes. Dixon et al. (2011) pointed out that 70% of all CL/P and 50% of CPs are non-syndromic and the rest are associated with around 500 Mendelian syndromic forms.

Environmental factors have been directly shown to result in orofacial clefting. Experiments in animal models to explore the etiopathogenecity (Lohnes et al., 1994) of OFC have yielded valuable information. Mouse embryos that were administered teratogenic doses of

phenytoin (Helms et al., 1997) were shown to have deficient growth of facial prominences and nasomaxillary area. Lohnes et al. (1994) pointed out that vitamin –A deficient mice during fetal development had abnormalities in structures originating from frontonasal prominences, 1st and 2nd branchial arches. However the question remains how much of this animal experimental data can be extrapolated towards humans. Retrospective studies on possible exposure to teratogenic factors stand a chance of recollection bias while prospective studies in humans on teratogenic effects of environmental agents pose ethical issues.

Maternal factors in the etiology of OFC have been painstakingly investigated (Shaw et al., 1996) and numerous agents (E.g.: Folic acid deficiency, Vitamin A, maternal alcoholism, maternal smoking etc.) are implicated in causing OFC in the offspring. A study in Sweden pointed out that maternal obesity could be associated with OFC. Even though exact linkage is not known, possible maternal type II diabetes could be an implicating factor (Cedergren and Kallen, 2005). Shaw et al. (1996) studied if maternal smoking is associated with increased risk of isolated CL/P and CP. They found out that maternal smoking of 20 cigarettes or more is associated with increase in risk of isolated CL/P and CP and this risk increased significantly (3 to 11 fold increase) in progeny who also had TGF α allele in addition to mothers who smoked. Wyszynski et al. (1997) in a meta-analysis of existing evidence up to date have concluded that maternal cigarette smoking during the first trimester of gestation is associated with a significant increase in incidence of CL/P or CP in the next generation. Jugessur and Murray (2005) in an insight into the cleft phenotype agreed with this finding.

Gene-environmental interactions resulting in OFC have been explored (Murray, 2002) and it is also noticed that the chances of developing OFC when exposed to a particular teratogen varies significantly based on genetic susceptibility (Zhu et al., 2010). This means that two

individuals with the same genetic make-up may not respond the same to an environmental factor resulting in OFC and vice versa.

Despite the above findings, OFCs with known etiology comprise only a minor portion of these phenotypes (Marazita et al., 2004) even though population groups with high recurrence risk of OFC have been identified. Numerous models have been proposed to explain the modus operandi and thus predict recurrence risk of OFC. The multifactorial threshold (MFT) model, which proposes that multiple genes with their additive affect in conjunction with environmental factors fails to explain the causality of CLP (Marazita et al., 2004). MFT model presumes that all genes have an equal, minor and additive role in the etiology of a disease process. This model espouses that individuals who have a higher genetic threshold will present with overt cleft manifestation. MFT model has also been refuted by Sivertsen et al. (2008) in a population based cohort study of first degree relatives of 4138 children born between 1967 and 2001 in Norway and treated for cleft deformities. Sivertsen et al. reiterated the fact that clefts are a result of multifactorial etiology and have a high recurrence rate in the families. They also added that the anatomic severity of facial cleft do not predict the recurrence risk of cleft in first-degree relatives. This means that severity of the cleft is independent of genetic predisposition of oral clefting, which again serves to discredit MF/T model as a possible explanation of OFC. Another critical observation of Sivertsen et al is that:

> *"Mildly affected members have recurrence risks similar to families with more severely affected members, with equivalent severity among recurrent cases"*

This observation is not only important in clinical counseling of the cleft phenotype but also in genetic counseling in predicting recurrence risk of cleft abnormality. This observation ties well into the existing evidence that in majority of multiplex families, unaffected individuals also

will possess cleft susceptibility loci. However with misplaced emphasis on obvious clinical presentation of cleft phenotype it is quite probable that we are missing latent genetic liability for CL/P. Hence, closer look at "clinically unaffected" first degree relatives may yield endophenotypes that will increase the power of genetic analysis and also in counseling of families on recurrence risk of OFC in the families.

2.2 THE RANGE OF CLEFT PHENOTYPES

Orofacial clefting is heterogeneous in presentation, therefore it is difficult to classify and categorize OFCs. Given the heterogeneity of phenotypic involvement, it is very important to look past the very obvious facial characteristics of cleft patients and identify subclinical phenotypes (endophenotypes) in order to complete the OFC spectrum. It is therefore important to describe all phenotypic markers for clefting, which has wide array of clinical / subclinical presentations (Neiswanger et al., 2007; Mossey et al., 2010).

2.2.1 Subclinical Phenotypes in CL/P (Endophenotypes)

Limitations of characterizing clefts based on qualitative (affected vs. unaffected) features have long been realized (Weinberg *et al*., 2006, Dixon *et al.*, 2011). Weinberg *et al*. (2008) pointed out that, with emphasis being placed on typical clinical presentation of craniofacial clefting, a large cohort of at-risk population with subclinical presentation and possibly cleft predisposing conditions (genome, environmental factors) is being missed. Completing the phenotypic spectrum of OFCs can potentially improve predictability of recurrence risk of this deformity.

Identifying reliable phenotype risk markers may not be used as a proxy but as an adjunct to genetic analysis. Given the current limitation of genetic analysis in predicting recurrence risk the former cannot be overlooked. Mossey *et al.* (1998b) reported that contemporary genetic counseling has serious shortcomings in predicting the recurrence risk of OFC in the offspring. They quoted that, the data genetic counseling is based on, is rather empirical and the recurrence risk is pegged at 2-6% if one parent or one child has OFC, 9% if two children already has OFC and 15-17% if both a parent and a child has already been affected with the cleft.

Mossey *et al.* (2010) recently did a systematic review of dentocraniofacial phenotype to identify microforms of orofacial clefting. They listed a myriad of craniofacial features claimed to be cleft microforms found in cleft patients or in first degree relatives and these microforms included: absent maxillary lateral incisor, high palatal vault, torus palatinus, V-shaped maxillary arch, premaxillary supernumerary teeth, congenitally absent anterior teeth, morphology of upper lateral incisor, altered craniofacial shape, palatal arch form, bifid uvula, submucous cleft palate, microform cleft lip/orbicularis oris discontinuity /fissure, nasal deformity, impacted maxillary canine, velopharyngeal variations and cervical spine anomalies. Dixon *et al.* (2011) also provided a succinct summary of anatomical (e.g.: lip pits, lip prints, brain variants analyzed using MRI), functional (e.g.: VPI) and biological (e.g.: cognitive ability, IQ) characteristics that could be subclinical phenotypes of orofacial clefting.

Weinberg *et al*. (2006) did an exhaustive literature review on completing the orofacial cleft spectrum with particular focus on subclinical phenotypes of orofacial cleft. Considering the fact that causative factors for NS CL/P is not known in most cases, even though a clear familial tendency is noticed, Weinberg *et al.* (2006) argued that subclinical phenotypes in unaffected relatives need to be discerned first to complete the orofacial cleft spectrum. Identifying those

subclinical phenotypes will enable better gene mapping, and also in predicting relative risk in the future generations. To address the ambiguity in defining the orofacial cleft phenotype, Weinberg *et al.* (2006) provided a comprehensive review of literature of subclinical phenotypes based on the knowledge acquired from the Pittsburgh Oral-Facial Cleft (POFC) study. They reviewed the possibility of certain phenotypes being subclinical phenotypes, those characteristics were: fluctuating and directional asymmetry, non-right handedness, dermatoglyphic patterns, craniofacial morphology, orbicularis oris muscle defects, dental anomalies, structural brain and vertebral anomalies and certain minor physical anomalies (MPAs).

Deviation from bilateral symmetry can be classified into two types: Fluctuating asymmetry (FA) and Directional asymmetry (DA). If a difference between anatomical characteristics of right and left sides of the body is noticed in an otherwise normal development process, FA is assumed. However in DA there is a consistent difference between anatomical parts and counterparts, i.e. each side of the body differs in size from its contralateral side.

It is believed that, since bilateral anatomical characteristics are coded and thus derived from the same genetic information, a breakdown in bilateral symmetry may therefore indicate a deviation from the normal development process that has not been compensated for. Consistent with this observation, FA has been noticed in experimental subjects with genetic, environmental and stressors (Weinberg *et al.*, 2006). Breakdown in normal developmental processes resulting in FA was linked to several factors including maternal obesity and smoking, length of gestation, Down's syndrome, fetal alcohol syndrome etc. (Weinberg *et al.*, 2006). Most common characteristics looked at to evaluate FA are dermatoglyphics and dentition the reason being; both characteristics are established early on during the embryonic development and the characteristics are unique so that valid and reliable measures on deviations can be performed. There is some

evidence that population with NS CL/P and their unaffected relatives demonstrate FA in dermatoglyphic pattern and dental traits.

The biologic rationale behind the association between NS CL/P and FA is that; both may be victims of the same genetic/environmental stressors (Weinberg *et al.*, 2006). The possibility that the genetic and environmental stressors associated with NS orofacial clefting are also seen in patients with FA (Weinberg *et al.*, 2006), makes a compelling case for identifying FA which may be a form of subclinical NS orofacial cleft. On the other hand developmental instability resulting in FA may also be associated with NS CL/P phenotypes (Neiswanger *et al.*, 2002) and the relationship is purely coincidental.

DA demonstrates a systematic left to right discrepancy. Considering the fact that NS CL/P demonstrates a left sided predominance it is hypothesized that DA may be a mild subclinical phenotype of NS CL/P and presence of DA may suggest that cleft susceptible genes are present in the proband. To evaluate if DA is associated with NS CL/P a closer look at the unaffected relatives of the patients is needed. DA of craniofacial anatomy has been studied. A cephalometric study of unaffected relatives of NS CL/P by Al-Emran *et al.* (1999) indicated the presence of a DA in unaffected relatives. McIntyre and Mossey's (2004) study of posteroanterior radiographs showed that unaffected parents of NS CL/P patients demonstrated a clear DA compared to controls. Yoon *et al.* (2004) also identified a clear increase in ipsilateral nasomaxillary width of unaffected parents of NS CL/P offspring, strongly suggestive of DA. Unilateral nasal asymmetry as a form of DA has been extensively studied (Pashayan and Fraser, 1971; Farkas and Cheung, 1979; Fukuhara, 1987).

Abnormalities in teeth size, shape, number, eruption timings have also been reported by Ranta (1986) as subclinical phenotypes of orofacial clefting. Considering the fact that tooth buds

are in the area of fusion of medial and lateral nasal prominences, it is reasonable to expect anomalies of teeth associated with buds from this region. However, generalized dental anomalies have been associated with CL/P and hence logically, dental anomalies as a form of FA have been extensively studied. Higher incidence of hypodontia is reported in CL/P population (Ranta, 1986; Shapira *et al.*, 1999); however this association has been refuted by Anderson and Moss (1996). Nevertheless, genetic basis for the linkage between hypodontia and CL/P population has been postulated. Genetic analysis of population groups with hypodontia showed defective MSX1 (Lidral and Reising, 2002) and PAX9 genes (Vieira, 2003); these gene defects are also seen in CL/P population (Vieira, 2003). Supernumerary teeth, enamel formation defects, increased asymmetry, delay in eruption have all been shown to be associated with CL/P (Weinberg *et al.*, 2006). Anderson and Moss (1996) suggested that certain dental morphological traits may be seen more commonly in cleft patients, these features include: talons cusp of maxillary lateral incisors, absent or altered cusp patterns of maxillary 1^{st} molars, mandibular 1^{st} and 2^{nd} premolars. Brain lateralization refers to the idea that two halves of the brain is dissimilar to each other and each half is responsible for specialized functions. Brain lateralization is seen in vertebrates and enables them to perform two functions at the same time (e.g.: writing with one hand and eating with another) (Rogers *et al.*, 2004). It is hypothesized that since facial structures are derived from neuroectoderm, there could be a biological rationale between abnormalities in brain development and function and breakdown of craniofacial development. It has been noticed that patients suffering with schizophrenia present with structural brain anomalies, atypical handedness and facial dysmorphology (Weinberg *et al.*, 2006). It has been debated that non-right handedness is related to orofacial cleft phenotypes; many claim is a form of developmental instability. The relationship of non-right handedness with FA and CL/P has been studied

extensively and the association was found to be unclear (Jeffrey and Boorman, 2000; Scott *et al.*, 2005; Weinberg *et al.*, 2006)

There is strong evidence that FA is strongly associated with changes in dermatoglyphic patterns (Bokhari *et al.*, 2002; Weinberg *et al.*, 2006; Neiswanger *et al.*, 2006; Scott *et al.*, 2005). Dermatoglyphic patterns (Arch, ridge, radial loop or whorl patterns) can be evaluated with validity and reliability and serve a valuable tool in assessing FA. These patterns have been studied extensively and it was noticed that slowly forming arch patterns and rarely present radial loops are more common in cleft patients (Bokhari *et al.*, 2002). Scott *et al.* (2005) pointed out that whorl patterns develop early in development while arch forms appear later during embryologic development. It has been shown that probands exposed to teratogenic agents during fetal development demonstrate an increase in arch pattern. Bokhari *et al.* (2002) evaluated dermatoglyphic patterns of 66 children exposed to teratogenic agents (phenytoin and phenobarbital) during prenatal development. They noticed that this cohort demonstrated an increase in arch pattern and subtle changes in ridge patterns of these people; more so in the subset that were exposed to multiple teratogenic agents. Scott *et al.* (2005) evaluated dermatoglyphic patterns in Filipino CL/P individuals and compared the patterns to those of unaffected relatives and also to control population. They noticed that CL/P population demonstrated a unique dermatoglyphic pattern dissimilar to those of unaffected relatives, and unaffected relatives showed a dermatoglyphic spectrum pattern different from controls. This difference in the dermatoglyphic presentation is more evident in female gender. Presence of a unique dermatoglyphic pattern in CL/P patients has been evaluated and confirmed in other ethnic groups as well.

Martin *et al.* (2000) studied orbicularis oris (OO) anatomy using ultrasonography in 21 cleft patients and their families and 52 control subjects with no history of clefting in their families. They identified that the prevalence of OO defects considerably increased in first-degree relatives of overt cleft patients. They also suggested that there is an increased risk of cleft incidence in the future generations in parents with OO abnormalities. The authors suggested that OO defects are a CL phenotype and ultrasonography is diagnostic in identifying those defects. Neiswanger *et al.* (2007) also studied to assess if OO discontinuity is a form of cleft phenotype using high-resolution ultrasonography. They pointed out that defects in OO can be a mildest form of cleft phenotype and also identified gender dimorphism in the appearance of these discontinuities. Even though both the male and female relatives of cleft patients have high incidence of OO discontinuities, the relationship is not statistically significant in female relatives. Neiswanger *et al.* (2007) suggested that OO defects can be an endophenotype of cleft phenotype and that identifying these OO defects can help in estimating recurrence risk of clefting in future generations.

Velopharyngeal mechanism is a group of naso/oropharyngeal muscles acting in concert to produce speech. Submucosal muscular defects involving levator palatini / musculus uvulae or neuromuscular defects in general may result in hypernasality, nasal air emissions or compensatory articulation disorders which is characterized as velopharyngeal insufficiency (VPI) (Weinberg *et al.*, 2006). Anatomical (scarring, size discrepancy between the nasopharynx and the palate) or neuromuscular deficit in velopharyngeal mechanism may cause VPI. Even though VPI is seen in 2.5% of normal population, the incidence increases in cleft palate (CP) patients (Weinberg *et al.*, 2006). It has been reported that VPI in unaffected population is associated with CP offspring in subsequent generations especially if they already have a history

of clefting in the family. In a pilot study of multiplex family sample derived from POFC Weinberg *et al.* (2006) noticed a 24% prevalence of VPI in unaffected relatives of NS orofacial cleft families suggesting that this deviation from the normal could in fact be a subclinical marker of OFC. Huston *et al.* (1985) studied variations of velopharyngeal mechanisms in cleft lip, cleft palate patients and their unaffected relatives and compared them with controls. They pointed out that incidence of clefting seem to increase in future generations of relatives who are affected by subclinical cleft manifestations.

2.2.2 Facial Form/Shape as a Subclinical Phenotype

It is well established that craniofacial shape is transmitted along the generations with the transfer of genetic material (Coccaro *et al.,* 1972). The question then arises: would it be possible for us to look at craniofacial features of the parents and predict relative risk of NS CL/P in the next generation? How much of these morphogenetic characteristics are transmitted into morphometric features? Are there certain craniofacial characteristics that are cleft markers? One way of finding out is by studying craniofacial morphology of the unaffected parents of cleft children and comparing the anatomy with the general population.

Based on strong epidemiological trends there have been studies to see if particular facial characteristics are associated more with CLP than others and if clinically unaffected relatives of CLP individuals demonstrated craniofacial characteristics not seen in normal population. Mossey *et al.* (1997) and Weinberg *et al.* (2008) indicated that unaffected relatives of CLP patients presented with strong craniofacial characteristics not seen in normal population. Based on these findings there have been suggestions that an evaluation of craniofacial morphology should be a

part of genetic counseling in predicting relative risk of the OFC (Suzuki *et al*., 1999; Mossey *et al.,* 1998b; Mossey *et al.,* 1997).

Before that can be achieved, specific facial forms in unaffected relatives of isolated CLP patients need to be identified that would function as risk markers of isolated CLP. Several studies tried to decipher the effects of OFC on facial morphology. The studies compared craniofacial forms of cleft individuals with those who had clefts and surgically corrected (e.g., Liao *et al*., 2006) and also to individuals without surgical correction (e.g.: Shetye and Evans, 2006), to avoid any morphological changes due to surgery *per se*. Even though that sounds logical, the later group may harbor dysmorphic features not only as a result of clefting but also the features that are predictors of cleft (cleft markers). There is a very good possibility we will miss these endophenotypes (cleft markers) if we compared cleft groups with and without surgery. On the other hand morphological comparison of cleft patients with controls would yield information on how OFC can affect facial shape. The controls being the unaffected relatives of the individuals with overt cleft defect. The idea being, unaffected relatives will have essentially the same genotype (except cleft susceptible loci) and the environment (to rule out any influence of possible effects of nurturing).

Based on this premise numerous studies compared craniofacial morphology of OFC patients with their unaffected relatives. These studies (e.g., Mills *et al*., 1968) however did not make a distinction between syndromic versus non-syndromic clefts or type of the clefts (CL/P vs. CP). Even if such distinction is made, it is not very helpful to have descriptive information on what comprises a dysmorphology; rather a quantitative approach at defining dysmorphology is desirable. Mossey *et al.* (2010) in a systematic review of the literature on parental craniofacial phenotypes in orofacial clefting concluded that the craniofacial phenotype of parents of cleft

patients is unique compared to the normal population, however there is "*insufficient consistency of evidence"* to create a phenotypic model to recognize orofacial cleft morphogenes. They attributed this to variation in methodology in most of the studies that looked into these phenotypes thus far. It is widely agreed that NS cleft patients and their unaffected relatives demonstrate a distinct phenotype but the characteristics have not been quantitatively defined yet (Weinberg *et al.*, 2006; Mossey *et al.*, 2010).

Several methods of studying craniofacial morphology have been used in discerning cleft markers in unaffected family members of orofacial cleft population.

2.2.3 Cephalometric studies

Niswander (1968) compared craniofacial anatomy of the parents of patients with CL/P using laminographs. Niswander noticed nasal cavity, nasal floor, palatal shelf abnormalities in parents of the CL/P and CP patients; he also noticed sexual dimorphism in appearance of these abnormalities in parents (Mossey et al., 2010).

Coccaro *et al.* (1972) at the National institutes of Health (NIH) studied craniofacial anatomy of parents of children with CL/P using lateral cephalograms and compared with normal population. They found out that the faces of parents of children with CL/P were less convex, had mandibular prognathism, vertical and horizontal measurements were also smaller compared to the normal population.

Nakasima and Ichinose (1983) conducted a cephalometric study to see if there was any in-between difference in craniofacial anatomy of parents of CL/P, CL, CP and normal children. They noticed that CL/P parents presented with a "significantly reduced head length and width, maxillary depth and upper face height, increased lower face height and various craniofacial

width measures (upper face, orbital, nasal and mandibular)". The authors pointed out that multiple discriminant function analysis using the aforementioned characteristics could differentiate between the control and the orofacial cleft population. When face width was used in multiple discriminant function analysis, different cleft groups (CL/P, CP and CL) can be clearly distinguished.

Ward *et al.* (1989) did a landmark study in which they introduced the concept of "hierarchical cluster analysis", based on cephalometric analysis of 82 unaffected relatives of CL/P individuals, and noticed considerable phenotypic heterogeneity. They categorized these unaffected relatives into three homogenous clusters of phenotypes and observed that among the three groups, one group had cephalometric measurements closest to standardized norms while the other two to overt cleft phenotypes.

The idea of "cluster analysis" is that some unaffected relatives may genotypically and phenotypically be related "***more***" to the clefting than the others. This is a direct rebuttal of MF/T model, which presumes that both the parents of the OFC individual have the same genetic burden resulting in clefting in their progeny. As a matter of fact, Ward *et al.* also noticed that the parents of the CL/P children could belong to different clusters of phenotypic homogeneity, which means that they do not share genotypic (possibly phenotypic) burden resulting in CL/P.

Raghavan *et al.* (1994) compared craniofacial anatomy of 38 parents of CL/P children with those of 24 parents with offspring with no such anomalies, using lateral cephalograms and frontal radiographs (124 lateral cephalograms and 124 frontal radiographs). The authors felt that the study group had a distinct morphology compared to the control population; study group presented with smaller facial dimensions transversely and also vertically. The authors also felt that cranial base angle was obtuse (N-S-Ba), upper and total facial height smaller, maxilla

forwardly placed with prominent ANS and increased palatal length. Raghavan *et al.* pointed out that even though bizygomatic, biperital, bigonial and bizygomaticofrontal widths were smaller in the parents of CL/P children, nasal width was found to be larger compared to the controls.

Mossey *et al.* (1998) studied craniofacial anatomy of unaffected relatives of 83 children using lateral cephalograms in a cohort of Scottish population. The findings were compared to age and gender matched controls and found that unaffected relatives demonstrated distinctive morphological features that were segregated along the gender lines. Based on the findings they used discriminant function analysis and noticed that they could identify unaffected male relatives in 80% and female relatives in 90% of the cases compared with controls. With the same sample they studied if any specific cephalometric characteristics are risk markers of CLP versus CP. They found out that mandibular ramus length is a predictor of CP in 71.4% and CLP in 62.5% of the times.

Al-Emran *et al.,* (1999) studied craniofacial anatomy of unaffected parents of CL/P children using frontal radiographs and found that unaffected fathers had significantly increased nasal cavity width and decreased maxillary alveolar width; while unaffected mothers also had significantly reduced maxillary alveolar width in addition to reduced head width and upper face width. They used these craniofacial anatomical features in stepwise logistic regression to correctly classify 74% of the male relatives and controls (based on nasal cavity and alveolar width) and 77% of the female relatives and controls (based on head width).

Suzuki *et al.* (1999) studied dentocraniofacial morphology of the parents of cleft lip and or palate patients and compared it with controls to see if there is any discrepancy in the measurements and if the data could be used in genetic counseling in predicting relative risk of CL/P. They took dental records, lateral cephalograms and posteroanterior head films in parents

with known CL/P and compared them with control group. No dental predictors in mesio-distal widths of teeth were noticed. However, inter-orbital distance, nasal cavity width and inter-coronoid distance, anterior cranial base length and overall cranial base length were found to be greater in affected parents compared to the controls. They concluded that their discriminant analysis was only accurate in 67.9% of the times in pooled experimental and control subjects hence is not reliable enough in genetic counseling. They suggested additional variables, like craniofacial morphology needs to be evaluated and incorporated in genetic counseling.

Perkiomaki *et al.* (2003) analyzed lateral cephalogram data of 28 Costa Rican families with a history of CL/P. The cephalometric data revealed that the anterior cranial base and the palate lengths were shorter in unaffected relatives of CL/P patients compared with age matched standardized norms.

McIntyre and Mossey (2004) did a retrospective analysis of PA cephalograms to see if there are craniofacial asymmetries in size or shape in parents of children with orofacial clefting (OFC). Conventional posteroanterior cephalometric tracings were done to evaluate size related asymmetry while Procrustes superimposition and Euclidean Distance Matrix Analysis (EDMA) was used to see if there was any shape related asymmetries. The authors noticed there was a statistically significant skeletal asymmetry in parental craniofacial complex in OFC patients; they also suggested a left sided directional asymmetry (DA) in these measurements based on the findings. Cephalometrics provide a method of calculating size but not the shape, but ratio's of the size adjusted standardized measurements may be able to give approximate shape description. Perhaps, McIntyre and Mossey's is the only 2D study to compare shape asymmetries in unaffected relatives.

Yoon *et al.* (2004) did a retrospective analysis of frontal cephalograms of 28 UCLP Costa Rican children and compared the frontal cephalogram findings with those of their parents. They found that parents demonstrated an increase in ipsilateral unilateral nasomaxillary width relative to their offspring's unilateral cleft lip and palate side. They also noticed a decrease in head width, mandibular width, total and lower facial heights in unaffected relatives of CL/P individuals. On the other hand, the data showed that total face width, interorbital distance, nasal cavity and maxillary width increased.

Maulina *et al.*, (2006) in a systematic review of all the literature published on craniofacial morphology of parents of CL/P children pointed out that, while there is enough evidence to show that the craniofacial phenotype of the unaffected parents is different from the normal population, there are inconsistencies in previous studies to localize these differences. They also pointed out that the inconsistencies in the study designs make it difficult to compare those studies.

Weinberg *et al.* (2006) conducted a meta-analysis of all the case-control cephalometric studies till date that quantitatively studied craniofacial features on unaffected parents of NS CL/P children and compared those to the control population. After a MEDLINE search to find out the relevant articles on topics in "craniofacial, cephalometrics, cleft and parent" the author found 34 relevant published articles of which nine studies met their inclusion criteria. The authors concluded that significant phenotypic heterogeneity was identified in at least half of the variables studied; however the overarching observation in all the studies is that, an increase in nasal width was noticed in unaffected relatives of NS CL/P patients compared to controls. The authors noticed gender dimorphism in craniofacial phenotype heterogeneity. The synopsis of the meta-analysis was that: unaffected relatives of the NS CL/P patients presented with

"*Wider faces, narrower cranial vaults, longer cranial bases, longer and more protrusive mandibles, shorter upper faces and longer lower faces compared with controls*".

Zandi and Miresmaeili (2007) used cephalometrics to find phenotypic markers that could be used to predict relative risk of cleft incidence in future generations. The authors did a retrospective case-control analysis of 22 pairs of lateral cephalograms of unaffected parents of cleft patients and compared them to the age, gender and race/ethnicity matched controls. They chose seven linear, two angular and five triangular measures to make the comparison and noticed that mandibular body length (Go–Gn) and posterior maxillary triangle (S-N-PNS) was larger in mothers and posterior cranial base (S-Ba) shorter in fathers in the study group. They also noticed that anterior maxillary triangle (SNA) was larger in both parents in the study group. Zandi and Miresmaeili concluded saying that even though there are inconsistencies in opinions on the validity of cephalometric studies in predicting relative risk; unaffected parents present with a distinct craniofacial anatomy that can be picked up by cephalometrics.

Lu *et al.* (2009) studied craniofacial anatomy of unaffected parents of NS CL and also NS CL/P using lateral cephalograms and compared the anatomy with control population. The authors compared cephalometric characteristics of 98 parents of NS CL and 207 parents of NS CL/P to 206 normal people. They noticed that unaffected parents of NS CL/P population present with a distinct craniofacial morphology predictive of NS CL/P and male parent's craniofacial anatomy might be more predictive of recurrent risk of NS CL/P. Even though unaffected parents of NSCL also demonstrated a distinct craniofacial anatomy, the cephalometric findings are not as diagnostic as in unaffected parents of NS CL/P offspring. However, both the unaffected parents of NS CL as well as NS CL/P groups presented with increased nasal and inter-orbital widths; findings consistent with Weinberg *et al.*'s (2006) observations. The cephalometric analysis of

showed that unaffected parents of NS CL/P had a different set of observations compared to unaffected parents of NS CL patients. The authors used stepwise discriminant function analysis to assess diagnostic value of cephalometric features studied and found that the analysis could correctly classify 82.7% of the fathers and 78.6% of the mothers of NSCL children using just the two variables of nasal width and cranial base angle. The same analysis when used with unaffected parents of NS CL/P children could correctly classify 84.2% of the fathers and 80.1% of the mothers using a series of variables, including nasal width, gonial angle, palatal length, and cranial base angle.

2.2.4 Soft tissue morphometric analysis

Soft tissue morphometric analysis includes direct anthropometry and indirect anthropometry techniques (2D and 3D photogrammetry). Traditionally direct anthropometry has been the foremost method to quantitatively study human morphological characteristics and variations. Several investigators studied variations in craniofacial anatomy using this technique. Principal advantage is that no special equipment is required for direct measurements. However there are several disadvantages of this anthropometry which include; patient compliance, communication issues with people with developmental disorders, language and social barriers, possible inconvenience to the patient. Methodological disadvantages include, data collection from direct measurements can be time consuming, data collection is a one-time affair and the future investigators looking for data has to depend on past available data, and archiving craniofacial morphology is not easily possible with this technique.

Mills *et al.* (1968) evaluated if unaffected members of family with one or more oral clefts presents with higher prevalence of “morphological aberrations” compared to normal population.

The authors, based on the existing literature considered *"nasal asymmetry, high arched palate, micromaxilla, V shaped maxillary arch, supernumerary maxillary incisors, peg shaped lateral, congenitally missing anterior teeth and palatal tori"* as morphologic aberrations and are forms of subclinical phenotypes of orofacial clefting. Mills *et al.*, obtained diagnostic records in the form of clinical examinations, color photographs, dental casts and frontal laminographs of families with one or more affected with oral clefts and also of normal population. The authors based their observations on qualitative traits and pointed out that except palatal defects and notches on the lips, there were no differences in morphological traits between study or control groups. The authors concluded that morphological traits themselves are "impossible" to use as tools in predicting orofacial cleft prevalence in the family.

Fraser and Pashayan (1970) used facial photographs, direct anthropometry and physioprints to quantitatively evaluate craniofacial morphology in unaffected relatives of CL/P patients. They compared craniofacial characteristics of 50 unaffected parents of NS CL/P patients and compared them with those of 50 controls. Fraser and Pashayan noticed that facial width and length increased in unaffected relatives while the mid face flattened. The study was thorough in that the raters were blinded as to who comprised the study or control population.

Pashayan and Fraser (1971) evaluated if nostril asymmetry is a microform of Cleft lip using facial photographs of 50 parents of children with CL/P and compared the findings to those of 50 normal people. All the photographs were taken with the same camera and used standardized techniques in image capture and processing. Pashayan and Fraser (1971) measured nostril length, width and symmetry of the unaffected parents of CL/P and compared with normal people and felt that there was no statistical difference in nostril anatomy between the study subjects and normal population.

Figalova and Smahel (1974) in the Czech Republic, studied soft tissue craniofacial features of unaffected relatives and extended family (grandparents as well as aunts and uncles) using direct anthropometry. The anthropometric measures were compared to 50 male and equal numbers of female controls. Fathers of the cleft offspring demonstrated reduced upper face width and increased nose length compared to male controls, while mothers showed reduced mandibular width and increased intercanthal distance compared to female controls. Both the parents were shown to have a significant increase in upper face height.

Farkas and Cheung (1979) used direct anthropometry to collect eight surface measurements and 2 qualitative examinations of the nose in 1312 healthy North-American Caucasians patients aged between six to eighteen years. The object of their study was to identify various forms of nostril asymmetries in healthy North-American Caucasians and also mild forms of cleft lip/palate. They concluded that mild to moderate nostril asymmetry is seen in 88.6% of the population and is considered to be a normal variation but severe asymmetry is seen in 1.6% of the study population and this could be a microform of cleft lip/palate anomaly.

Fukuhara (1989) in a descriptive article to evaluate nostril asymmetry as a microform of cleft lip and palate makes a strong case that soft tissue drape may be concealing skeletal information that could be a microform of cleft. He refutes Pashayan and Fraser's study saying that nostril length and width are poor markers of asymmetry and the sample size of 50 is too small to identify this kind of nostril asymmetry. He recommends that nostril asymmetries should be considered a "*forme fruste*" or microform of cleft lip and palate especially of unilateral type.

Sigler and Ontiveros (1999) studied nasal anatomy of the parents of children with CL, and among the 1000 parents they evaluated came across three parents who had noticeable nostril anatomy and the family was not aware of it. The study falls short to be valid at several levels: it

is a case report; qualitative traits were assessed and obvious bias of evaluating anatomy in known cleft families.

Over the past two decades, the use of surface imaging methods to facilitate soft tissue morphometric analysis has become more common. Stereophotogrammetry is a non-invasive imaging technique gives a 3D depiction of the facial surface, and allows us to calculate linear distances, 3D angular measurements, surface areas and volumes significantly increases statistical power in shape analysis. 3D photogrammetry allows researchers to carry out objective craniofacial measurements with great degree of precision with quick image acquisition and minimum discomfort to the patient (Weinberg and Kolar, 2005).

Weinberg *et al.* (2008) were the first group to apply 3D stereophotogrammetry in the soft tissue analysis of a sample of CL/P unaffected relatives. They evaluated craniofacial shape of unaffected relatives of non-syndromic orofacial cleft patients using both 3D photogrammetry and direct anthropometry. Weinberg and colleagues pointed out the shortcomings of many prior studies on the craniofacial phenotype in cleft families, leading to inconsistent and contradictory findings: primary dependence on 2D cephalometric data, lack of standardization in measurements, failure to address gender dimorphism, failure to address shape versus size discrepancies and statistical errors.

To address the deficiencies of previous studies the authors compared the craniofacial shape of this population with demographically matched normal population and noticed facial shape differences in unaffected family members. Facial landmarks were collected from the 3D surfaces and subjected to statistical shape analysis. It is apparent that clear sexual dimorphisms exist in craniofacial shape of both the study and control population. The authors noticed that the shape differences were localized to specific regions of the face**:** unaffected female relative's soft

tissue anatomy displaying an increased nose width, increased upper face width and excess midface retrusion and in males, unaffected relatives demonstrated increased lower face height, decreased upper face height (mostly right side), and increased upper face and cranial base width compared to controls. Based on these observations Weinberg *et al.* performed Discriminant function analysis (DFA) of all the variables studied. DFA of this data was able to classify 70% of female unaffected relatives, 73% of female controls, 86% of male unaffected relatives and 93% of male controls. Another significant contribution of this study is an attempt to predict if certain unaffected family members posed an elevated risk of CL/P incidence in the next generation, based on the assumption that population with greatest susceptibility show a greatest phenotypic deviation from the normal. This risk allocation approach can correctly classify one third of female and 80% male relatives into at-risk category.

In a follow up study using 3D surface imaging, Weinberg *et al.* (2009) evaluated shape differences in unaffected parents in multiplex cleft families and compared the findings with normal population to see if there are any meaningful shape differences in unaffected parents. Weinberg *et al.* studied soft tissue morphology of 80 unaffected parents and compared those with 80 matched controls using Procrustes analysis of geometric morphometric data. They found the unaffected parents with a positive family history for clefting presented with a distinct facial shape compared to the control population. The authors noted the presence of mid face retrusion, increased lower anterior face height and a decrease in upper face height and increased interorbital width. They also reported gender dimorphism in nasolabial width morphometric variation in the study group when compared to matched controls.

3.0 PURPOSE OF THE PRESENT INVESTIGATION

There is ample evidence in the published literature that differences in craniofacial morphology exist between the unaffected relatives of NS OFC individuals and controls. However, significant contradictions in the description of these craniofacial characteristics exist owing to methodological inconsistencies, as clearly elucidated by Weinberg *et al.* (2006). Also, most of the data from the existing literature is derived from either 2D cephalometric data, which requires radiation exposure. This is difficult to justify in clinically unaffected individuals, and also carries a disadvantage of largely limiting to hard tissue imaging data. Although 3D stereophotogrammetry addresses many of the methodological inconsistencies, this advanced imaging technique has its own shortcomings: it is expensive, not readily available in many parts of the world, and adequate normative population based control data is also not available at the moment. Direct anthropometry, therefore, still remains an attractive alternative for many investigators. At present, however, there is very little direct anthropometric data addressing the question of facial form as a risk factor for clefting.

The purpose of the present study is to further our understanding of the craniofacial phenotype of unaffected relatives within NS OFC families through a rigorous quantitative assessment of craniofacial form/shape using direct anthropometry. This study attempts to address inconsistencies in defining the craniofacial characteristics of this population via direct anthropometry through comparison to a well-matched control population. The use of direct

anthropometry as a tool for characterizing craniofacial morphology in this ‘at-risk’ population has practical applications in settings where the use of advanced 3D imaging technology or 2D cephalometry is not practical.

4.0 MATERIALS AND METHOD

4.1 SAMPLE DESCRIPTION AND RECRUITMENT STRATEGY

The study sample was comprised of clinically unaffected mothers and fathers of children affected with nonsyndromic OFC and a set of population-based healthy controls recruited as part of an international collaboration between the University of Pittsburgh and the Foundation for the Community Control of Hereditary Diseases in Budapest, Hungary. A total of 67 unaffected fathers and 37 male controls were available for study. Likewise, 76 unaffected mothers and 59 female controls were included. All subjects in this study were recruited and seen in Budapest, Hungary between the years 2007 and 2010. Research ethics committee approval at both sites was obtained prior to the start of this study.

The unaffected mothers and fathers of OFC children were identified through probands contained within the Hungarian National Registry of Congenital Anomalies, which has been amassing comprehensive data on all Hungarian children born with birth defects since 1970. All families in the registry have been evaluated by a medical geneticist in order to determine syndromic versus nonsyndromic forms of clefting. Only nonsyndromic individuals were included in this study. All unaffected parents were above the age of 18 and were of European-Caucasian ancestry. The 67 unaffected father and 76 unaffected mothers in the sample were from a total of 78 families identified through the registry. The vast majority of parents (87.4%)

were from simplex families, with no prior history of clefting in the family on either side. The remaining 12.6% of parents were from multiplex families. Broken down by type of cleft, 37.1% of parents had a child with a cleft of the lip only (CL), while 62.9% had a child with a cleft affecting the lip and palate (CLP). For the present study, parents from both CL and CLP families were treated as a single sample. Parents from families with a history of isolated cleft palate were excluded.

Healthy controls were recruited through several mechanisms including a public health nurse service network (The Hungarian Association of Mother and Child and Public Health Nurses) and a temporary staffing agency that contacted individuals throughout Hungary to inform them of the study. Local advertisements were also used. Interested individuals were screened via telephone to determine eligibility. Exclusion factors included any personal or family history of craniofacial syndromes or congenital birth defects and European-Caucasian Ancestry. Eligible individuals were then invited to participate in the study. The 37 male and 59 female controls used in this study were selected from a larger sample of 213 possible controls in order to demographically match as closely as possible the ages of the parental sample (see Table 4-1). Controls were not included if they were either well outside of the age range of the parent sample of the corresponding sex or were biologically related to another control subject.

Table 4-1 Age Statistics for the Unaffected Parent and Control Samples

		N	*Min*	*Max*	*Mean*	*SD*	*t*	*Sig*
Male	*Parent*	67	27	68	42.6	8.8	2.34	0.022
	Control	37	25	65	38.3	9.1		
Female	*Parent*	76	26	63	40.2	7.7	0.67	0.504
	Control	59	25	67	39.3	8.9		

As Table 4-1 indicates, male parents were still significantly older compared to male controls, even after matching by age. Because the craniofacial complex is largely finished growing by the early 20's and the two groups were still quite close in age (only about 4.3 years different) it was determined that any stratification effects would be minimal and not likely to have any appreciable impact on the morphological comparison.

4.2 DATA COLLECTION

Following informed consent, study subjects took part in a phenotyping protocol carried out by trained foundation staff members. All subjects in the study underwent a series of structured interviews designed to capture demographic and medical history information about themselves and their family. Subjects then underwent a craniofacial anthropometric evaluation to capture quantitative measures directly on their head and face (Farkas, 1994; Kolar and Salter, 1997). Using commercially available anthropometric instruments (GPM, Switzerland) a series of 26 standard craniofacial soft-tissue measurements were taken (see Table 4-2 and 4-3). These variables were chosen based on two non-exclusive criteria: evidence of prior positive findings in the literature and capturing information across various regions of the craniofacial complex.

Table 4-2 List of Craniofacial Anthropometric Measurements Collected (Sliding caliper)

Anthropometric measure	*Landmarks involved*	*Instrument used*
Upper Facial Depth (Left)	t-n	Sliding caliper
Upper Facial Depth (Right)	t-n	Sliding caliper
Midfacial Depth (Left)	t-sn	Sliding caliper
Midfacial Depth (Right)	t-sn	Sliding caliper
Lower Facial Depth (Left)	t-gn	Sliding caliper
Lower Facial Depth (Right)	t-gn	Sliding caliper
Intercanthal Width	en-en	Sliding caliper
Outercanthal Width	ex-ex	Sliding caliper
Nasal Width	al-al	Sliding caliper
Subnasal Width	sbal-sbal	Sliding caliper
Philtum Width	cph-cph	Sliding caliper
Labial Fissure Width	ch-ch	Sliding caliper
Morphological Face Height	n-gn	Sliding caliper
Nasal Height	n-sn	Sliding caliper
Upper Lip Height	sn-sto	Sliding caliper
Upper Face Height	n-sto	Sliding caliper
Lower Face Height	sn-gn	Sliding caliper
Mandibular Height	sto-gn	Sliding caliper
Nasal Ala Length (Left)	ac-prn	Sliding caliper
Nasal Ala Length (Right)	ac-prn	Sliding caliper

Table 4-3 List of Craniofacial Anthropometric Measurements Collected (Spreading caliper)

Anthropometric measure	*Landmarks involved*	*Instrument used*
Maximum Cranial Width	eu-eu	Spreading caliper
Maximum Cranial Length	g-op	Spreading caliper
Minimum Frontal Width	ft-ft	Spreading caliper
Maximum Face Width	zy-zy	Spreading caliper
Cranial Base Width	t-t	Spreading caliper
Mandibular Width	go-go	Spreading caliper

Each individual's measurements were recorded on Teleforms, scanned and securely transferred to the University of Pittsburgh, where they were verified, error-checked and saved into a relational database.

4.3 STATISTICAL ANALYSIS

Two separate group comparisons were performed: (1) unaffected fathers were compared to male controls and (2) unaffected mothers were compared to female controls. This decision was based on numerous previous reports that have identified gender-specific facial differences in the parents of cleft-affected offspring (see earlier review). A stepwise discriminant function analysis (DFA) was performed for each comparison in order to identify the combination of craniofacial measures most important for distinguishing between unaffected parents and controls. DFA involves (1) constructing and testing the significance of a discriminant function model comprised of a set of weighted linear continuous variables for distinguishing between two or more groups,

and (2) the classification of individuals into these groups based on the function. The first step maximizes between-group variance and minimizes within-group variance, resulting in the maximal separation between groups. In the context of this study, such a method could be theoretically used to identify 'at-risk' parents by virtue of their craniofacial features, which may have direct relevance for recurrence risk estimation and for identifying potential etiological sub-classes within orofacial cleft population. Multivariate methods, such as DFA, are appropriate in this type of study because craniofacial anthropometric variables are likely to exhibit strong co-variance patterns (i.e., they do not vary independently). Failure to take this co-variance into account can mask the unique contribution and importance of each variable for distinguishing between groups (Meyers *et al*. 2006). DFA is also a valuable tool because it allows for the development of a classification method.

DFA assumes that variables display linearity, normality, an absence of excess multicollinearity and the analysis can be influenced by a presence of outliers. No major problems with linearity or normality were detected by inspection of scatterplots and histograms in this study. Seven variables were dropped due to redundancy with other variables: right upper face depth, right mid-face depth, right lower face depth, right nasal ala length, morphological face height, nasal height, and mandibular height. Dropping these variables had an added benefit of reducing the variable-to-subject ratio. The remaining 19 variables were inspected for evidence of excess multicollinearity. First, bivariate correlations were inspected for values in excess of 0.80, followed by inspection of tolerance and variation inflation factor scores (Meyers *et al*. 2006). No evidence of multicollinearity was detected, thus no additional variables were dropped from the analysis. The presence of multivariate outliers was tested by computing and inspecting Mahalanobis distance scores for each of the four groups included in the present

analysis. A single outlier was detected in the unaffected mother group; this individual was excluded from further analyses. All statistics were carried out in SPSS v19 (IBM Corp, New York).

5.0 RESULTS

5.1 UNAFFECTED FATHERS COMPARED TO MALE CONTROLS

Descriptive statistics for the 19 craniofacial measurements from 37 male controls and 67 unaffected fathers are provided in Table 5-1. A quick inspection of the means revealed a lack of systematic directional differences between the two groups (i.e., no group has universally larger or small measures).

Table 5-1 Descriptive Statistics for the 19 Anthropometric Variables included in the Analysis of Unaffected Fathers and Male Controls

Measurement	Male Controls (n = 37)		Unaffected Fathers (n = 67)	
	Mean	sd	Mean	sd
Maximum Cranial Width	155.68	6.12	159.51	5.99
Minimum Frontal Width	115.26	7.18	121.94	10.65
Maximum Face Width	138.80	8.37	135.48	10.42
Cranial Base Width	139.85	5.80	143.12	6.44
Mandible Width	112.34	6.95	112.49	8.19
Upper Face Depth (left)	117.99	4.50	119.31	4.87
Mid-Face Depth (left)	119.19	5.55	120.24	4.86
Lower Face Depth (left)	140.15	6.74	140.84	7.01
Maximum Cranial Length	191.14	7.51	189.69	6.99
Intercanthal Width	31.55	3.26	32.06	3.53
Outercanthal Width	96.19	8.42	99.99	6.60
Nasal Width	36.97	3.20	37.60	2.63
Subnasal Width	25.77	3.03	27.13	2.83
Philtrum Width	11.12	2.34	12.04	2.28
Labial Fissure Width	54.30	3.85	55.16	4.41
Upper Face Height	75.61	5.51	78.46	5.28
Upper Lip Height	21.47	3.44	20.84	3.42
Lower Face Height	68.89	6.83	69.18	6.37
Nasal Ala Length (left)	36.82	1.86	36.01	2.67

Stepwise DFA was run on the above 19 craniofacial characteristics. After four steps the discriminant model could no longer be improved statistically, indicating that a four-variable solution provided the most parsimonious model. The variables included in the model were: maximum cranial width, minimum frontal width, maximum facial width, and nasal ala length (left). A single statistically significant discriminant function was derived ($p < 0.001$), indicating that the combination of these four predictor variables was capable of differentiating unaffected fathers from male controls. Table 5-2 provided the details of the final discriminant model. Of note, the squared canonical correlation indicated that the discriminant function was able to account for 29.3% of the variance in the outcome variable.

Table 5-2 Statistics for the Final Discriminant Function Model Separating Unaffected Fathers from Male Controls

Eigenvalue	r_{cc}	r_{cc}^2	Wilks' Λ	χ^2	df	*p*
0.414	0.541	0.293	0.707	34.614	4	< 0.001

r_{cc} = canonical correlation

Looking at the variables included in the model and the sign of their coefficients (Table 5-3), it was clear that a combination of increased maximum cranial width and minimum frontal width combined with decreased maximum face width and nasal ala length (left) characterized the unaffected father group. The magnitude of the discriminant loadings provided in Table 5-3 can provide insight into the relative importance of each predictor variable to the discriminant function. In the present case, minimum frontal width was the most important variable in the discriminant model (most important for separating fathers from controls), followed by cranial width, maximum face width and nasal ala length (left).

Table 5-3 Variable Coefficients for the Final Discriminant Function Model Separating Unaffected Fathers from Male Controls

Predictor Variable	Unstandardized coefficients	Discriminant loadings
Minimum Frontal Width	0.054	0.525
Maximum Cranial Width	0.147	0.478
Maximum Face Width	-0.074	-0.256
Nasal Ala Length (left)	-0.198	-0.254
Constant	-12.445	

Discriminant loadings represent the simple linear correlation between each predictor variable and the discriminant function. Positive loadings indicate that predictor variable is greater in unaffected fathers compared to male controls

A discriminant score was calculated for each individual in the sample and these scores were then used to classify members into one of the two original groups: unaffected fathers or male controls. Figure 5-1 shows a set of aligned histograms of discriminant scores for the father and male control group; it is clear from the distribution of scores in the two groups that the discriminant function is effectively able to differentiate fathers from male controls. The discriminant model was able to correctly classify 62.2% male controls and 88.1% of unaffected fathers into their respective groups, for an overall correct hit rate of 78.8% (Table 5-4). The correct classification rate was significantly better than predicted by chance alone (Press's $Q = 34.615$; $p < 0.001$). Jack-knife cross-validation showed no loss of classification accuracy (Table 5-5).

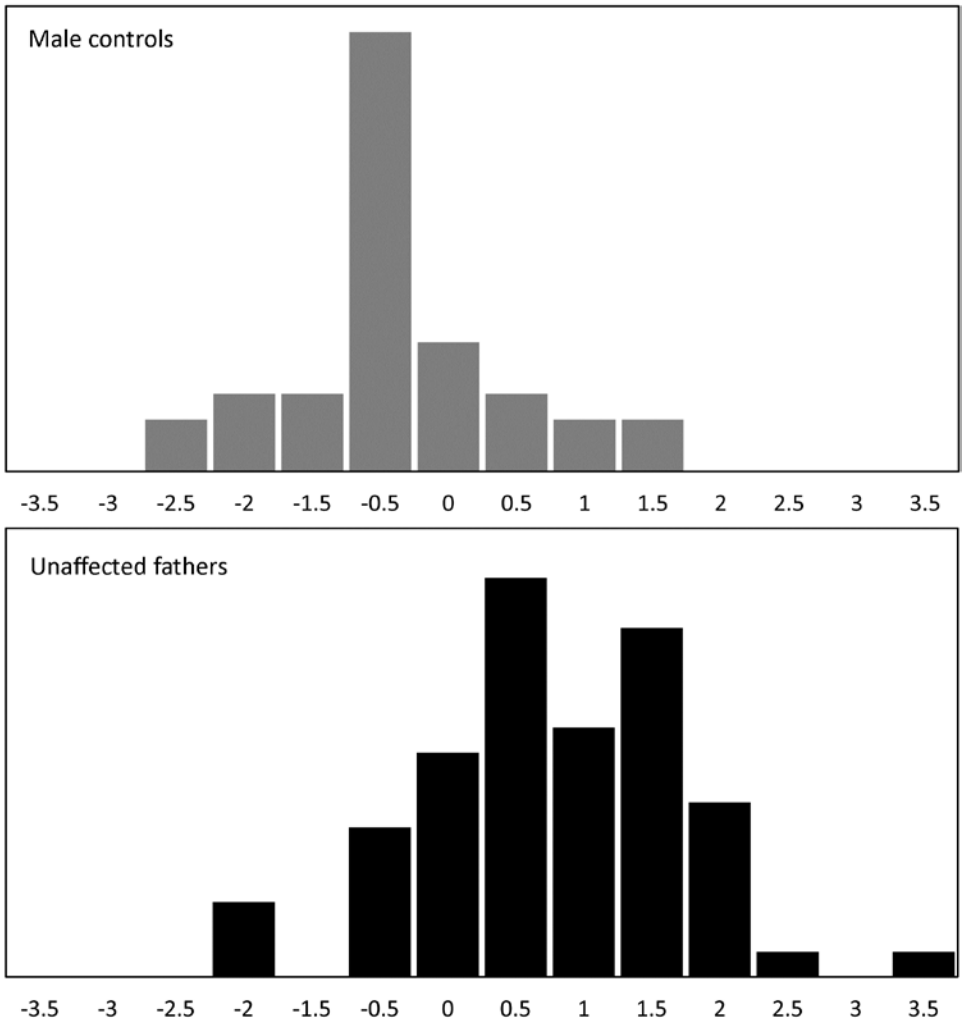

Figure 5-1 Stacked Histograms Showing the Distribution of Discriminant Scores for the Unaffected Father and Male Control Groups

Table 5-4 Classification Statistics for Male Relatives and Controls

		Predicted Group	
		Male Control	Unaffected Father
True Group	Male Control	23 (62.2%)	14 (37.8%)
	Unaffected Father	8 (11.9%)	59 (88.1%)

Overall correct classification rate = 78.8%

Press's Q = 34.615 ($p < 0.001$)

Table 5-5 Cross-Validated Classification Statistics for Male Relatives and Controls

		Predicted Group	
		Male Control	Unaffected Father
True Group	Male Control	23 (62.2%)	14 (37.8%)
	Unaffected Father	8 (11.9%)	59 (88.1%)

Overall cross-validated correct classification rate = 78.8%

Press's Q = 34.615 ($p < 0.001$)

5.2 UNAFFECTED MOTHERS COMPARED TO FEMALE CONTROLS

Descriptive statistics for the 19 craniofacial measurements from 59 female controls and 75 unaffected mothers are provided in Table 5-6. Inspection of the means revealed that the vast majority of measures (all but maximum facial width) were slightly larger in the unaffected mothers compared with female controls.

Table 5-6 Descriptive Statistics for the 19 Anthropometric Variables included in the Analysis of Unaffected Mothers and Male Controls

Measurement	Female Controls (n = 59)		Unaffected Mothers (n = 75)	
	Mean	sd	Mean	Sd
Maximum Cranial Width	148.07	5.48	151.53	6.23
Minimum Frontal Width	110.43	6.21	118.65	10.15
Maximum Face Width	130.87	7.77	129.01	9.17
Cranial Base Width	130.73	5.69	132.81	6.44
Mandible Width	104.07	6.15	106.46	6.99
Upper Face Depth (left)	111.13	4.62	112.49	4.32
Mid-Face Depth (left)	110.39	6.07	112.64	5.40
Lower Face Depth (left)	127.35	7.98	129.29	8.03
Maximum Cranial Length	181.02	6.45	180.87	6.34
Intercanthal Width	29.80	3.10	31.19	2.96
Outercanthal Width	92.69	7.15	96.42	5.96
Nasal Width	33.17	3.34	33.79	2.61
Subnasal Width	23.57	3.24	23.99	2.76
Philtrum Width	10.18	2.21	10.34	1.67
Labial Fissure Width	51.41	4.07	52.13	3.67
Upper Face Height	71.15	4.45	73.67	3.45
Upper Lip Height	19.50	2.75	19.06	2.44
Lower Face Height	61.64	5.45	61.65	4.68
Nasal Ala Length (left)	32.77	2.17	32.89	2.08

As with the male sample, stepwise DFA was run on the same 19 craniofacial characteristics. After five steps the discriminant model could no longer be improved statistically, indicating that a five-variable solution provided the most parsimonious model. The variables included in the model were: minimum frontal width, upper face height, maximum face width, philtrum width, and upper face depth (left). A single statistically significant discriminant function was derived ($p < 0.001$), indicating that the combination of these five predictor variables was capable of differentiating unaffected mothers from female controls. Table 5-7 provided the details of the final discriminant model. The squared canonical correlation indicated that the discriminant function was able to account for 38.1% of the variance in the outcome variable.

Table 5-7 Statistics for the Final Discriminant Function Model Separating Unaffected Mothers and Female Controls

Eigenvalue	r_{cc}	r_{cc}^2	Wilks' Λ	χ^2	df	*p*
0.615	0.617	0.381	0.619	62.063	5	< 0.001

r_{cc} = canonical correlation

Considering the five predictor variables included in the model and the sign of the discriminant loadings (Table 5-8), it was clear that a combination of increased minimum frontal width, upper face depth (left), philtrum width and upper face height coupled with decreased maximum face width characterized the unaffected mothers. The magnitude of the discriminant loadings revealed that minimum frontal width was the most important variable for group discrimination, followed by upper face height, upper face depth (left), maximum face width and philtrum width.

Table 5-8 Variable Coefficients for the Final Discriminant Function Model Separating Unaffected Mothers from Female Controls

Predictor Variable	Unstandardized coefficients	Discriminant loadings
Minimum Frontal Width	0.128	0.607
Upper Face Height	0.092	0.410
Upper Face Depth (left)	0.099	0.196
Maximum Face Width	-0.102	-0.139
Philtrum Width	-0.254	0.054
Constant	-16.576	

Discriminant loadings represent the simple linear correlation between each predictor variable and the discriminant function. Positive loadings indicate that predictor variable is greater in unaffected mothers compared to female controls

Figure 5-2 shows a set of aligned histograms of discriminant scores for the mother and female control group; it is clear from the distribution of scores in the two groups that the discriminant function is effectively able to differentiate mothers from female controls. The discriminant function was able to correctly classify 79.7% female controls and 82.7% of unaffected mothers into their respective groups, for an overall correct hit rate of 81.3 (Table 5-9). The correct classification rate was significantly better than predicted by chance alone (Press's Q = 52.657; $p < 0.001$). The classification accuracy was slightly reduced in the cross-validated results, with an overall correct hit rate of 78.4% (Table 5-10).

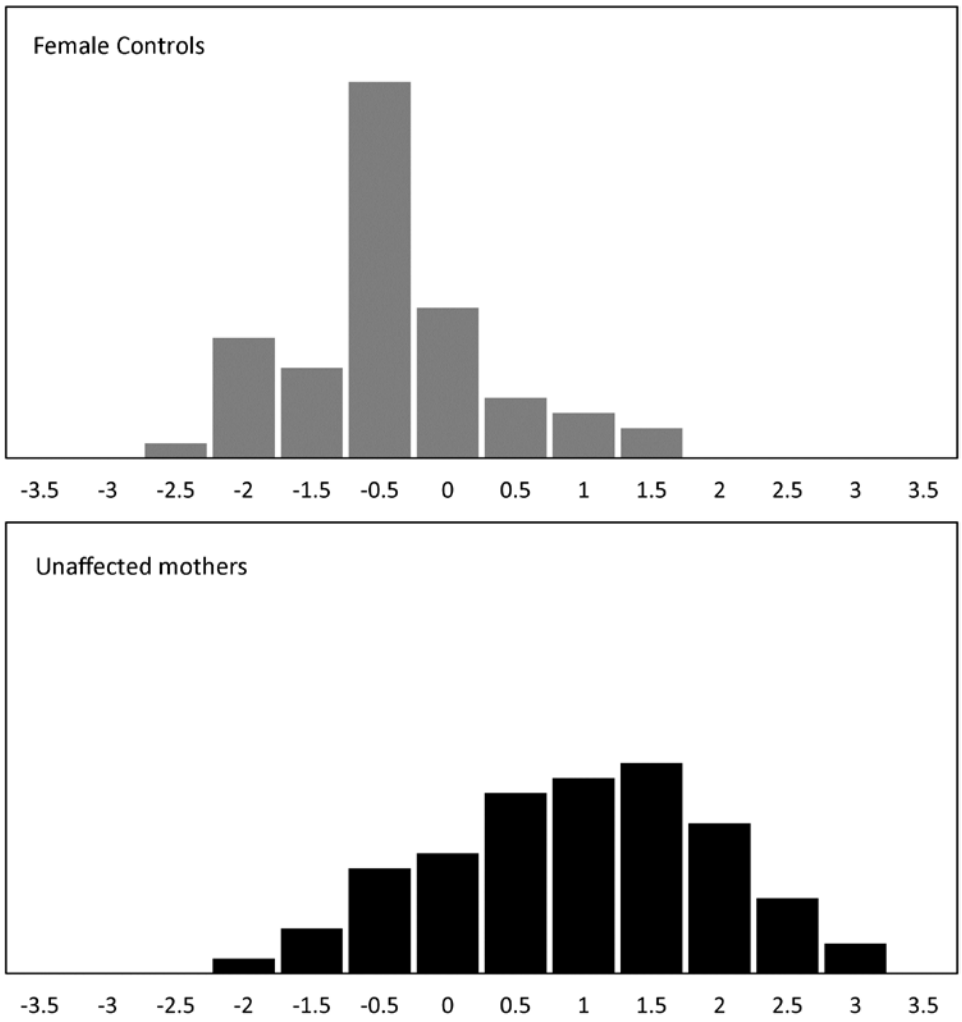

Figure 5-2 Stacked Histograms Showing the Distribution of Discriminant Scores for the Unaffected Mother and Female Control Groups

Table 5-9 Classification Statistics for Female Relatives and Controls

		Predicted Group	
		Female Control	Unaffected Mother
True Group	Female Control	47 (79.7%)	12 (20.3%)
	Unaffected Mother	13 (17.3%)	62 (82.7%)

Overall correct classification rate = 81.3%

Press's Q = 52.657 ($p < 0.001$)

Table 5-10 Cross-Validated Classification Statistics for Female Relatives and Controls

		Predicted Group	
		Female Control	Unaffected Mother
True Group	Female Control	45 (76.3%)	14 (23.7%)
	Unaffected Mother	15 (20%)	60 (80%)

Overall cross-validated correct classification rate = 78.4%

Press's Q = 43.104 ($p < 0.001$)

6.0 DISCUSSION

6.1 SUMMARY OF CURRENT FINDINGS

Direct anthropometry was used to compare craniofacial form/shape of unaffected parents of known NS OFC patients to a demographically-matched unaffected control population, in a gender-specific manner. Nineteen linear direct anthropometric measurements were evaluated using a stepwise DFA in males and females separately. The derived discriminant function (DF) models were highly significant ($p < 0.001$). The DF models showed that a combination of craniofacial measures were capable of effectively distinguishing unaffected family members from controls and that the salient discriminating features are localized to specific regions of the face in a partly gender-specific manner.

In males, the discriminant function included four variables and was able to correctly classify 78.8% of controls and unaffected fathers. Examining the pattern of discriminant loadings, the four craniofacial characteristics for group discrimination were minimum frontal width, maximum cranial width, maximum facial width and nasal ala length. The model showed that unaffected fathers tended to have wider foreheads and skull vaults, narrower facial width and shorter ala (left). In females, the discriminant function performed slightly better, correctly classifying a total of 81.3% of unaffected mothers and controls. The final model included five variables, which included minimum frontal width, upper face height, upper face depth, maximum

face width and philtrum width. Like unaffected fathers, unaffected mothers tended to have wider foreheads and narrower upper faces. In addition, unaffected mothers displayed longer and more protrusive upper faces along with wider philtrums.

The findings illustrate that a discriminant model constructed from a small subset of craniofacial measures collected via direct anthropometry can effectively discriminate unaffected parents from controls, providing further evidence that the identification of phenotype markers indicative of latent orofacial cleft susceptibility is possible using low-tech methods.

6.2 COMPARISON OF RESULTS TO EARLIER STUDIES

There were significant discrepancies between the current study and earlier literature on the nature of craniofacial characteristics in the unaffected relatives from NS OFC families (see Table 6-1). The current study pointed out that minimum frontal width was shown to be increased in unaffected relatives(both mothers and fathers) compared with matched controls, which contradicts several earlier studies (Figalova and Smahel, 1974; Raghavan *et al.*, 1994; Al-Emran *et al.*, 1999 and Weinberg *et al.*, 2006). However, Fraser and Pashayan's (1970) study was in agreement with the current study's findings. Another significant contradiction between the current study and earlier studies was that maximum cranial width was found to be increased in unaffected fathers; a finding not supported by any other published study that evaluated this characteristic.

Upper anterior face height was shown to have increased in mothers, which agreed with several earlier observations (Figalova and Smahel, 1974; Ward *et al.*, 1989) but disagreed with many others (see Table 6-1). The current study also pointed out that maximum facial width

decreased in unaffected parents (fathers and mothers) of NS OFC patients, which was what Coccaro *et al.* (1972) and Raghavan et al. (1994) noticed with their sample. However, several other studies contradicted our study's observation on this measure (Yoon *et al.*, 2004; Weinberg *et al.*, 2006). The current study also noticed an increase in depth of upper face (left); and the findings of Raghavan *et al.* (1994) agreed with this observation. However, Fraser and Pashayan (1970) and Nakasima and Ichinose (1983)'s studies disagreed with the current study on this measure.

Table 6-1 Comparison of the Current Findings with those of Prior Studies

Studies	***Upper face width***	***Max cranial width***	***Upper face height***	***Max face width***	***Upper face depth***	***Philtrum width***	***Alar length (Left)***
Present analysis	↑♂ ↑♀	↑♂	↑♀	↓♂ ↓♀	↑♀	↑♀	↓♂
Fraser and Pashayan, 1970	↑♂ ↑♀			↑♂ ↑♀	↓♂ ↓♀		
Coccaro *et al.*, 1972			↓♂ ↓♀	↓♂ ↓♀			
Figalová and Šmahel, 1974	↓♂	↓	↑♂ ↑♀				
Nakasima and Ichinose, 1983		↓♂ ↓♀	↓♂ ↓♀		↓♂ ↓♀		
Ward *et al*., 1989			↑♂ ↑♀				
Raghavan *et al.*, 1994	↓	↓	↓	↓	↑		
Mossey *et al.*, 1998a			ns				
AlEmran *et al.*, 1999	↓♂ ↓♀	↓♂ ↓♀					
McIntyre and Mossey, 2003a	ns		↓				
Perkiomaki *et al.*, 2003			↓				
Yoon *et al.*, 2004	↑	↓	↓	↑			
Weinberg *et al.*, 2006b	↓		↓	↑			

The current study agreed with the existing evidence (Mossey *et al.*, 1998, Neiswanger *et al.*, 2007, Weinberg *et al.*, 2006 and Weinberg *et al.*, 2008) on gender dimorphism in craniofacial

characteristics of unaffected relatives compared to the control population, and also to the fact that these characteristics are localized to specific regions of the face. The current study pointed out that minimum frontal width increased and maximum face width decreased in both genders. On the other hand, maximum cranial width was shown to be increased only in males, while upper face height and upper face depth was shown to be increased only in females.

Significant differences in findings between the current and previous studies may be explained by methodological inconsistencies across all the available literature. One major inconsistency relates to the method of data collection. Most of the previous studies (e.g., Coccaro *et al.,* 1972, Perkiomaki *et al.,* 2003; Yoon *et al.,* 2004) used cephalometric imaging to evaluate craniofacial characteristics. Even though there was some data available from direct anthropometry, most of the findings were based on qualitative observations (Mills *et al.,* 1968; Fukuhara, 1989); however Figalova and Smahel's (1974) study was based on quantitative analysis of traits and this study is closest in observations to the current study. 3D photogrammetry (Weinberg *et al.,* 2008 and Weinberg *et al.,* 2009) also was used for quantitative assessment of craniofacial characteristics, and the observations contradicted our study's findings.

Disagreements in the available literature may also be because of significant differences in the ethnicity of study population. None of the existing studies except Figalova and Smahel (1974) looked exclusively at a central European population. Other studies used data collected from American (Mills *et al.,* 1968; Coccaro *et al.,* 1972; Ward *et al.,* 1989; Weinberg *et al.,* 2008 and Weinberg *et al.,* 2009), Japanese (Fraser and Pashayan, 1970; Nakasima and Ichinose, 1983), Chilean (Perkiomaki *et al.,* 2003 and Yoon *et al.,* 2004) or East Indian (Raghavan *et al.,*

1994) samples. It is possible that the etiological characteristics of these various populations differ, which would lead to increased heterogeneity.

Also, there is no mention of the nature of cleft family composition in most of the earlier studies. Our study had a mix of both simplex and multiplex families (87.4% simplex families). Weinberg *et al.* (2008; 2009) study looked exclusively at multiplex families, while other studies did not provide a good description of family composition in this aspect. This is important because, the proportion of simplex families in the sample is likely to significantly increase the etiological heterogeneity, and subsequently may explain some of the phenotypic discrepancies present in the literature. Another shortcoming of the current study is that unaffected relatives of both NS CL and NS CL/P were lumped into the same group, which could influence the outcome of our study.

It is difficult to offer a biological rationale for unique craniofacial characteristics observed in the current analysis, particularly given the small sample sizes used in this study. Clearly, the results here contradict many prior findings. Although arguments can be made for biological relevance of almost any trait, at this point it is essential to both independently verify the reliability of the data collection methods and independently confirm the results of the present study through replication. Therefore, the results of the current study should be approached with caution before any overarching interpretations can be made.

6.3 POTENTIAL IMPLICATIONS

Identifying subclinical risk phenotypes in unaffected parents of NS OFC children has the potential to improve the correlation between genotype and phenotype in families with the trait.

One possible net outcome of this improvement may be increased power for mapping the genes that underlie NS OFC liability. It would also be possible to more accurately predict recurrence risk of OFC in the context of genetic counseling.

The above statements assume that unaffected parents can be accurately sorted into risk classes, based on aspects of their phenotype. Although the statistical models produced here show good ability to discriminate unaffected parents from population-based controls, the validity of the models used in the study must be established first. Replicating the study with a larger independent sample is essential if the aforementioned promises are to ever become a reality.

6.4 STRENGTHS AND LIMITATIONS OF THE STUDY

The primary strength of the study is that data collection was done using direct anthropometry, which is a low-tech method of data collection that can be implemented almost anywhere and has the potential to be translated into a useful clinical tool if independent validation of the current approach can be done. The study was conducted with data gathered from a relatively homogenous population (Hungarian population, European-Caucasian). Further, male and female samples were analyzed separately to account for gender dimorphism. There were also several limitations. For example, the reliability of the data collected at the clinical setting is unclear; as no intra- or inter-observer measurement error data were available. Further, the sample sizes were still relatively small, eliminating the possibility of using a 'hold-out' sample for validating the results.

7.0 CONCLUSIONS

While it is widely accepted that the craniofacial morphology of unaffected parents of NS OFC is different compared to the normal population, understanding this phenotype has proven to be complicated. The current study identified that there is a difference in craniofacial morphology of unaffected parents of NS OFC children. The study found out that there is an increase in cranial width and minimum frontal width, while face width and nasal ala length (left) decreased in unaffected fathers compared to the male controls. The study showed that salient discriminating features are localized to specific regions of the face in a partly gender-specific manner. Unaffected mothers were shown to have an increase in minimum frontal width, upper face height and upper face depth (increased protrusion) and philtrum width, while the maximum face width decreased compared to the controls. The study showed that a model derived using a small subset of direct anthropometrically measured craniofacial features can be used to discriminate unaffected parents from the controls. The accuracy of this model is excellent in identifying at risk female and male parents, in this study population.

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