OLFACTORY DEFICITS IN CLEFT LIP AND PALATE

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Cleft lip with or without an associated cleft palate (CL/P) is one of the most common congenital birth defects. Both the frequency with which it occurs and the high psychosocial and financial costs associated with CL/P contribute to a significant public health interest in the condition. Defining the spectrum of disability associated with CL/P will serve to improve treatment planning and caregiver education and may inform efforts to describe the etiology of this complex trait.

The principal aim of this project was to test the hypothesis that significant olfactory dysfunction exists in individuals with repaired orofacial clefts (OFCs; cleft lip, with or without cleft palate ((CL/P) and isolated cleft palate (CP)) and their first degree relatives (FDRs). Two small studies and anecdotal reports suggest impaired olfaction in individuals with OFCs, but this is the first investigation of the olfactory phenotype in both cases and relatives. Genetic, physiologic, and developmental features of individuals with OFCs provide plausible explanations for this poorly explored phenomenon.

Methods: The widely used and extensively validated University of Pennsylvania Smell Identification Test (UPSIT) - a "scratch and sniff" 40 item odor discrimination test - was employed to describe olfactory ability in a sample of 12 subjects with non-syndromic CL/P or CP and 39 of their unaffected FDRs. Control data was obtained from published norms on over

2000 individuals. Standard non-parametric and categorical statistics were used to test for group differences in olfactory performance.

Results: The likelihood of having a smell deficit was increased nearly fivefold in the cases (OR=4.94; 95% CI 1.56-15.65) compared with controls. Similarly, the likelihood of having a smell deficit was increased nearly fourfold in the FDRs (OR=3.87; 95% CI 1.99-7.52) compared with controls. Cases scored significantly lower on the UPSIT compared with their FDRs ($p<0.043$), indicating that the olfactory deficit was greater in cases. This study provides the first evidence of olfactory deficits in the FDRs of OFC cases and confirms the existence of olfactory deficits in OFC patients.

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PREFACE

I am grateful first and foremost to the families who have been willing to open their lives to yet another research study. Their participation reflects a selfless desire to move the field forward for the benefit of families yet to come.

Family and dear friends have gone above and beyond in supporting my career change and sudden reentry into academic life after years of independence. I literally could not have pursued this dream without them and am deeply grateful for their love and encouragement, not to mention a warm bed and regular meals.

To Raj especially I owe many, many hours of walks in the park, movies watched to completion, and conversation uninterrupted by talk of genes, papers, exams, and exhaustion. I'm continually inspired by his faith in me and words of wisdom, both of which have kept me on this path innumerable times.

My advisor and mentor, Seth Weinberg, has my eternal thanks for having confidence in my abilities and allowing me the flexibility to complete this project. His never-ending energy and ability to guide without dictating the course enabled me to push myself beyond what I thought possible and learn more than I would have believed about this complex field. The entire staff of the CCDG was invaluable in keeping this project organized and on-track.

Finally, thanks to my committee for their careful review and thoughtful comments. It was a pleasure and a privilege to work with them.

1.0 INTRODUCTION

Little has been published about possible impairment of the sense of smell in individuals with non-syndromic cleft lip with or without cleft palate (CL/P) or isolated cleft palate (CP). Genetic, physiologic, and neuroanatomic features of individuals with CL/P or CP provide plausible explanations for this under recognized phenomenon. Neuroimaging studies, for example, have shown morphological abnormalities in the ventral frontal cortex of individuals with facial clefts, a brain region critical to the processing of olfactory information. Additionally, both orofacial clefts and anosmia (total inability to detect odor) are a major features of the genetic disorder Kallmann Syndrome. Finally, regardless of repair status considerable dysmorphology is likely to be present in the internal nasal architecture of those with OFCs; such changes can impact airflow, nasal volume, and airway dynamics, all of which can have a functional impact on olfaction. Given that both OFCs and the associated corrective surgeries typically impact the nasal cavity, it is expected that olfaction will be either reduced (hyposmia) or absent (anosmia) in OFC individuals at higher rates than in the general population.

Two previous studies as well as anecdotal evidence suggest that CL/P patients may have a diminished (hyposmia) or absent (anosmia) sense of smell. In a study of 15 subjects (nine boys, six girls) with CL/P, Richman et al demonstrated a reduced ability to discriminate between odors in fully 50% of affected males, compared with only 9% of unaffected male controls (Richman et al. 1988). There was no difference in odor discrimination ability between affected

and control females. In a group of 25 patients, Grossmann et al (2005) obtained mixed results in olfactory assessment of CL/P subjects. This study detected reduced airflow and a higher threshold for odor detection in CL/P subjects, but in direct contrast to Richman and colleagues, found no evidence of difficulty with odor discrimination (Grossmann et al. 2005). It has been established by several groups (Fukushiro and Trindade 2005; Grossmann et al. 2005) that CL/P patients have changes in nasal airflow and total nasal cavity volume, including a reduction in both airflow and nasal volume on the cleft side in unilaterally affected (UCLP) subjects (Pirvola et al. 2002; Hebert et al. 2003). Gross anatomic differences leading to reduced airflow are an attractive explanation for olfactory deficits but are likely to be overly simplistic given these mixed results.

The results of several additional areas of inquiry provide insight into other possible underlying causes for olfactory dysfunction with OFC. Kallmann Syndrome (KS), a genetic disorder characterized by facial clefting, anosmia and idiopathic hypogonadotropic hypogonadism, has causative *FGFR1* mutations in common with non-syndromic (NS) CL/P. Dodé and others (Dode et al. 2003; Murray and Schutte 2004) have noted that KS patients with *FGFR1* mutations have a relatively high (~25%) prevalence of orofacial clefts, an intriguing overlap given the established role of *FGFR1* in NS clefting (Riley et al. 2007; Riley and Murray 2007). FGFR1 expression has also been shown to play an important role in olfactory bulb morphogenesis (Pirvola et al. 2002; Hebert et al. 2003; Dode et al. 2007). A further line of evidence implicates changes in brain structure. Traumatic brain injury (TBI) experience confirms that damage to the ventral frontal cortex (VFC, containing the olfactory bulb) frequently results in a reduced ability to discriminate between odors (Yousem et al. 1999).

Intriguingly, Boes and Nopoulos have discovered structural abnormalities in the ventral frontal cortex (VFC) in male CL/P patients (Nopoulos et al. 2005; Boes et al. 2007).

The majority of evidence to date suggests that olfaction is in some way compromised in individuals with OFCs. Various biologically plausible explanations exist. Exploring each of these possible mechanisms was beyond the scope of this project. The goal of this project is to determine whether olfactory deficits are indeed part of the orofacial clefting phenotype. Positive results in this inquiry are expected to lead to a more rigorous investigation of the underlying causes of reduced olfaction. Given that a variety of subclinical phenotypes have been identified in the relatives of those with OFCs, we proposed that unaffected first degree relatives of OFC cases would also show some level of olfactory dysfunction. This study marks the first time that these relatives have been assessed for the olfactory phenotype.

Olfaction is of great importance in social, emotional, and environmental health. If reduced olfactory ability is indeed a part of the orofacial cleft phenotype, it is deserving of attention in its own right given the negative consequences associated with smell problems.

Of more academic interest is the ability of expanded phenotypes (often referred to as "subclinical phenotypes" because they may appear in individuals not visibly affected with an overt defect) to inform our search for candidate genes and improve estimation of recurrence risks. With the recognition that clefting appears multiple times in some pedigrees, numerous centers around the world sought to discover the genetic basis for OFCs and to identify predisposing features in unaffected family members. In the early 1960's, Fukuhara and colleagues at Tokyo Medical and Dental University became interested in what they called a "carrier status" for cleft lip and palate. Through a series (Fukuhara and Saito 1962; Fukuhara and Saito 1963; Fukuhara 1965) of radiographic studies, they discovered a variety of

abnormalities in the bony nasal structures, nasal septum, and palates of unaffected relatives of CL/P individuals. Two-thirds of unaffected monozygotic twins of individuals with CL/P were found to have a reduction in nasal cavity width in a later study (Johnston and Hunter 1989); similar findings were described in affected individuals (Liu et al. 1992). As previously discussed, the structure of the nasal cavity is of critical importance in olfaction. This, along with previously described genetic contributions to both olfactory system development and palate morphogenesis, suggests that any olfactory deficits identified in OFC patients may extend to their visibly unaffected relatives.

More recently, the Center for Craniofacial and Dental Genetics (CCDG) at the University of Pittsburgh in particular has had success in identifying several diverse subclinical phenotypes in family members of OFC patients (Weinberg et al. 2006) in numerous ethnic groups. Subclinical phenotypes described in relatives of CL/P patients by the CCDG and others include subepithelial defects in the orbicularis oris muscle (Klotz et al. 2010), whorls on the lower lip (Neiswanger et al. 2009), altered dermatoglyphic patterns (Neiswanger et al. 2002), an excess of non-right handedness (Scott et al. 2005), and a specific pattern of facial proportions in the parents of children with OFCs (Weinberg et al. 2009).

Identification of a new subclinical phenotype will aid in providing genetic counseling to families, improve treatment and management, and possibly lend power to genetic association studies in CL/P and CP populations. The presence of familial morphologic changes and specifically the presence of familial changes in nasal cavity structure provided support to our hypothesis that olfactory deficits may in fact be a previously unrecognized subclinical feature of the non-syndromic orofacial cleft phenotype.

2.0 SPECIFIC AIMS

2.1 SPECIFIC AIM 1

Assess and compare odor discrimination ability in CL/P and CP cases using the 40-item University of Pennsylvania Smell Identification Test.

Hypothesis relating to Aim 1. Individuals with non-syndromic CL/P and CP will demonstrate deficits in odor discrimination compared with both published norms and unaffected relatives on the Smell Identification Test.

2.2 SPECIFIC AIM 2

Assess and compare odor discrimination ability in the unaffected relatives of CL/P or CP cases using the 40-item University of Pennsylvania Smell Identification Test.

Hypothesis relating to Aim 2. Unaffected relatives will demonstrate deficits in odor discrimination compared with published norms on the Smell Identification Test.

2.3 SPECIFIC AIM 3:

Assess sex differences in odor discrimination among cases and unaffected relatives as compared with age-matched norms using the University of Pennsylvania Smell Identification Test.

Hypothesis relating to Aim 3. Compared with females, males affected with non-syndromic CL/P and CP and their male unaffected relatives will show a greater reduction in ability to discriminate between odors compared with age and gender matched controls.

3.0 BACKGROUND AND SIGNIFICANCE

3.1 CLEFT LIP AND PALATE

3.1.1 Epidemiology

Cleft lip, with or without cleft palate (CL/P) and cleft palate only (CP) may occur as an isolated birth defect or as part of a larger sequence or syndrome. Only individuals with nonsyndromic (isolated) CL/P or CP were considered in the course of this study; all epidemiologic data therefore refer to the isolated CL/P and CP population only. Of the two conditions, CL/P is more common with CP occurring half to one-third as frequently (Spritz). An estimated 70% (Spritz) of CL/P is of the non-syndromic variety, while between 15-50% of CP occurs in the absence of a syndrome or sequence (Croen et al. ; Spritz).

Cleft lip and/or cleft palate are common, pan ethnic conditions. A large study of over 2,000 ethnically diverse CL/P and CP patients in California demonstrated an incidence of 1.5-2 per 1,000 live births, with the incidence varying by population (Croen et al. 1998). In this particular cohort, the prevalence of CL/P was highest among Native Americans, followed by Whites, Japanese, and Chinese and lowest in African Americans. CP showed a similar distribution, with the highest rates in Native Americans followed by Whites, Hispanics, and African Americans.

3.1.2 Developmental origin of orofacial clefting

It is useful to consider the normal progression of midface development in order to fully appreciate the developmental origin of OFCs. At week 4 of embryonic development (Cohen 2006) just after closure of the anterior neural tube, the foundation of future midface structures arises in the form of five prominences (Figure 1) or swellings: a single frontonasal prominence and paired bilateral maxillary and mandibular prominences (Bender). These structures originate from migrated neural crest cells.

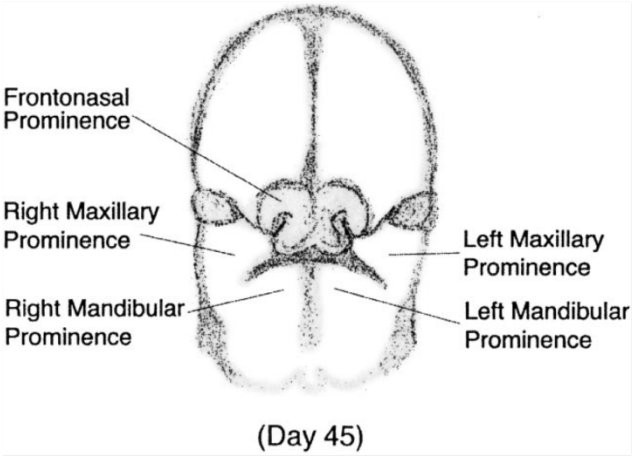

Figure 1: Facial Features of Embryo[i]

Later in the fourth week, the mandibular prominences merge to form the lower jaw (mandible), lower lip, and lower cheeks while the maxillary prominences continue to grow towards each other. By week 6-7, nasal pits from nasal placodes surrounded by the frontonasal swellings have formed nasal pits (later the nares) and the medial (inner) portions of the frontonasal prominence have fused with each other and with the maxillary prominences, giving rise to the upper lip. This allows for development of the intermaxillary segment, which will form

the small primary palate, philtrum, and dental arch. During this same time, shelf-like structures extend from the maxillary process then merge following programmed cell death and subsequent fusion along the advancing edges. These shelves form the secondary palate, which comprises the majority of the adult hard palate. Each stage of this process requires carefully orchestrated bilateral symmetric cell migration, differentiation, growth, and apoptosis to allow for the complete fusion of homologous structures into single unified midface structures. Numerous proteins are involved in this complex process. Of particular note to this investigation, FGFR1 participates in apoptosis at the medial edge epithelium of the palatal shelves- a critical antecedent to the fusion of the bilateral shelves to form an intact hard palate; (Britto et al. 2002) the role of this protein in olfaction will be discussed in section 1.2.2. An error in the control or execution of any part of this process can lead to failure of paired structures to unite and result in an orofacial cleft. A variety of cleft configurations ranging from a slight unilateral cleft of the lip to a bilateral, completely open lip and palate are possible (Figure 2), depending on which processes are impacted (Cohen 2006 ; Mossey et al. 2009).

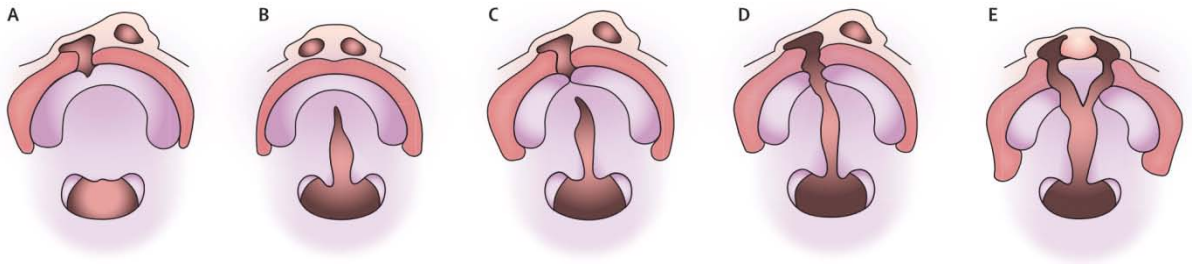

Figure 2: Non-syndromic orofacial clefts[ii]

A. Cleft lip and alveolus. B. Cleft palate. C. Incomplete unilateral cleft lip and palate. D. Complete unilateral cleft lip and palate. E. Complete bilateral cleft lip and palate.

3.2 OLFACTION

3.2.1 Anatomic and molecular basis of olfaction

Olfaction (the process of detecting and identifying odors) relies on processes in both the nasal cavity and the brain. For the purposes of this study the primary process of interest is odor detection, which occurs in the nasal cavity and during the earliest stages of brain involvement.

The first stage in the process of olfaction is detection of an odorant. In order for detection to occur, air containing volatile aromatic compounds must be taken in (inhaled) through the vestibule of the nose- that is, the area just inside the nostrils. This area is divided by the nasal septum, which separates air intake from the left and right nostrils and supports the nasal cartilage (Sahin-Yilmaz and Naclerio). Several components of the nasal architecture interact at this point to create individual variations in airflow volume and patterns. Congenital differences such as thickness of the nasal cartilage or variations in septum or alae shape along with acquired factors such as congestion, polyps, and trauma all create subtle (or not so subtle) differences in the initial phase of olfaction (Pinto 2011).

Once air has entered the nose, three bony structures called turbinates direct its flow through the remainder of the nasal cavity. Approximately 10% of all air inhaled through the nose is eventually directed to the olfactory region (Sahin-Yilmaz et al.) by these structures. The turbinates are described by their position relative to the vestibule and are referred to as the inferior, middle, and superior turbinates. The uppermost (superior) and middle turbinate surfaces along with the roof of the nasal cavity and a small portion of the septum are comprised of

olfactory epithelium (Pinto 2011; Sahin-Yilmaz et al.). In a unique projection of the nervous system into the environment, olfactory sensory neurons are present in this lining and receive direct stimulation from inhaled odor molecules (Hoover). The olfactory neurons project through the skull at the cribiform plate to the olfactory bulbs, paired structures located above the olfactory epithelium and just below the frontal lobe of the human brain (Hoover). As with any neuron, the basis of olfactory neuron function is the fitting of a stimulant molecule, in this case an odorant, with an appropriately shaped receptor located in the plasma membrane of the neuron (Hoover ; Pinto 2011). In the final stage of odor detection, the fitting of odorant to receptor triggers a biochemical response and subsequent nerve impulse, which in turn stimulates the olfactory bulb.

Each olfactory neuron expresses one just type of odorant receptor (OR); 10-20 million of these cells are required for discrimination of odors (Pinto). It is likely that each odorant stimulates a unique and characteristic pattern of receptors rather than only a single receptor type (Holbrook and Leopold 2006). Reflective of this complexity is the large number of genes in the human genome responsible for the development of ORs; the OR gene family numbers 900 in humans and represents 3-5% of the entire genome (Baghai et al. ; Young and Trask), although it bears mentioning that over half of these genes have degenerated into pseudogenes (Young et al.).

Within the olfactory bulb, the individual olfactory neuron axons converge into glomeruli representing one type or category of receptor (odorant), allowing for the efficient transduction of information to the olfactory cortex for processing (Figure 3). Several types of cells provide output from the olfactory bulbs, with mitral cells being the primary connection from the olfactory bulbs to the cortex (Reed 2004). Information from the olfactory bulb is processed in a

complex fashion in the olfactory cortex of the cerebrum. It is at this stage of the process that detection ends and perception begins.

The hypotheses tested in this project are suggested and supported by phenomena related to the detection phase of olfaction. A thorough review of perception is therefore beyond the scope of this project. It is however interesting to note that perception of odors takes place in the limbic and paralimbic regions of the brain- the same regions involved in the processing of emotions and formation of memories. Perhaps it is this connection which underlies the reported reduced quality of life in those who suffer from olfactory dysfunction (Smeets et al. 2009).

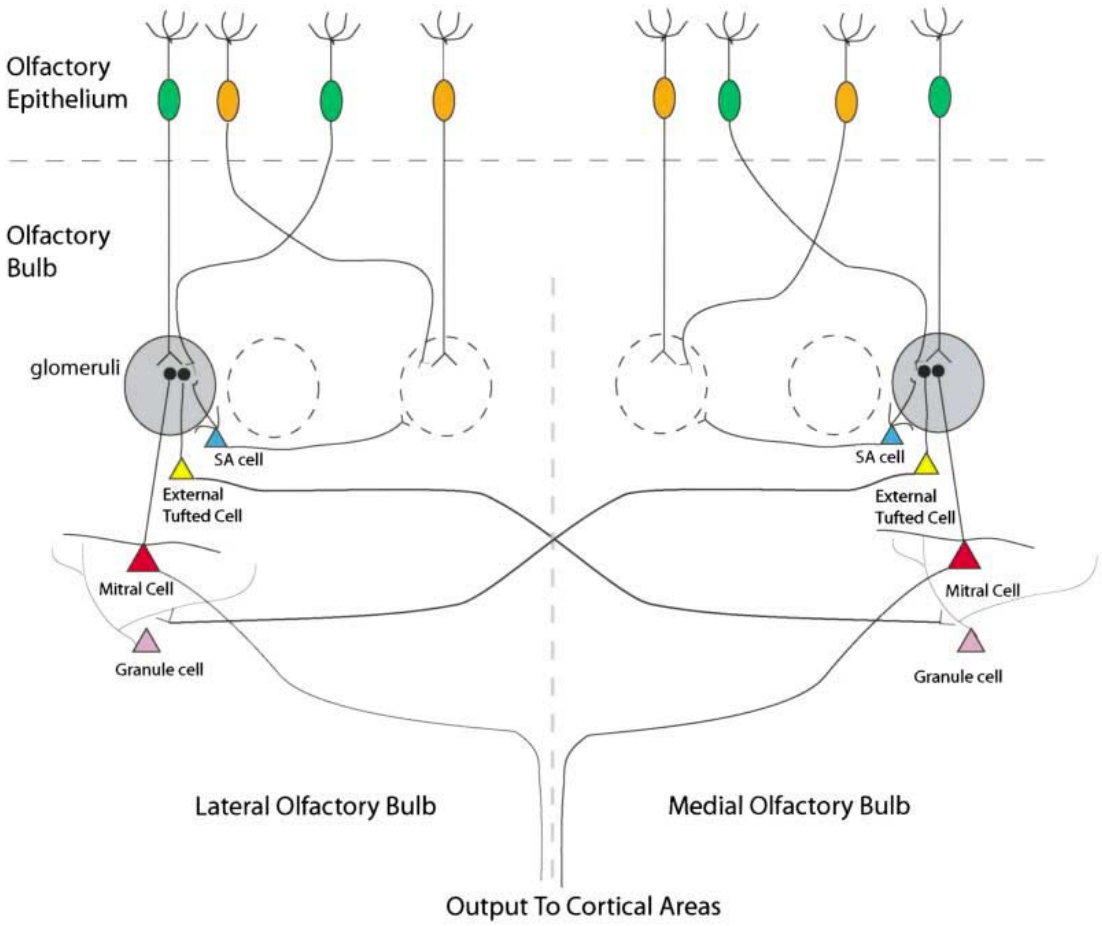

Figure 3 Schematic of connections in the olfactory bulb[iii]

3.2.2 Prevalence of olfactory deficits in healthy populations

Olfactory deficits are not unheard of in the general population, whether they are congenital or the result of facial trauma, inflammation of the oral mucosa, polyps, or structural abnormalities. Formal investigations of this problem in otherwise healthy individuals are rare, but a population-

based study (Bramerson et al. 2004) conducted by Brämerson and colleagues in Sweden suggested that 19.1% of healthy persons have some decrease in their ability to identify odors. The finding was somewhat stronger in males and older individuals as well as in people with nasal polyps or diabetes. Smoking did not negatively impact olfactory ability, although other groups (Frye et al. 1990) have found a dose-dependant decrement in the odor identification ability of smokers. The use of published, validated normative scores (Doty 2008) for 3,928 healthy individuals by sex and age was used in this investigation in order to eliminate the possibility that any deficits detected in our sample were related to this apparently high prevalence of deficits in the population.

3.3 FUNCTIONAL IMPORTANCE OF OLFACTION

While it may appear at first glance to be a trivial or minor deficit, reduced olfactory ability in fact places a considerable burden on those affected. Loss or reduction in the sense of smell interferes with environmental cues warning of fire, spoiled foods, dangerous chemicals, or noxious gasses. Olfaction is also intrinsically linked to memory formation and learning (Walla 2008) and plays a vital role in social and emotional exchanges (Zhou and Chen 2009). Smeets and colleagues found that anosmic and hyposmic patients experience significantly more depression and report an overall reduced quality of life compared with their peers (Smeets et al. 2009). One survey cited by Smeets (Smeets et al.) found that fully half of over 100 individuals with smell dysfunction would be willing to spend at least 20% of their annual income to correct the problem, indicating the large impact that loss of smell has on perceived quality of life.

A recent investigation (Sobin et al. 2006) of olfactory deficits in children with 22q11 deletion syndrome found significant deficits in the affected children compared with their healthy siblings. Most striking were the results for individual odor discrimination. Over half of the affected children failed to correctly identify the odor of smoke compared with fewer than 9% of their siblings. These children also struggled (41% error rate) to identify paint thinner, considered by the authors to be a proxy for the larger category of toxic fumes. These data support concerns that important safety hazards exist in children with olfactory dysfunction. In addition to several other structural abnormalities, individuals with 22q11 frequently present with clefts of the secondary palate, lending additional support to the notion that cleft and olfaction may be related.

Determination of any reduced olfactory ability CL/P patients experience provides an important target for psychosocial and patient education interventions aimed at improving patient safety and quality of life.

3.4 PHYSIOLOGY OF OLFACTORY DYSFUNCTION

3.4.1 Olfaction and nasal anatomy

Adequate olfaction depends on structures in both the nasal passages and the brain. Nasal turbinates direct odorant molecules to neurons embedded in the olfactory epithelium. The olfactory neurons project to the olfactory bulb and converge into mitral-cell interfacing glomeruli in the olfactory bulb, allowing for the efficient transduction of information through the olfactory cortex for processing. Nasal passage structure is also an important component of olfaction.

Unilateral obstructions have been shown to affect uninasal olfaction, although in general overall olfactory ability tends to reflect the ability of the higher-functioning nostril (Hornung and Leopold 1999).

Several groups have examined the relationship between nasal structure, airflow, and olfaction in both cleft and typical populations. Hornung and Leopold (1999) found relationships between the volume of some nasal compartments and olfactory ability, with larger posterior (upper nasal chamber) compartments and smaller volume at the nostril compartments being associated with satisfactory olfactory performance (Hornung et al. 1999). Interestingly, Fukushiro & Trindade observed that posterior rhinomanometry (PR) measurements in 16 UCLP subjects did not differ significantly from unaffected controls, although PR measures did differ in BCLP subjects (Fukushiro et al. 2005). These results can be contrasted with an evaluation of olfactory ability in CL/P by Richman et al (1988) in which more than half (57%) of boys with ULCP but no boys with BLCP performed poorly (<60% correct) on a ten-item odor discrimination test (Richman et al. 1988). The discordance been these results may be a function of small sample size or specific sample characteristics, but they may also indicate that some developmental process beyond simple disruption of normal nasal architecture is at work.

More recently, Grossmann and colleagues detected significantly decreased nasal airflow in both UCLP and BLCP subjects but no difference in odor detection threshold in BLCP subjects as compared with controls. Conversely, they did find a statistically significant correlation between reduced airflow and higher smell threshold in UCLP patients (Grossmann et al. 2005). This failure to demonstrate a consistent relationship between cleft type, nasal airflow, and smell threshold indicates that nasal airflow changes alone are not sufficient to explain the odor discrimination deficits found in CL/P populations.

Although mixed, these findings are of interest given the previously described morphologic changes in the family members of CL/P and CP individuals. If anatomy-related changes in airflow are even partially responsible for differences in odor discrimination ability, it would be expected that anatomic differences in family members would lead to a similar olfactory phenotype in them.

Establishing the true extent of the olfactory deficits in CL/P populations will allow researchers to begin to interpret the results of nasal airflow studies and may eventually inform repair techniques that will best restore normal nasal anatomy.

3.4.2 Brain structure, olfaction, and orofacial clefts

Studies in traumatic brain injury (TBI) patients have confirmed the vital role of the olfactory bulb and olfactory tract in the detection of and discrimination between odors. Multiple groups have demonstrated a correlation between decreased volume in the ventral frontal cortex (VFC), a region that includes the olfactory bulb, and poor scores on the University of Pennsylvania Smell Identification Test (SIT) (Yousem et al. 1999; Fujiwara et al. 2008). It is clear from these works that structural integrity of the VFC is essential for satisfactory olfaction.

Neuroimaging studies in CL/P populations have demonstrated a pattern of VFC abnormalities in affected males. Specifically, they have revealed an overall decrease in orbitofrontal cortex (OFC) volume in CL/P adult males (Nopoulos et al. 2005) and a reduction in straight gyrus (a VFC structure) volume in a pediatric CL/P population (Boes et al. 2007) previously shown to have a variety of other morphologic brain abnormalities (Nopoulos et al. 2007). A recent study by van der Plas and colleagues revealed that boys with right-sided cleft lip have global reductions in white matter volume (van der Plas et al.). The volume reductions were

most striking in the cerebrum, particularly in the occipital and frontal lobes. While the olfactory bulb was not specifically mentioned, the proximity of the olfactory bulb to the regions shown to be reduced in size as well as the overall effects in the cerebrum (site of higher-level odor processing) is an interesting possible contributor to olfactory deficits.

Taken together, the TBI and CL/P neuroimaging literature suggest the presence in of significant structural brain abnormalities in regions likely to affect olfaction in individuals with CL/P.

More work is necessary to understand the association between these abnormalities, OFCs, and olfaction and to determine whether similar changes are present in family members of those with OFCs.

4.0 SPECULATIVE GENETIC CONTRIBUTIONS TO ANOSMIA IN OFC

4.1.1 Developmental origins of olfaction

Of special relevance to any investigation of olfaction in OFC populations is the critical role played by *FGFR1* in the development of the olfactory system. This gene, also implicated in orofacial clefting, is critical for morphogenesis of the olfactory bulbs (Riley et al.). Hebért et al demonstrated in 2003 that mouse embryos without functional *Fgfr1* do not develop a working olfactory bulb (Hebert et al. 2003). Dodé (Dode et al. 2007) and Pitteloud (Pitteloud et al. 2006) then demonstrated that *FGFR1* expression is similarly essential for olfactory bulb morphogenesis in humans.

A genetic condition called Kallmann Syndrome (KS) will be discussed in more detail in section 4.1.2 of this document**.** Briefly, these individuals have hypogonadism with anosmia. Based on the discovery that some cases of KS are caused by mutations in anosmin-1 encoding *KAL1*, Hardelin and colleagues conducted a series of immunohistofluorescence experiments on human embryos and confirmed that anosmin-1 is an essential component of olfactory bulb development. Later investigations described positive regulation of FGFR1 by anosmin-1, confirming the primary role for both of these proteins in the development of functional olfactory bulbs (Ayari and Soussi-Yanicostas 2007; Hu et al. 2009).

Outside of the olfactory bulb, the olfactory sensory neurons play a critical role in olfaction. Sonic hedgehog (*SHH),* a gene widely implicated in a broad phenotypic spectrum of craniofacial abnormalities including OFCs, has additionally been shown to play a critical role in olfactory sensory neuron functioning (Lipinski et al. ; Hu and Helms 1999; Jiang et al. 2006). Gong et al recently found that Shh is involved in olfactory axon growth, branching, and glomerular interfacing in mice (Gong et al. 2009). When Shh was blocked, olfactory neuron axons were immature and failed to project into the glomeruli, despite the fact that the structural integrity of the olfactory bulb and glomeruli was preserved.

While the present study is not able to assess the relative contribution of any of these genes to smell deficits in OFC populations, defining the olfactory phenotype in cases and relatives is a first step in designing experiments designed to do so.

4.1.2 Syndromic occurrence of anosmia and CL/P

FGFR1 has a role in ~3-5% of NS CL/P cases (Murray 2004). Kallmann syndrome (KS), a genetic disorder characterized by anosmia in the presence of idiopathic hypogonadotropic hypogonadism, has causative *FGFR1* mutations in common with non-syndromic (NS) CL/P. KS may be inherited in an autosomal dominant (Kallmann syndrome 2, KS2) or X-linked (KS1) manner. Kallmann syndrome 1 is caused by mutations in *KAL1,* whereas KS2 results from mutations in *FGFR1*. Together KS1 and KS2 account for only ~25% of patients (Pallais et al. 2007); additional clarification regarding the genetic status of other patients is needed. The olfactory deficits present in KS1 and KS2 patients result from both *KAL1* (anosmin-1 encoding) mutations and from *FGFR1* mutations. This overlap is explained by the fact that anosmin-1, crucial for olfactory bulb development in embryos, (Hardelin et al. 1999) is known to act as a

regulator of FGF signaling (Ayari et al. 2007). Accordingly, KS patients with an anosmin-1 (*KAL1)* mutation have a failure of olfactory bulb morphogenesis secondary to altered anosmin-FGF interaction, while KS2 patients have FGF deficiencies which lead to failed olfactory bulb morphogenesis despite the presence of normal anosmin-1.

Orofacial clefting occurs in about 5% of all KS patients, much higher than the population prevalence of 0.12%. Compared with the larger KS population, KS patients with *FGFR1* mutations (KS2) have a relatively high (25-30%) prevalence of orofacial clefts, (Dode et al. 2003; Albuisson et al. 2005; Dode et al. 2007) an intriguing overlap given the established role of *FGFR1* in non-syndromic clefting (Riley et al. 2007; Riley et al. 2007). In addition to overt clefts, KS patients frequently have other midline facial defects including high-arched palate and dental agenesis. Palatal form changes, CL/P, and dental agenesis have also been reported in KS patients with *KAL1* (anosmin-1) and unidentified mutations, although to a lesser degree than in KS2 patients (Versiani et al. 2007). Interestingly, at least one study of KS patients has identified a family history of hyposmia and/or anosmia (in 8 families of 10 patients) in family members without an overt KS phenotype, suggesting reduced penetrance and variable expressivity for these mutations (Versiani et al. 2007).

The Kallmann syndrome population provides intriguing insight into the shared genetic pathways for olfactory system and craniofacial development. Identifying those families with olfactory deficits as part of the phenotypic spectrum of OFCs may lend additional power to candidate gene studies in the future.

5.0 MATERIALS AND METHODS

5.1 THE UNIVERSITY OF PENNSYLVANIA SMELL IDENTIFICATION TEST

The University of Pennsylvania Smell Identification Test (UPSIT or SIT) is an extensively validated 40 item forced-choice test designed to evaluate odor discrimination ability in subjects as young as 5 years of age (Doty et al. 1984). The test consists of four booklets containing 10 odorants each, with one odorant challenge per page. Each odorant is embedded in microcapsules and released only upon scratching with a pencil tip. A multiple choice question with four possible answers is presented with the odor, for example "This odor smells most like a) chocolate b) banana c) onion or d) fruit punch" (Doty et al. 1984).

An odor identification test was chosen over smell threshold detection tests for several reasons. First is the ease of administration; the test is durable, relatively easy for staff to learn and administer consistently, and can be completed in a small amount of time as part of a larger protocol. In addition to ease of use, forced-choice odor identification is known to be as effective as more burdensome threshold tests in detecting olfactory deficits (Richman et al. 1988). Given that at least some evidence for reduced odor discrimination exists while the evidence for increased smell threshold in CL/P populations is mixed at best, it was determined that the SIT discrimination test would be most informative for the purposes of this study.

5.2 DATA COLLECTION

5.2.1 Recruitment

5.2.1.1 Subject ascertainment

Our goal was to enroll 50 CL/P individuals and 50 unaffected family members through recontacting of previous Center for Craniofacial and Dental Genetics (CCDG, University of Pittsburgh) study participants and a large, multi-day "research blitz" at The Children's Hospital in Denver. CL/P subject status included bilateral and unilateral cleft lip, with or without cleft palate, as well as isolated cleft palate. Only non-syndromic individuals were recruited.

Pittsburgh subjects were recruited via mailings to previous participating families with at least one eligible member as determined by a CCDG database query. Additionally, families participating in the overall CCDG Orofacial Cleft Study completed the UPSIT protocol. Colorado families were recruited by the craniofacial clinic staff at The Children's Hospital in Denver.

5.2.1.2 Screening procedures

Subjects at the University of Pittsburgh underwent phone screening prior to scheduling a study appointment. This screening determined eligibility based on age, personal or family history of non-syndromic CL/P or CP, and absence of exclusion criteria discussed below. These subjects underwent an additional round of on-site screening on the day of the study to verify the absence of exclusion factors (for example, the subject having developed rhinitis following the phone screen).

Subjects at The Children's Hospital in Denver were recruited and screened as part of a larger research study of which the smell test was a small portion. These subjects were screened on-site for appropriateness, again based on age, affected status, and absence of any exclusion criteria. This was accomplished by administering the questions included on the back of booklet one of the UPSIT test kit.

5.2.1.3 Inclusion/Exclusion Criteria

In order to be included in this study, subjects: (1) were 10-59 years old, (2) had a personal or family history (first degree relative) of non-syndromic (isolated) cleft lip with or without cleft palate or cleft palate only, (3) had no history of facial or head trauma, (4) had no current rhinitis, allergies, or upper respiratory infection, and (5) could not have a family history of syndromic orofacial clefts. Males and females were included in the study. Subjects of all ethnicities were eligible provided they spoke English or Spanish, the two languages available as validated forms of the UPSIT tool.

5.2.2 Data collection procedures

All recruitment and data collection procedures were conducted with the prior approval of the University of Pittsburgh Institutional Review Board (IRB) and the Colorado Multiple Institutional Review Board (COMIRB).

The UPSIT is widely used in both academic and clinical settings to test olfactory function.(Doty et al. 1995) The UPSIT has been extensively validated, provides age-adjusted normosmic, hyposmic, and anosmic scores, and shows high test-retest reliability.(Doty et al. 1995) It consists of a 40-item, forced-choice odor identification test and is designed to detect

reduced ability to discriminate between familiar odors at normal concentrations. Each odor is presented on a "scratch and sniff" style card with a four answer, multiple-choice question requiring the subject to select the odor being presented. The expected time required to complete this test is 20 minutes. Prior to administering each test, Pittsburgh subjects completed the "Olfaction On-Site Interview Form" to ensure eligibility. Pittsburgh subjects did not complete the screening questions provided on the test booklets because identical information was collected elsewhere. Denver subjects completed the screening questions provided with the test. Instructions were given as described in the test manual. Subjects were instructed to sit apart from family members in an odorless room and were only permitted to address questions to the administrator. A list of standard prompts or definitions was developed at the Center for Craniofacial and Dental Genetics (CCDG) by a multidisciplinary team of staff experienced in administering the test to children; only prompts from this list were used to answer questions from subjects in order to prevent the introduction of bias from multiple administrators.

Following administration of the test, Pittsburgh subjects completed a short demographic form intended to capture information usually collected on the test booklet; this was done to allow the information to be collected on a scanable Teleform rather than on the test booklet. As several years had elapsed since many of these subjects had been seen at the CCDG, the demographic form also contained questions regarding items which may be expected to change over time such as family history, smoking and medication status. This step was not necessary for the Denver subjects because all data was collected at the time of UPSIT administration as part of the larger protocol.

5.3 SAMPLE CHARACTERISTICS

A total of 58 subjects completed the protocol. Five unaffected siblings and one grandparent were excluded from the final analysis for a total of 51 analyzed subjects from 25 families (12 cases and 39 relatives). The unaffected relative group was comprised entirely of the parents of affected children. Three affected siblings from one family (Figure 4) were included in the analysis. This interesting family was comprised of two sets of twins, one monozygotic and one dizygotic, as well as a singleton child. The monozygotic twins did not take the UPSIT due to young age.

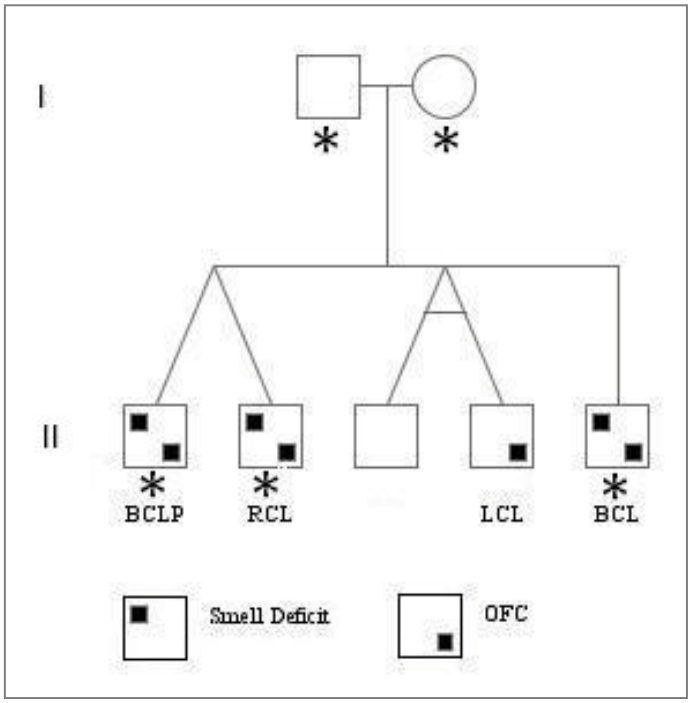

Figure 4. Family with affected hyposmic twins

* indicates UPSIT was administered to subject

Controls were not collected. Nearly 4,000 healthy individuals have completed the UPSIT in the process of validating the test and developing normative scores; these data are available in the UPSIT test manual (Doty 2008) provided with the purchase of UPSIT test booklets. Normative UPSIT data were available for 2762 individuals between the ages of 10 and 59 (males, n = 1302; females, n = 1460). The term "controls" is used here in reference to data derived from this population.

Table 1 Group demographic composition

Parameter	*Orofacial cleft*	*First-degree relative*
N	12	39
Gender (female/male)	4F/8M	24F/15M
Ethnicity (Hispanic/Non -Hispanic)	1H/11N	14H/25N
Smoking (self/household/no)	0/2/10	14/13/12
Primary Language (Spanish/English)	0/12	9/30
Age (years)	18.6 ± 13.8	33.4 ± 7.7

5.4 DATA ANALYSIS

Tests were scored by two independent raters using a template provided with the UPSIT test; incongruent results were reconciled by having both raters re-score the test. Consensus was easily reached with this method. Briefly, subjects indicate their answer by filling in a bubble corresponding to their selection for each of 40 items. A scoring template with only the correct answer bubble punched out is provided. Scoring is completed by placing the template over the subject's answer sheet and manually counting the number of missed items.

The UPSIT is scored by comparing the subject's number correct (out of 40 items) to the performance of age and sex matched controls. The version of the test kit used for this project offered 1,302 male and 1,460 female controls for subjects in the 10-59 age range. Controls are divided first by gender and then into groups spanning five years (i.e., males 5-9 years of age) and the percentile rank for each possible score is provided within these groups. Clinical categories consist of normosmia, mild microsomia, moderate microsomia, severe microsmia, anosmia, and malingering and are determined based on the distribution of scores within each gender/age subcategory, allowing for subjects of different ages and genders to be compared with each other. For our sample, each subject was compared with the appropriate gender/age subcategory and assigned a status of "normosmic" for all subjects scoring in the normosmic clinical range or "deficit" for those subjects scoring in any of the remaining clinical categories.

Statistical analysis was somewhat limited by the small number of OFC cases in the sample. Chi-square tests were used to compare the proportion of deficit in unaffected relatives to control norms. The more conservative Fisher's exact test was used to compare OFC cases with control norms and to compare male and female first degree relatives (FDRs) with each other and with control norms due to the small number of subjects in each category. Results of the chi-

square analyses were verified using the Z-ratio independent proportions test. Odds ratios were calculated for each of these comparisons. The Mann-Whitney *U* nonparametric test was used to rank and compare the raw UPSIT scores of FDRs with OFC cases and to compare the raw UPSIT scores of female and male FDRs. All results were considered statistically significant if p-values were at or below 0.05 and were reported as one-tailed values.

Among cases and FDRs who either smoked or were exposed to household smoke, the number of subjects identified with and without an olfactory deficit was roughly equal (Table 2). Due to the similar distribution of smoking in each group and a lack of information regarding the smoking status of the UPSIT control population, smoking was not used as a variable in any analyses.

Table 2: Summary of smoking rates

Group	*Exposure*	*Total (n)*	*Deficit (n)*
OFC	Household	2	1 (50%)
	Smokers	0	0
FDR	Household	4	2 (50%)
	Smokers	13	6 (46%)
Total (OFC + FDR)	Household	6	3 (50%)
	Smokers	13	6 (46%)

None of the study participants admitted to having bipolar disorder, schizophrenia, major depressive disorder or alcoholism, all of which are known to affect or be associated with changes in olfaction. None of the participants reported taking any medications known to affect olfaction.

6.0 RESULTS

6.1 SPECIFIC AIM 1

Individuals with OFCs demonstrated a higher frequency of deficits in olfactory discrimination compared with both matched controls and unaffected first degree relatives (FDRs) on the Smell Identification Test.

Of the 2,762 controls provided, 349 were classified as having some level of olfactory deficit. The frequency of olfactory deficits was significantly higher in the 12 OFC cases, with five achieving scores in the deficit range (41.7% vs. 12.6%; $p = 0.012$). The likelihood of having a smell deficit was increased nearly fivefold in the cases (OR = 4.94; 95% CI: 1.56-15.65) compared with controls. These results are presented in Table 3.

Subjects with OFCs also performed significantly worse on the UPSIT test than did their unaffected first-degree relatives. The median case scores was 33.5 compared with 35 for FDRs ($p = 0.043$), indicating overall poor performance for the cases even compared with the deficit-prone FDR group. These results are presented in Table 4.

Table 3: Comparison of olfactory deficits in cases vs controls

	Cases (n=12)	*Controls (n=2762)*	*p1-p2 (95% CI)*	*Z*	*p*
Deficit	5 (41.7%)	349 (12.6%)	0.29 (0.04-0.59)	3.008	0.001
No Deficit	7	2413			
	$\chi 2 = 9.045$; df = 1; p = 0.012 (one-tailed)				
	OR (95% CI) = 4.94 (1.56-15.65)				

Table 4: Comparison of UPSIT scores between cases and unaffected first-degree relatives

Cases (n = 12)		*Relatives (n = 39)*			
Median Score	*Mean Rank*	*Median Score*	*Mean Rank*	*U*	*p*
33.5	19.58	35	27.97	157.000	0.043

6.2 SPECIFIC AIM 2

As hypothesized, unaffected first-degree relatives of OFC cases demonstrated a higher frequency of deficits in odor discrimination as compared with matched controls on the Smell Identification Test. These results are presented in Table 5.

The sample included 39 unaffected FDRs, 14 of whom were classified as having some level of olfactory deficit. The frequency of olfactory deficits was significantly higher in the relatives than in the control population (35.9% vs. 12.6%; $p < 0.001$; one-tailed test). The likelihood of having a smell deficit was increased nearly fourfold in the FDRs (OR = 3.87; 95% CI: 1.99-7.52) compared with controls.

Table 5: Comparison of olfactory deficit rates in first-degree relatives vs controls

	Relatives (n=39)	*Controls (n=2762)*	*p1-p2 (95% CI)*	*Z*	*p*
Deficit	14 (35.9%)	349 (12.6%)	0.23 (0.09-0.40)	4.295	< 0.001
No Deficit	25	2413			
	$\chi 2 = 16.443$; df = 1; $p < 0.001$ (one-tailed) OR (95% CI) = 3.87 (1.99-7.52)				

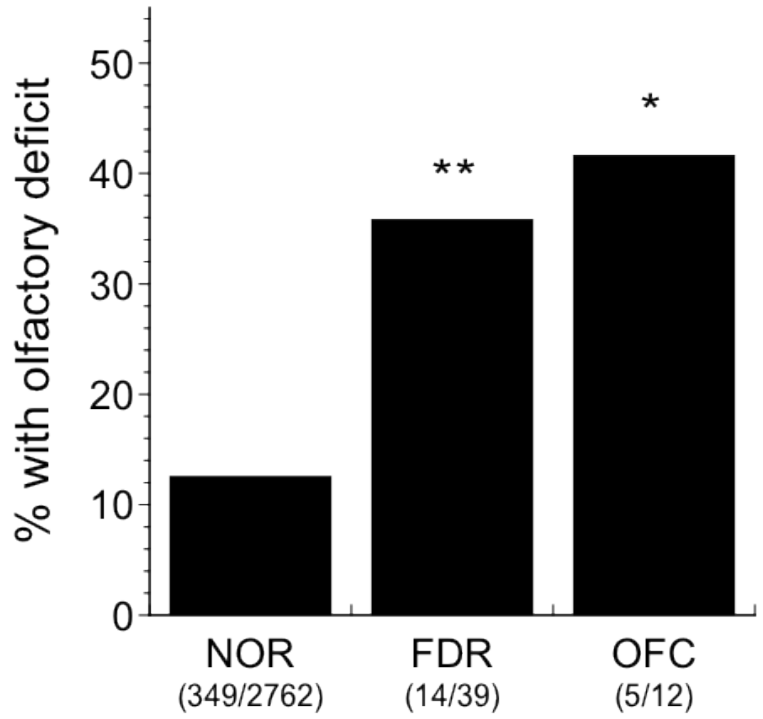

Figure 5. Prevalence of olfactory deficits in all groups

NOR: Normal controls

FDR: First-degree relative

* p = 0.012 for OFC vs. NOR

** p < 0.001 for FDR vs. NOR

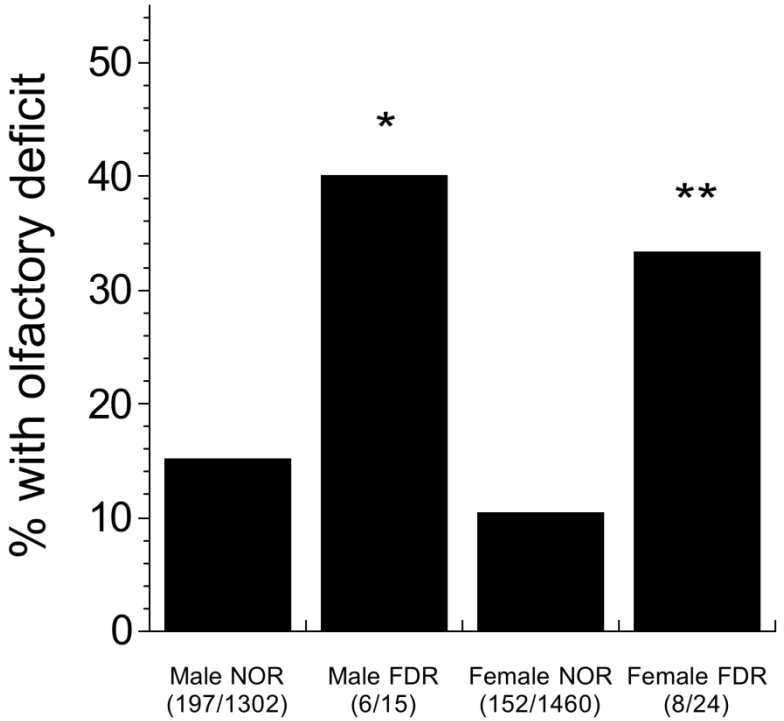

Figure 6. Prevalence of olfactory deficit by sex

NOR: Normal controls

FDR: First-degree relative

* p = 0.018; male NOR vs. male FDR

**p = 0.002; female NOR vs. female FDR

6.3 SPECIFIC AIM 3

A small case sample prevented direct testing of the hypothesis that males with OFCs have a greater magnitude of olfactory deficits than do females with OFCs. The deficits found in male versus female FDRs was used as a proxy for this comparison.

When compared with control norms, male FDRs demonstrated a significantly higher frequency of smell deficits (Figure 8). The control population offered 1,302 healthy males, 197 of whom were classified as having some level of smell deficit. Of the 15 male FDRs who completed the UPSIT, 6 scored in the deficit range. The difference between these frequencies indicated a significantly higher prevalence of smell deficits in the male FDRs (40.0% vs. 15.1%; $p = 0.018$). Male FDRs were almost four times more likely to have a smell deficit than were male controls (OR = 3.74; 95% CI: 1.32-10.62). These results are presented in Table 6.

Female FDRs showed a similar effect to male FDRs (Figure 8). Of the 24 female FDRs completing the UPSIT, 8 (33.3%) had a deficit. This was significantly higher than observed in the female control population of 1,460 of which 152 (10.4%) had a deficit ($p = 0.002$). The likelihood for female FDRs to have a smell deficit was over fourfold higher than for female controls (OR = 4.30; 95% CI: 1.81-10.22). These results are presented in Table 7.

Finally, female FDRs were compared with male FDRs. Contrary to the hypothesis that smell deficits would be more prevalent in males, these two groups showed similarly high rates of olfactory deficits. There was a slight but statistically insignificant increase in the frequency of

smell deficits in male vs. female FDRs (40.0% vs. 33.3%; $\chi 2 = 0.178$; df = 1; $p < 0.466$). There was a small reduction in the median UPSIT scores for male vs. female FDRs, but this again failed to reach significance (; p = 0.066). These results are presented in Table 8.

Table 6: Comparison of olfactory deficit rates in male first-degree relatives and male controls

	Relatives (n=15)	*Controls (n=1302)*	*p1-p2 (95% CI)*	*Z*	*p*
Deficit	6 (40%)	197 (15.1%)	0.25 (0.02-0.52)	2.652	0.004
No Deficit	9	1105			

$\chi 2 = 7.035$; df = 1; p = 0.018 (one-tailed)
OR (95% CI) = 3.74 (1.32-10.62)

Table 7: Comparison of olfactory deficits in female first-degree relatives and female controls

	Relatives (n= 24)	*Controls (n=1460)*	*p1-p2 (95% CI)*	*Z*	*p*
Deficit	8 (33.3%)	152 (10.4%)	0.23 (0.06-0.45)	3.591	< 0.001
No Deficit	16	1308			

$\chi 2 = 12.898$; df = 1; p = 0.002 (one-tailed)
OR (95% CI) = 4.30 (1.81-10.22)

Table 8: Comparison of UPSIT scores between male and female unaffected first-degree relatives

Male Relatives (n = 15)		*Female Relatives (n = 24)*			
Median Score	Mean Rank	Median Score	Mean Rank	U	p
34	16.53	36	22.17	128.000	0.066

7.0 DISCUSSION

The results obtained by this study demonstrate a clear association between orofacial clefting and olfactory deficits. The olfactory deficits first identified by Richman (Richman et al. 1988) in the OFC population were confirmed and expanded upon with an added comparison between unaffected first degree relatives (FDRs) and individuals with OFCs (cases). Richman and colleagues detected deficits in 10/20 (50%) of cases while the current study detected a deficit in 5/12 (41.7%) of cases. These results stand in contrast to the somewhat equivocal findings obtained by Grossmann et al (Grossmann et al.), in which only deficits in odor detection threshold but not odor discrimination were detected. It should be noted that neither group used a standardized olfactory assessment tool for their work; Richman utilized 10 "common" odors suggested by a previous work (Wright 1987; Richman et al. 1988), while Grossmann used only four odors (including water) deemed "common household odors" and selected by an undisclosed method.

Perhaps the most striking result of the current work was the unequivocal presence of smell deficits in the FDRs. A variety of subclinical phenotypes have been described in the unaffected relatives of those with OFCs (Neiswanger et al. 2002; Scott et al. 2005; Neiswanger et al. 2009; Weinberg et al. 2009; Klotz et al. 2010); the present study strongly suggests that

there is an additional, previously undescribed phenotype involving a compromised ability to discriminate between odors.

Several explanations are possible for the presence of impaired olfaction in the OFC cleft population. These include genetic, physiologic, and structural factors as well as the possibility that defects occur secondary to invasive restorative surgeries and other interventions. The recent discovery of smell deficits in the 22q11 deletion syndrome population (Sobin et al.) led to speculation by the authors that medical procedures such as the insertion of nasogastric tubes and repair of the pharyngeal flap may lead to olfactory compromise. The current findings in first degree relatives strongly suggest that there may be some effect beyond simple disruption of the gross anatomy or treatment-induced trauma mediating the smell deficits found in people with OFCs.

With regards to the genetic, physiologic, and structural contributions to olfactory functioning, the current study provides only indirect insight into the complex interactions between these factors. Given that FDRs show a magnitude of deficits nearly matching that observed in cases it seems likely that genetic factors at least partially explain the link between clefting and olfaction. The aforementioned studies demonstrating changes in the face shape and brain structure of FDRs make it difficult to speculate about a straightforward explanation such as a shared genetic pathway for olfactory development and clefting as opposed to a less direct heritable mechanism like subtle anatomic changes as the source of shared deficits.

If larger studies find that smell deficits in parents indicate a predisposition towards orofacial clefting, evaluating functional olfaction in parents of an OFC-affected child may offer a cost-effective, simple, objective way to refine recurrence risks for future children.

The calculation of accurate, personalized recurrence risks is critical for genetic counseling purposes. If the degree to which olfactory deficits segregate with OFCs in multiplex families is well quantified, administration of the UPSIT could serve as a rapid assessment of parents of an affected child who are seeking information about their future risks. Olfactory assessment in this case could also be utilized in extended family, serving as a marker of a predisposition to having a child of their own with an orofacial cleft. Even more promising is the possibility of developing a battery of assessments to evaluate the full spectrum of subclinical phenotypes in family members; this would give the most accurate recurrence risk provided that the degree to which each serves as a marker of liability to clefting is carefully quantified.

Aside from speculation about the underlying cause of the family-wide olfactory dysfunction identified, the results of this study provide useful clinical information. Identifying the possibility of olfactory compromise in OFC patients is a first step in reducing any associated morbidity. For example, awareness that a child with a CL/P is at risk to struggle with identifying the odor of smoke may prompt recommendations that families increase the number of smoke detectors in their home and use extra caution when allowing their child to be at home unsupervised or begin to cook independently. Caution should also be used with paints or chemicals producing noxious odors and persons with reduced olfaction should be trained to be vigilant about ventilation given that they may lack awareness of toxic or combustible fumes in their environment. Psychosocial interventions such as extra attention to personal hygiene and grooming habits may reduce the anxiety around body odor cited by many anosmic individuals (Miwa et al. 2001).

In conclusion, the present study confirms the presence of olfactory deficits in individuals with CL/P or CP at nearly a fivefold higher prevalence than in the general population.

Furthermore, this study suggests for the first time that these deficits are found in at least a third of unaffected first degree relatives of persons with an OFC.

Given these findings, psychoeducational interventions for individuals with an OFC may need to evolve to include training in awareness of smoke, gasses, or other environmental hazards. Families may appreciate counseling regarding some of the physical and emotional sequelae of olfactory dysfunction, including higher rates of depression, concern about personal hygiene, altered perception of food taste, and excessive anxiety about failure to detect dangerous odors (Miwa et al. 2001; Smeets et al. 2009). These findings may also inform candidate gene studies, attempts to clarify inheritance patterns for OFCs, and refinement of recurrence risks in families. Future studies to confirm and expand these results are the first step in this process.

7.1 LIMITATIONS

One limiting factor in our analysis was the lack of control data such as raw scores and exact ages at the individual level. Only percentile rankings and clinical categorizations were possible for the controls, making direct comparison of UPSIT scores impossible.

The small size of the OFC-affected sample was another imitation of this study. Too few cases were available to conduct a meaningful analysis of the relationship between factors such as cleft type, repair/surgical history, the spectrum of cleft types in a family, or the presence of other subclinical phenotypes and smell deficits. Furthermore, too few parent-child duos were available to draw any conclusions about recurrence risk related to the absence or presence of smell deficits or to detect patterns of concordance or discordance for the olfactory phenotype within families.

The inclusion of multiple family members in one case raises the possibility that some other factor present in this family (heritable or otherwise) could be influencing the anosmia identified in multiple members. Given the robust finding in the unaffected relative group as a whole, we think this is unlikely but it cannot be completely eliminated. Again, a larger sample will allow for more aggressive exclusion of subjects while still maintaining an adequate sample size.

Many of these limitations are addressed by the recent inclusion of the UPSIT protocol in the larger Center for Craniofacial and Dental Genetics Orofacial Cleft study. Over time, enough cases will become available to allow for finer comparisons such as the aforementioned ones.

7.2 FUTURE LINES OF INQUIRY

Several interesting lines of inquiry remain to be pursued. Of utmost interest would be obtaining magnetic resonance imaging (MRI) scans of the nasal cavity and olfactory bulbs of OFC cases and FDRs. This would allow for direct testing of the hypothesis that structural alterations in the nasal cavity and/or of the olfactory bulbs are related to the observed dysfunction. Nasal airflow analysis would be particularly helpful in such investigations.

Also interesting would be genetic studies to determine whether OFC individuals with smell deficits are more likely to have mutations in *FGFR1, SHH,* or as-yet undetermined genes related to both clefting and olfaction. Additional information about the genetic causes of Kallmann syndrome may provide candidate genes beyond *FGFR1* and *SHH.*

The emerging literature regarding changes in the three-dimensional facial structure of family members of OFC cases also provides fertile ground for future investigations. It would be interesting to test whether a particular pattern of facial measurements is more or less associated with smell deficits in these families.

Finally, an expanded population of individuals with CL/P or CP is essential to determine whether the type of cleft is related to olfaction; this would provide guidance for genetic counselors, occupational therapists, and cleft-craniofacial teams in counseling families and would potentially further refine the phenotypes of individual families, increasing the power of genetic association studies.

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