Evidence of Olfactory Deficits in Isolated Orofacial Clefting

by

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ABSTRACT

Orofacial clefting is one of the most common congenital malformations with a high morbidity and mortality rates. Clefts have a complex etiology and a wide range of phenotypic expression and subclinical features associated with it. Subclinical features occur at a higher rate in the unaffected first-degree relatives of the affected cleft patients compared to the normal population. Few examples of subclinical features include orbicularis oris muscle defects, facial dysmorphology, dermatoglyphic patterns. These subclinical features may represent an incomplete phenotypic expression. Identification of such traits in the families can help in understanding the genetic etiology. Reduced olfaction has been seen in patients affected with orofacial clefting and their unaffected relatives. However, it is not known whether these deficits were present in affected subjects and their families with different cleft types.

The aim of this study is to investigate the prevalence of olfactory deficits in cases with different types of orofacial clefting (Cleft lip CL, cleft of the lip with or without palatal involvement CL/P, and cleft palate CP) and their unaffected first-degree relatives.

The University of Pennsylvania Smell identification test (UPSIT) was administered to 32 cases and 100 unaffected relatives. 447 people served as controls who had no history of orofacial clefting. The data was obtained from the larger Pittsburgh Orofacial Cleft Cohort. Only White, Non-Hispanic and subjects between the ages 10 and 59 years were included in the study. Exclusion criteria was based on nasal congestion, loss of smell due to trauma, depression (major and current depression), anti-depressants (Rolipram, Clomipramine, and Citalopram).

Chi-square test and Fishers exact test was used for categorical outcomes. Non parametric tests such as Shapiro Wilk and Mann Whitney test were used for quantitative scores. These tests were used to compare olfactory performance across groups.

There is a significant difference in the olfactory deficits between the cases and controls (p-value: 0.00004). There is a significant different in the different types of cleft among the cases (p-value: 0.0002 for CL, 4.41E-07 for CL/P, 0.002 for CP). There is a prevalence of olfactory deficits among the unaffected relatives belonging to the CL/P family type (p-value 0.03).

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Preface

My sincere thanks to all the participants of this study without whom this study would not have been possible.

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I thank my committee members for their careful review and thoughtful comments.

1.0 Introduction

Orofacial clefting is one of the most common congenital conditions with high morbidity and mortality rates. It can be defined as the improper fusion of the orofacial structures and presents as cleft of the lip (CL), cleft of the palate (CP) or cleft involving both the lip and palate (CLP) (Keteyian and Mishina 2017). The prevalence of orofacial clefts (OFC) is 1 in 940 live births in the United States, making it the second most common congenital defect (Parker et al 2010).

1.1 Epidemiology

The worldwide prevalence rate of Orofacial clefts is 1 in 700 live births (World Health Organization [WHO], 2001). The prevalence of Orofacial clefts varies according to the population. The Asian and American Indian populations have the highest prevalence rate at 1 in 500 live births. African population have the lowest prevalence rate at 1 in 2500 live births (Dixon et al 2011). Differences also exist in regard to sex and laterality. CP is about twice as common in females, while the opposite is true for CL/P (Dixon et al., 2011; Mossey et al., 2009). Left sided unilateral CL cases are more common than right sided CL cases. (Dixon et al 2011).

About 30% of the cleft cases are syndromic; i.e., syndromes that have cleft of the lip with or without palatal involvement (CL/P) or CP as one of the features, among other symptoms. 70% of the cases are non-syndromic and are thus isolated orofacial clefts. 50% of the patients with CP are thought to be syndromic, while only 5-10% of the CL/P cases are associated with a syndrome ((World Health Organization [WHO], 2001).

1.2 Embryology

The study of embryology has not only helped us in understanding the normal embryonic development of structures but has also helped us in tracing the pathogenesis defects associated with structures (Carlson, 2009).

The embryological development of the face is quite complex. The major events in the formation of the face take place between the 4th and 8th weeks of development. The neural crest mesenchyme and the head ectoderm contribute to the formation of face and oral cavity (Som and Naidich, 2013). Five facial process/prominences make up the face: the frontonasal process (FNP), paired maxillary processes (MXP), and paired mandibular processes (MP).

The FNP emerges in the later part of third week. The forebrain enlarges and pushes the ectoderm overlying it forward resulting in FNP formation. The stomodeum or the primitive mouth form during the beginning of 4th week below the developing forebrain and FNP. Lateral to the stomodeum is the MXP and caudally is the MP (Fig 2), which both derive from the first pharyngeal arch. The orbital floor, upper lip, inferior portion of lateral nasal wall are all formed from MXP. The MP eventually forms the lower jaw.

1.2.1 Midface and Primary palate formation

Around the end of the 4th week a pair of ectodermal thickenings called nasal placodes appear on the FNP. The mesenchyme that is present medially and laterally to each nasal placode form the medial nasal processes (MNP) and lateral nasal processes (LNP), respectively. The nasal groove is formed when the nasal placodes go below the surface and the groove gets deepened

further as the LNP and MNP develops. The deepening of nasal grooves leads to nasal pit formation (Fig 3). The pits continue into stomodeum to form nasal sacs. By the beginning of the 5th week the olfactory epithelium is formed due to the thickening of the ectoderm in the upper one third of the nasal sac. By the end of the fifth week there is vascular and sensory innervation formed between the nasal sac and the olfactory bulb.

During the fifth week the MNP from each side fuses to form the intermaxillary segment (Fig 4). The intermaxillary segment forms the philtrum, premaxilla and the middle portion of the upper lip and jaw (Fig 5). The MXP first fuses with the LNP and then the MNP resulting in upper lip formation. The lateral part of the upper lip is formed by MXP. The cheek is formed when the lateral parts of MXP and MP fuse. (Warbrick, 1960; Tepper and Warren 2010; Mitchell *et al,* 2010).

The maxillary, medial and lateral nasal process forms the lip. A summary of lip development is given below (fig 1).

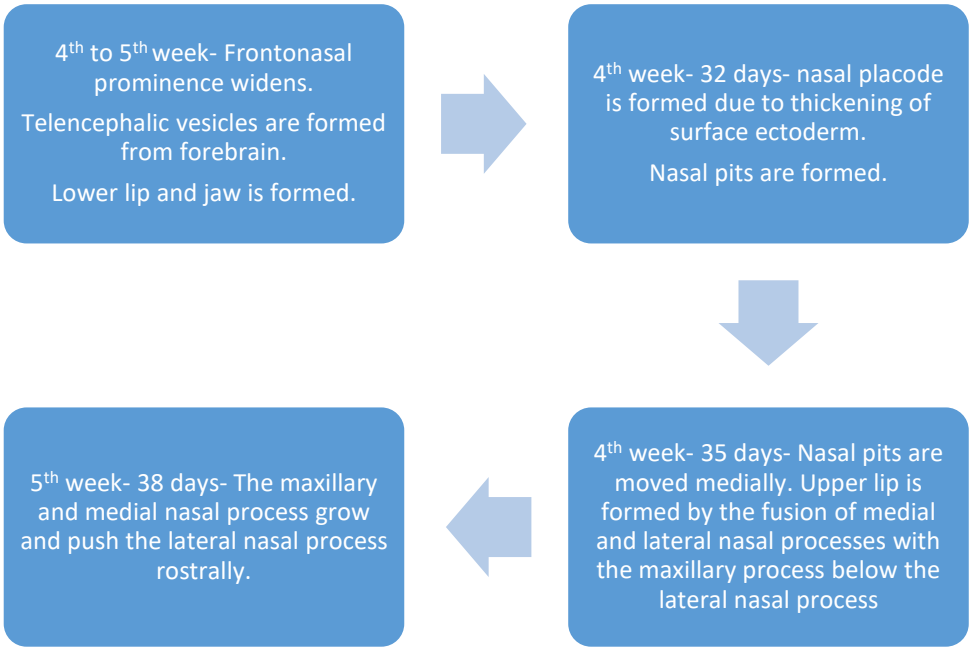


Figure 1 Summary of Lip formation

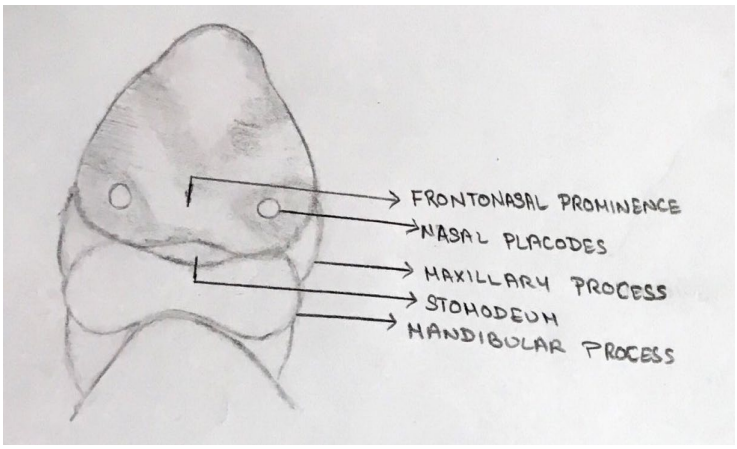


Figure 2 Development of face 4th week

Ref: Facial Embryology Honrado et al., (2018)

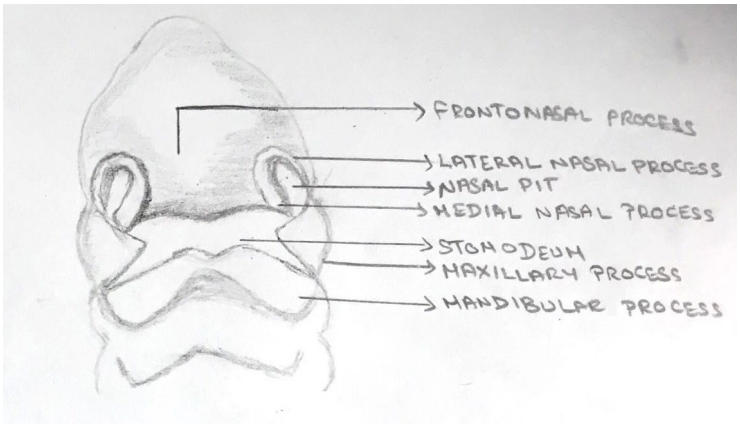


Figure 3 Development of face: End of 4th week.

Ref: Human Embryology and Developmental Biology Carlson *et al.,(2019)*

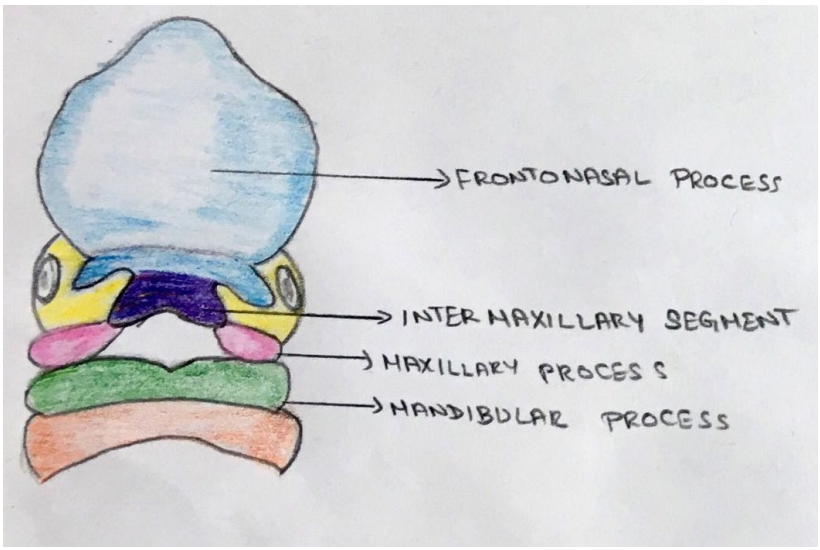


Figure 4 Development of face: 5th week

Ref: Embryology- Development of the Head and neck, the eye and ear Mitchell *et al.*, (2009)

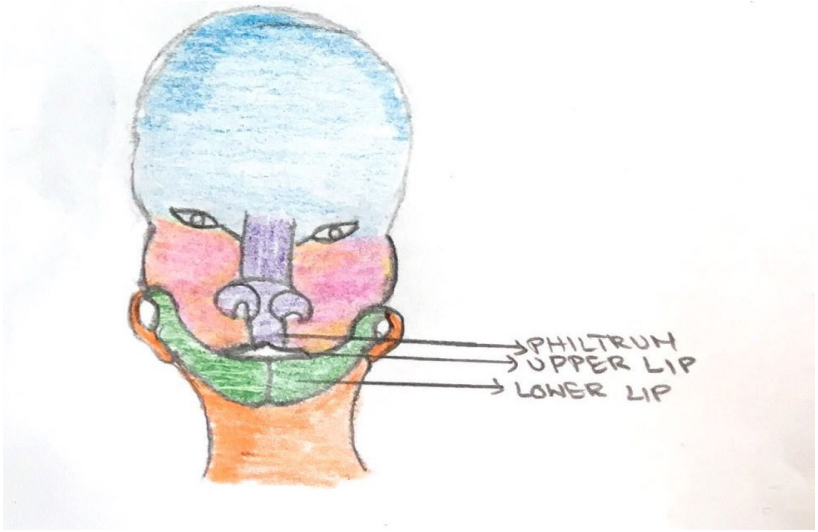


Figure 5 Development of face

Ref: Embryology- Development of the Head and neck, the eye and ear Mitchell *et al* .,(2009)

1.2.2 Secondary palate formation:

The secondary palate is formed by fusion of the lateral palatine processes, which derives from the MXP. Membranous bone is formed in the pre-maxilla and extends to the lateral palatine process, thus forming the hard palate. The posterior portions of lateral palatine process do not get ossified and forms the soft palate (Pansky, 1982). A summary of the formation of palate is given below (Som and Naidich, 2014). (Fig 6 and fig 7).

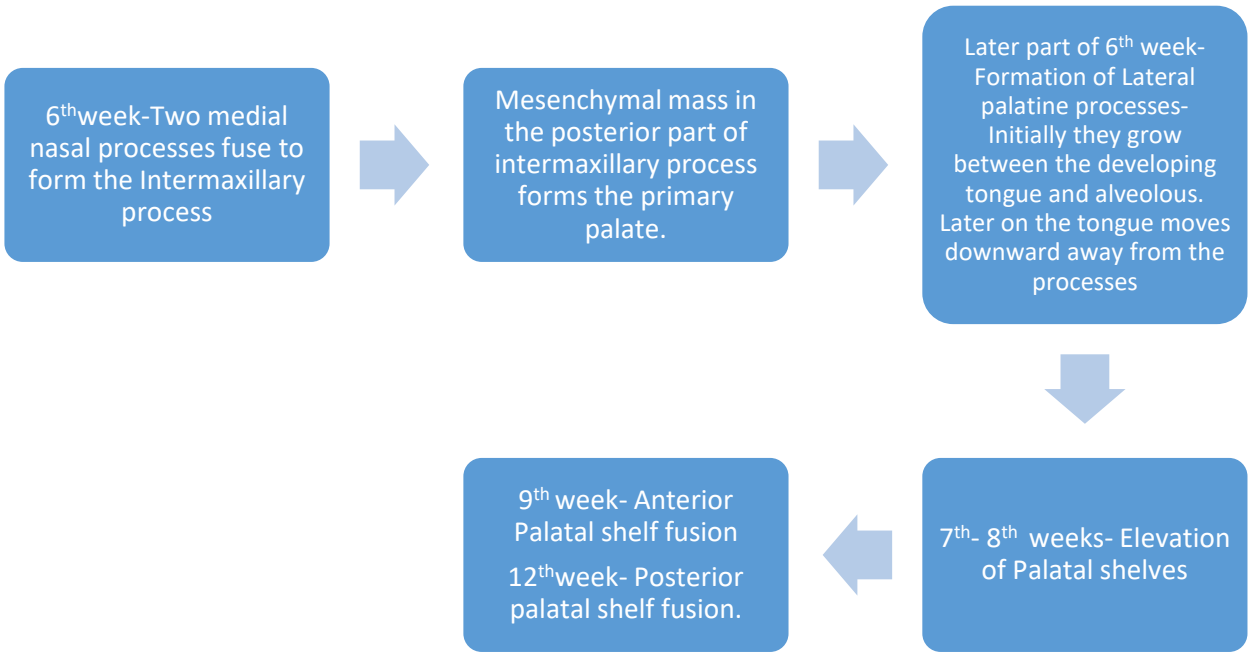


Figure 6 Summary of palate formation

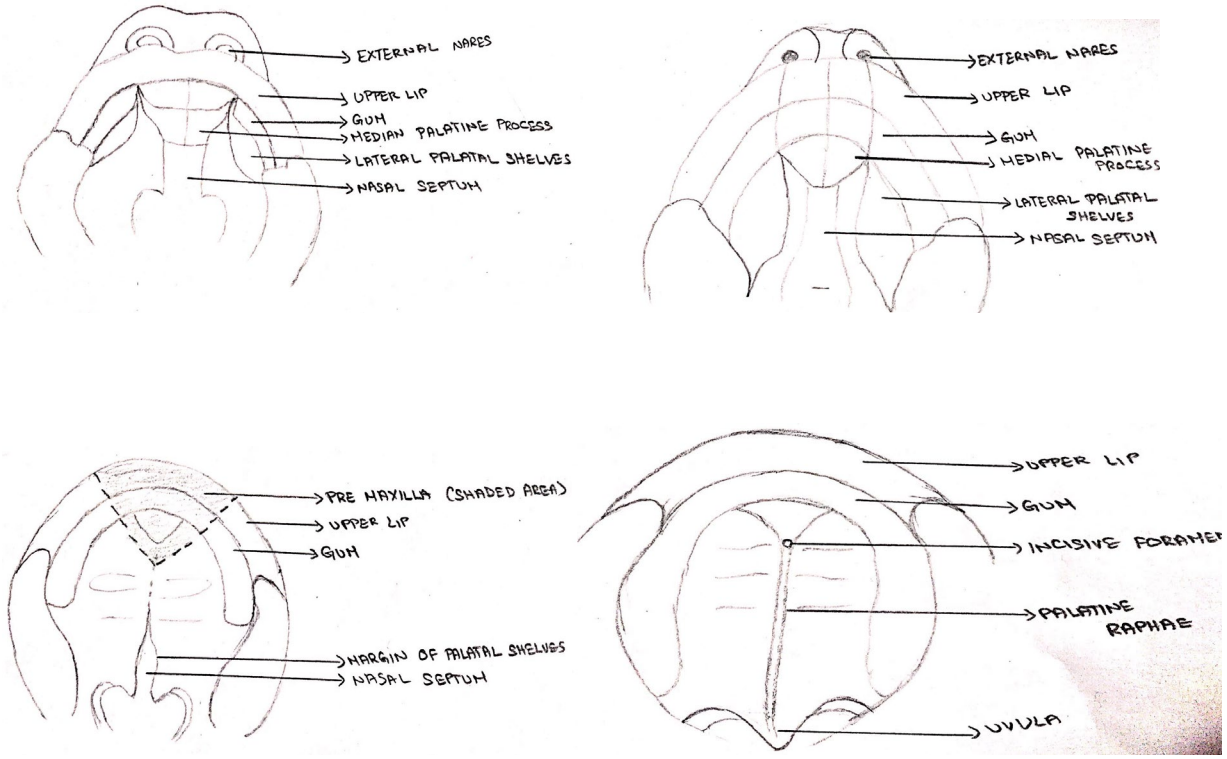


Figure 7 Development of palate

Ref: Human Embryology and Developmental Biology Carlson *et al.*,(2019)

1.3 Orofacial Clefting

The formation of face is complex and there are many ways this highly regulated process could go wrong. Failure of fusion of any of the processes mentioned above may result in OFC. Failure of fusion of the MNP with MXP results in CL/P. CL/P can be unilateral or bilateral. Failure of fusion of the MNP results in median cleft (Koh *et al*, 2016). Failure of fusion of lateral parts of MXP and MP results in lateral cleft. Median and lateral clefts are rare. CP results when the two lateral palatine processes fail to fuse (Pansky, 1982).

Failure of the facial prominences to fuse can be due to genetic causes and/or environmental factors.

More than 400 syndromes have been found to be associated with CL/P, about 75% have a known genetic cause (Leslie and Marazita, 2013). In most of the cases the syndromes are inherited in a Mendelian manner. Van der Woude syndrome (VWS) (OMIM #: 119300) is the most common form of syndromic CL/P. Back in the 1950s, Van der Woude reported an autosomal dominant condition that was characterized by lower lip pits and CL/P or CP (Van Der Woude, 1954). Kondo *et al* (2002) carried out direct sequence analysis in the 350-kb critical region of VWS and identified mutations in the *IRF6.* When studying a pair of monozygotic twins, they had identified a nonsense mutation in the exon 4 of *IRF6* region of the affected twin.

70% of OFC do not occur with other abnormalities and are classified as non-syndromic. Mapping these complex traits are difficult since the affected family members don't necessarily share a single genetic variant. Several approaches have been applied to identify the genetic causes of non-syndromic clefting

1. Genetic Linkage Analysis: It is one of the commonly used method to map a chromosomal location to a diseased gene (Pulst; Cantor). In genetics the likelihood of two genes or a gene and a diseased gene to be located near each other and thus be inherited together is given by the LOD (Logarithm of Odds score). If everyone in the family has the same mutation, then the LOD score can be summed across the families thus increasing the power of linkage analysis. But in complex traits the affected members of the family have different mutations and summation of LOD score gives a negative result. Some family members don't express the trait but will

still carry a mutation due to decreased penetrance. This breaks the linkage between the gene and the trait (Lidral et al, 2008).

2. GWAS: This method tests millions of SNPs spread across the entire genome for association with a trait (Norrgard, 2008). Unlike the candidate gene approach, GWAS is agnostic. Because of the high number of SNPs tested, the threshold for statistical significance is very high. GWAS has now identified dozens of variants associated with non-syndromic OFC (Birnbaum *et al*., 2009; Beaty *et al*., 2010; Mangold *et al*., 2010). A GWAS can identify variants that may be a risk factor for the disease, but it does not necessarily identify the causal variant. Additional experimental testing is often needed to confirm a variant's role in the pathogenesis of a disease or trait.
3. Candidate Gene studies: Candidate genes can be selected based on animal studies and Mendelian clefting syndromes.
    i) Animal studies: Transgenic mouse models or mouse with spontaneous clefts are used to study candidate genes causing clefts. These are excellent models to study clefting as there are craniofacial developmental similarities between the humans and mouse models. Cleft palate phenotype in the mouse is more common than cleft lip phenotype (Schutte and Murray, 1999; Jugessar and Murray, 2005).
    ii) Mendelian forms of cleft: Sometimes Syndromic clefting which has a mendelian form of inheritance gives clues to isolated forms of clefting. VWS has a similar phenotype to isolated clefting. Mutations in *IRF6* has been implicated in both VWS and isolated clefting (Zucherro *et al*., 2004)

A few of the major genes that have been implicated in isolated OFC include:

1. *IRF6*: It was found that when disequilibrium testing that was carried at V274I position, there was an overtransmission of valine allele. A SNP of *IRF6* results in this transmission. It was concluded that variation in *IRF6* results in 12% of the CL/P (Zucherro *et al.,* 2004).
2. *MSX1:* It is a non-clustered homeobox gene that is a strong candidate gene in both syndromic and isolated forms of clefting due to the results of animal studies and linkage studies. It is strongly associated with tooth agenesis (Modesto, Krahn, Lidral *et al.,* 2006)
3. *Fgfr:* Rice *et al* (2004) studied that early palate formation is due to epithelial mesenchymal interactions. He studied the role of *Fgf10, Fgfr2b and Shh* signaling in the palate development. They had observed that mice which did not have *Fgf10* and its receptor *Fgfr2b* developed cleft palate. The differentiation process and the cell turnover rate was affected in these mice. *Fgf10* signaling is in the palatal mesenchyme and Shh is in the epithelium. *Fgf10* regulates the expression of Shh and the *Fgf10* and *Fgfr2b* deficient mouse was shown to downregulate the expression of *Shh* (Murray and Schutte, 2004).

   Apart from genetics, environmental factors also play a significant role in OFC. Cigarette smoking has an effect on orofacial clefting. Development of CL/P and CPO has been associated with maternal cigarette smoking (Beaty *et al*., 1997) (Chung *et al*., 2000). Romitti *et al* (2007) reported that there was an association between alcohol intake and orofacial clefts. This association was influenced by the type of alcohol and folic acid intake. Folic acid deficiency has been shown to cause

facial clefts in rodents (Munger 2002) but the results are inconsistent in human (Gildestad *et al*., 2015; Shaw *et al*., 1995).

1.4 Subclinical phenotypes in OFC

In most genetic studies, OFC has been conceptualized as a simple binary trait – affected or unaffected. However, there is a wide range of phenotypic expression in OFC, including certain subclinical manifestations, which may be present at a higher than normal frequency in non-cleft relatives within affected families (Weinberg *et al*., 2006). Some of the subclinical phenotypes that have been documented in OFC include increase fluctuating asymmetry (Neiswanger *et al*., 2005), increased non-right-handedness (Scott *et al*., 2004), altered dermatoglyphic patterns (Neiswanger *et al*., 2002; Scott *et al*., 2005), subtle changes in craniofacial morphology (Raghavan *et al*.,1994; Perkiomaki *et al*., 2003), increased frequency of orbicularis oris muscle defects (Neiswanger *et al*.,2001), and minor dental defects (Eerens *et al*., 2001). The presence of these features is hypothesized to reflect underlying genetic risk factors for OFC. Thus, expanding the definition of OFC to include these subclinical expressions may aid in gene mapping efforts.

1.5 Olfactory deficits as a subclinical phenotype in OFC

About 10.6% of the US population over the age of 40 have reported olfactory dysfunction making it one of the most prevalent defects (Battacharya, 2005). There is evidence that individuals

with OFC may have higher than normal rates of olfactory deficit. For example, some Mendelian syndromic involve both orofacial clefting and olfactory deficits.

Kallmann syndrome is a hypogonadotropic hypogonadism condition characterized by anosmia (total loss of olfaction) or hyposmia (reduced olfaction), CL/P, unilateral renal agenesis and abnormalities of bones in fingers and toes (OMIM: 308700). It can be inherited as X- linked recessive when there is a mutation in *KAL1* gene, autosomal dominant due to mutations in *FGFR1, PROKR2, PROK2, CHD7 or FGF8*, or autosomal recessive due to mutations in *PROKR2* and *PROK2.*

Olfactory dysfunction associated with Kallmann syndrome is observed only in KAL2 type where there is *FGFR1* mutation. It was observed that FGFR1c isoform is important for the development of olfactory system (Dode *et al*., 2007). Cleft palate occurs in 25-30% of the KAL2 cases (Dode *et al*., 2003). *FGFR1* is important for cell proliferation, migration and differentiation thus playing a crucial role in embryological development (Thisse B and Thisse C, 2005).

It has been shown that anosmin-1 encoded by *KAL1* and *FGFR1* encoded by *KAL2* colocalized in olfactory bulb of rats during its development. Moreover, it was seen that anosmin-1 was positive extracellular regulator of *FGFR1* signaling suggesting that anosmin-1 plays a crucial role in olfactory bulb morphogenesis (Ayari *et al.*, 2007).

CHARGE syndrome (OMIM# 214800) is another syndrome that shares traits with Kallman syndrome (KAL2). Its characteristic features are coloboma, heart anomalies, choanal atresia, retardation of growth and/or development, genital and ear anomalies. CL/P occurs in 20-30% of the KAL2 and CHARGE syndrome. There is a loss of function mutation in *CHD7*. It is speculated that *CHD7* mutation may be involved in the *FGFR1* signaling pathway (Hardelin and Dode, 2008).

There have been a few studies that support the fact that olfactory deficits are seen in OFC affected cases and their UR.

There have also been several studies documenting various degrees of olfactory deficit in non-syndromic cleft cases and their unaffected family members. These studies suggest that children with clefts have nasal vestibule abnormalities or nasal septum deviations secondary to surgery may lead to reduced olfaction (or hyposmia). The presence of similar deficits in unaffected relatives, however, suggests that there may be a root biological cause.

***Richman et al.*, (1988):** The olfactory response of 35 subjects (20 boys and 15 girls) affected with CL/P were studied along with 68 controls (34 boys and 34 girls). Ten common household odorants were asked to be identified by the subjects and controls. 50% of the boys affected with CP with or without CL had 60% less olfactory scores when compared to 9% of the boys without CP, 20% of girls with CP and 15% of girls without CP. The olfactory deficits were seen to be higher in the affected boys compared to the control group. This was not the case in the females. It was also seen that olfactory function increases with age.

***Grossmann et al.*, (2005):** This study was done to assess the nasal airflow and olfactory function in patients affected with CP with or without CL after they underwent surgery. The affected group had 15 patients with unilateral cleft palate and lip (UCLP), 2 with only CP (UCLP subgroup), 8 with bilateral cleft lip and palate (BCLP subgroup). There were 20 nonaffected orthodontic patients in the control group. The nasal airflow was reduced for all CP patients ($p<0.02$). The airflow on the affected side of UCLP group was lower than control ($p<0.02$). In the BCLP group the airflow was reduced and symmetrical in both nostrils. The smell threshold was higher in the UCLP group ($p<0.01$). No significant difference was found in the BCLP group.

Significant correlation between the smell threshold and nasal airflow was found in the UCLP group ($r = -0.33$, $P = 0.05$).

Even though the above studies indicate that there is some level of olfactory deficits present in the OFC groups, they don't specify the nature of the defects. The question arises if the olfactory deficits are innate to the clefting process or if they are secondary to surgical procedures. The following studies prove that olfactory deficits are inherent to orofacial clefting.

***Roosenboom et al*., (2015):** Roosenboom et al had studied the facial characteristics and reduced olfaction related to NSCL/P. She concluded that the non-affected first-degree relatives of patients with non-syndromic CL/P showed - reduced smell capacity when compared with the control group who did not have a family history of Nonsyndromic CL/P. In a follow-up study, Roosenboom *et al.,* (2018) administered the sniffin' sticks olfactory test to 54 NSCL/P patients, 44 unaffected first-degree relatives of these patients and 35 patients from the control group with a negative family history. It was seen that patients with NSCL/P and their unaffected relatives have an increased prevalence of hyposmia and anosmia when compared to the control group. It was also seen that there was reduced olfactory function among CP patients. The olfactory bulb (OB) volume and olfactory sulcus (OS) depths were measured. It was found that NSCL/P patients with hyposmia had reduced OB volume. The left OS depth was smaller in NSCL/P patients with hyposmia.

There was age bias in the study. Even though they compared the different phenotypes of clefting in the affected individuals, they could not do the same for the unaffected relatives since the sample size was small.

***May et al*., (2015):** 60 unaffected parents of the patients with OFC were administered the UPSIT. Their olfaction scores were compared with 2762 controls. 41.7% of the parents displayed olfactory deficits when compared to the 12.6% of controls. 41.7% of the unaffected father

displayed olfactory deficits compared to 15.1% of the male controls ($p=0.001$). Olfactory deficits were present in 41.7% of unaffected mothers when compared to 10.4% female controls ($p<0.001$). There was no difference in olfactory deficits between the unaffected fathers and mothers.

This study concluded that the olfactory deficits were prevalent among the unaffected relatives of the individuals affected with clefting. But there was no comparison of the olfactory deficits between different subtypes of clefting. Also, the controls used in the study were from the UPSIT test manual.

The above studies suggest that olfaction can be considered as a subclinical feature of clefting, but several limitations and biases are present in prior research. In the current study, we are trying to overcome the above-mentioned limitations by using a set of carefully collected controls, correcting for possible age and sex bias, and comparing olfactory deficits between different subtypes of cleft (CL, CLP and CP).

2.0 Specific Aims

Specific Aim1:

Assess and compare the prevalence of olfactory deficits in affected subjects with CL, CLP and CP using the 40 item University of Pennsylvania Smell Identification test. It is hypothesized that the individuals affected with CL, CLP and CP will exhibit a higher prevalence of olfactory deficits when compared to the control population.

Specific Aim 2:

Assess and compare the prevalence of olfactory deficits in the unaffected relatives of cases affected with CL, CLP and CP using the 40 item University of Pennsylvania Smell Identification test. It is hypothesized that the first-degree unaffected relatives of patients affected with CL, CLP and CP will exhibit a higher prevalence of olfactory deficits when compared to the control population.

Specific Aim 3:

Assess and compare the prevalence of olfactory deficits in different cleft types and the first-degree unaffected relatives based on their family type. Based on the association of olfactory deficits with syndromic CLP, it is hypothesized that there will be reduced olfactory function in relation to palate (i.e., CLP and CP) compared to isolated Cleft lip CLO).

3.0 Materials and Methods

3.1 University of Pennsylvania Smell Identification Test

The University of Pennsylvania Smell Identification Test (UPSIT) was developed to assess quantitative olfactory levels of a patient presenting with olfactory deficits. The test is a self-administered 40 multiple-choice questionnaire which takes around 10-15 minutes and can be scored by non-medical personnel in less than 1 minute. It consists of 4 booklets with 10 questions (odorants) per booklet. The odorant in question is placed in a 10-50μm diameter microencapsulated crystals which is located on a brown strip (Doty *et al*., 1984). One has to scratch the paper identify the odorant and choose an option. The questions may be framed like "This odor smells most like a (a) Chocolate (b) Banana (c) Onion or (d) Fruit punch". This test is a forced choice method since one has to choose an answer even if an odor cannot be perceived. The rate of reliability is high r=0.94 (Doty *et al*., 1989a).

The UPSIT scoring manual is based on the olfaction data from 4000 control individuals. Individuals who take the UPSIT are scored based on these norms. Since the scoring in the manual is adjusted for age and sex, it also indicates how a tested individual has performed when compared to his age group and gender (Doty, 2008).

Ease of administration, reliability, less time that is required to complete the test, durability of the test makes it one of the most sought-after tests. Despite its advantages there is also a cultural bias. It is said that the test is based mostly on American culture and might not be applicable to other cultures. Also, the test only detects smell deficits above the threshold level. It does not test for Odor detection threshold or odor identification threshold. They can only identify the different

odors. Odor detection threshold can be defined as the lowest concentration of an odorant which could be perceived by humans. Odor identification threshold is the concentration at which 50% of the human population will be able to discriminate between odors.

3.2 Data Collection

3.2.1 Subject Recruitment

Individuals with OFC, their unaffected relatives (UR) and unaffected controls were recruited from Pittsburgh, Lancaster, Puerto Rico, and Colombia as part of the larger Pittsburgh Orofacial Clefting study. OFC affected individuals included those affected with only unilateral or bilateral cleft lip, cleft lip without or without cleft palate and only cleft palate.

3.2.2 Inclusion and Exclusion criteria

Subjects between 10 and 59 years of age, who were white, non-Hispanic were included. USPIT scores for this age range have been reported to be the most reliable (Doty *et al*., 1984a). Other racial and ethnic groups were not included in the current study since our samples are too small in these groups and there are known ethnic differences in olfactory ability (Hoffman *et al*., 2010) and potential testing biases (Doty *et al*., 1985a).

The effect of smoking on olfactory function is dose related. In a study where UPSIT was administered to 638 people with a history of smoking, it was seen that long-term smoking had a reversible effect on olfaction (Frye, Doty *et al*., 1990). In our study 3 out of 32 cases, 56 out of

100 UR and 199 out of 447 controls were smokers. Excluding them will result in small sample size (Table 1). Hence, they were included in the study. Smoking bias is another limitation for our study.

Table 1 Group Demographic Composition

PARAMETER	CASES	UNAFFECTED RELATIVES	CONTROLS
N	32	122	447
AVERAGE AGE	20.0 yrs.	31.9 yrs.	32.4 yrs.
SEX	17M/15F	48M/74F	165M/282F
SMOKERS	3	56	199
NON-SMOKERS	29	66	248

Allergic rhinitis or nasal sinus disease may cause nasal congestion. It has been suggested that the destruction of the nasal mucosa due to inflammation or other obstructions may cause significant olfactory deficit (Cowart *et al*., 1993). Head injury is one of the important reasons for olfactory deficit. Approximately 5-20% of the patients presenting with olfactory deficit have a history of trauma. The shearing of olfactory nerve fibers and their connections to the olfactory bulb during the accident may cause olfactory deficits (Muller and Hummel 2009). The limbic system is responsible for our emotional behavior. The olfactory bulb has projections into the limbic system structures such as amygdala, hippocampus, insula, anterior cingulate cortex and orbitofrontal cortex. This shared network may be responsible for olfactory deficits seen in patients suffering from depression (Kohli *et al*., 2016). Citalopram, clomipramine and Rolipram are all antidepressants that has the same target receptor- ADRA1A (adrenoceptor α_{1A}). Blocking this

receptor has shown to inhibit the GABAergic inhibition of mitral cells present in the olfactory bulb (Lötch *et al*., 2015). Hence subjects were excluded based on nasal congestion, history of loss of smell due to trauma, depression and anti-depressants.

3.2.3 Study sample

After applying inclusion/exclusion criteria, a total of 32 individuals with OFC, 100 unaffected relatives (UR) and 422 controls were included. Among the 32 cases 8 were affected with cleft lip (CL), 15 were affected with cleft lip with palate (CLP) and 9 were affected with only cleft palate.

Among the 100 unaffected relatives 14 belong to the cleft lip family type, 66 belong to cleft lip and palate family type and 20 belong to cleft palate family type.

3.3 Data Analysis

The statistical analysis for this study was done using GraphPad PRISM version8 and R. Two types of comparisons were made between the cases and the controls, and (UR) and controls. The first analysis approach divided individuals into clinically-defined deficit or no deficit categories based the UPSIT score. The second approach involved comparisons based on the raw UPSIT scores.

Based on the UPSIT norms the olfactory deficits had been scored as Normosmia, mild microsmia, moderate microsmia, severe microsmia and total anosmia. For comparison purposes normosia is considered as the “No deficit” group. Mild, moderate, severe microsmia and total

anosmia as a whole is considered as “Smell Deficits” group. There was no age or sex bias when comparisons were made based on “Smell deficits: and “No deficits”. This is because when the subjects are scored and assigned an olfactory status, this is based on UPSIT norms which have already been adjusted for age and sex. The Chi-Square test was used to compare categories (deficit vs no deficit) between the cases/UR and controls. The fisher exact test was used to compare smaller sample size.

For the first and the second part of the specific aim the following comparisons were made (figure 8).

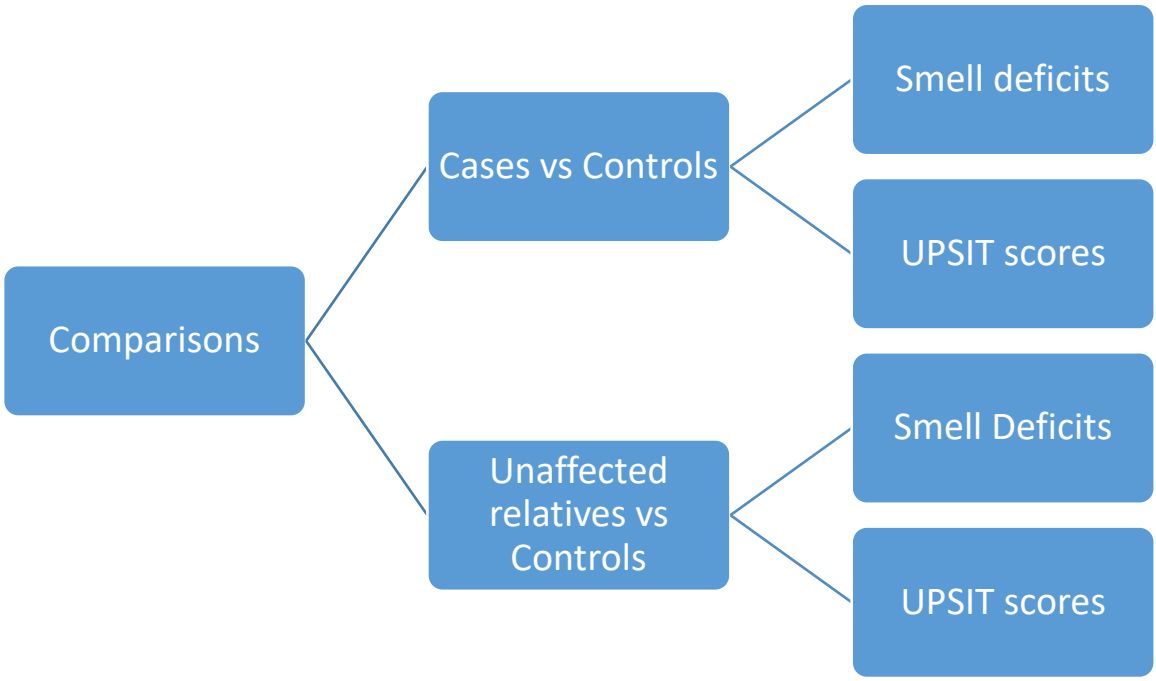


Figure 8 Comparisons for specific aim 1 and 2

For the third part of the specific aim the following comparisons were made (figure 9).

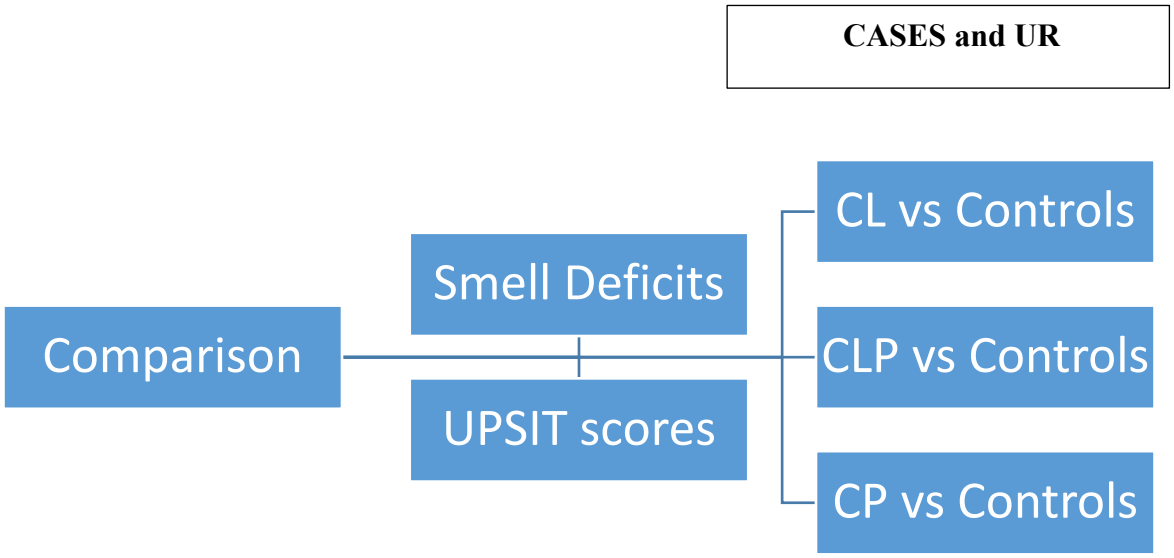


Figure 9 Comparisons for specific aim 3

For the raw UPSIT score comparison, age and sex effects had to be taken into account between these can have an effect on olfactory function (Doty *et al*., 1984a; Doty *et al*., 1985a). To solve for the potential age and sex bias when comparing the cases and UR to controls based on UPSIT scores, each case and UR were matched to two controls by age and sex. For example, if there was a male who was 10 years old in the case cohort, he was matched to two males in the control group who are of the same age. The UPSIT score distribution deviated from normality based on the Shapiro Wilk test. Thus, the non-parametric Mann Whitney U test was used for all UPSIT score between comparisons.

All statistical tests were considered one-sided due to the direction of effect indicated in the hypotheses. All results were considered statistically significant at $p \leq 0.05$.

4.0 Results

Olfactory dysfunction is categorized into 5 clinical categories based on the UPSIT scores- Normosia, Mild microsmia, Moderate microsmia, severe microsmia and total anosmia. Table 2 describes the distribution of olfactory deficits between the cases, UR and controls. It could be observed that most of the subjects are in the mild microsmia and normosia category. 25 of the 32 cases fall in the normosia and mild microsmia category. 108 of the 122 UR falls in the normosia and mild microsmia category. 419 of the 447 controls fall in the normosia and mild microsmia category.

Table 2 Distribution of smell deficits in cases, UR and controls

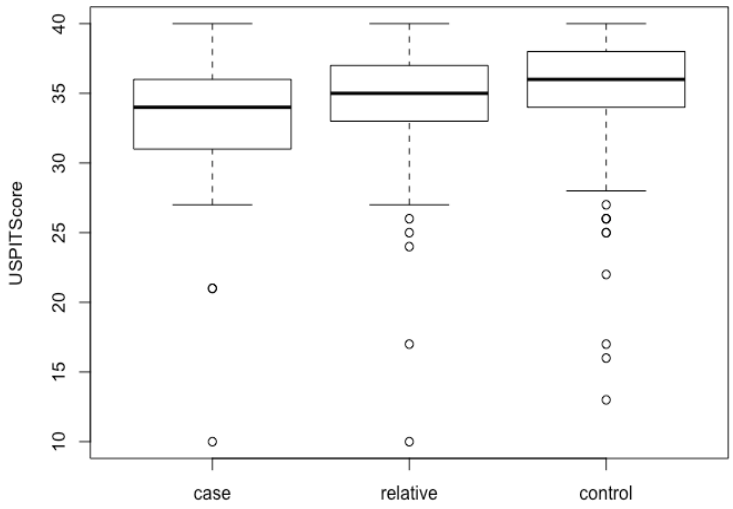
	Normosia	Mild microsmia	Moderate microsmia	Severe microsmia	Total anosmia
Cases	15	10	4	2	1
UR	78	30	10	2	2
Controls	333	86	22	3	3

It was not possible to compare between different levels of olfactory deficits such as normosia, mild microsmia, moderate microsmia and severe microsmia since the sample size of each of these groups was too small. Hence normosia was considered the “no deficit” group and the other categories of olfactory deficit were considered as one single “smell deficit” group (table 3). It could be observed from table 3 that 17 of the 32 (more than 50%) cases have smell deficits. 44 of the 122 UR (37%) have smell deficits compared to 114 of the 447 controls (24.4%).

Table 3 Frequency of smell deficits and no deficits in the Cases, UR and Controls

	Cases N=32	Unaffected Relatives N=122	Controls N=447
Smell Deficits	17 (53%)	44 (37%)	114 (24.4%)
No deficits	15 (47%)	78 (63%)	333 (75.6%)

The distribution of raw UPSIT scores in cases, UR and Controls is presented in figure 10. The average UPSIT score is low in cases compared to UR and Controls. Similarly, the UPSIT average of UR is lower compared to controls.



X axis: Cases, UR and Controls

Y axis: UPSIT scores

Figure 10 Distribution of UPSIT scores in Cases, UR and Controls

The distribution of UPSIT scores for the cleft type of cases and family type of UR and controls is described in figure 11. It could be seen that the average UPSIT score for CLP cleft type of cases is lower compared to CL and CP type. The UPSIT average of CLP family type is lower compared to CL and CP family type.

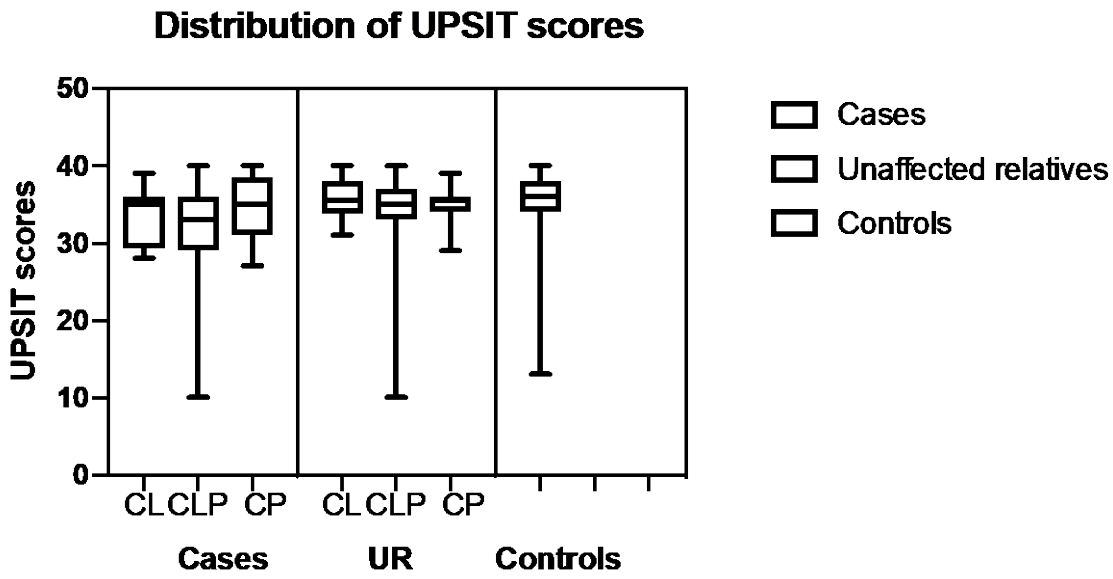


Figure 11 Distribution of UPSIT scores based on each cleft type

The distribution of UPSIT scores in Cases, UR and controls between smokers and non-smokers are described in figures 12, 13 and 14. In figure 12 it could be observed that 3 of the cases were smokers and their UPSIT scores were between 37 and 39.

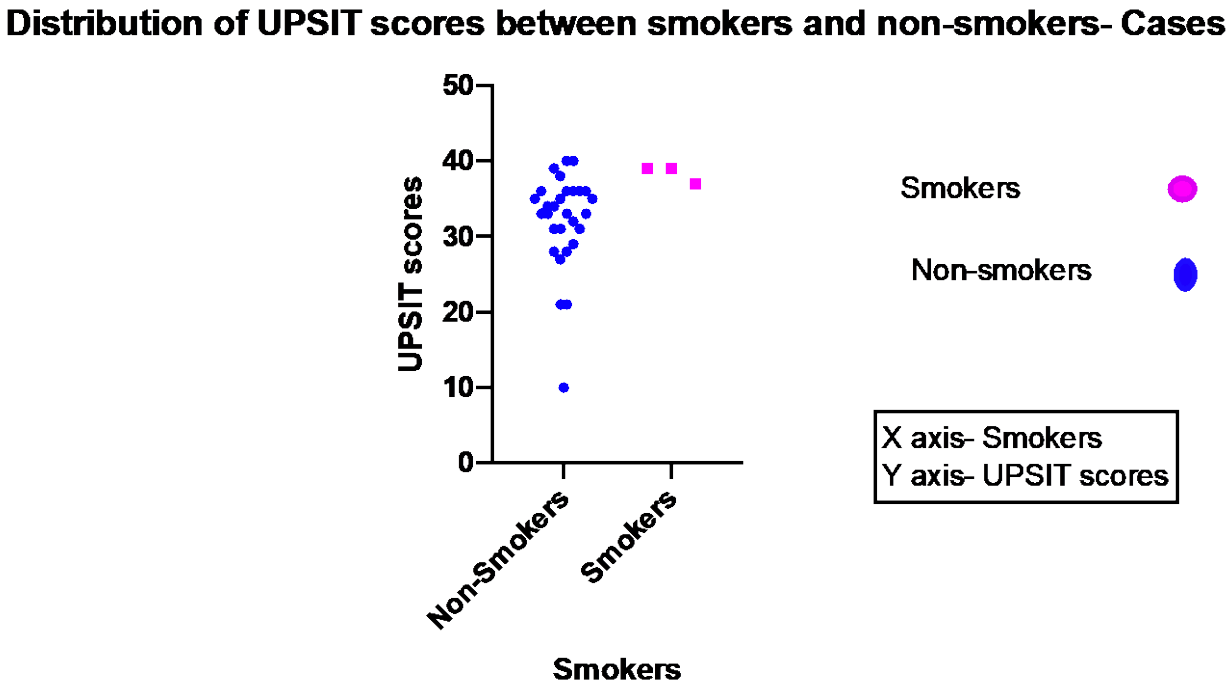


Figure 12 Distribution of UPSIT scores in cases between smokers and non-smokers

In figure 13 it could be observed that 56 of the 122 UR were smokers. Their UPSIT scores were between 26 and 40.

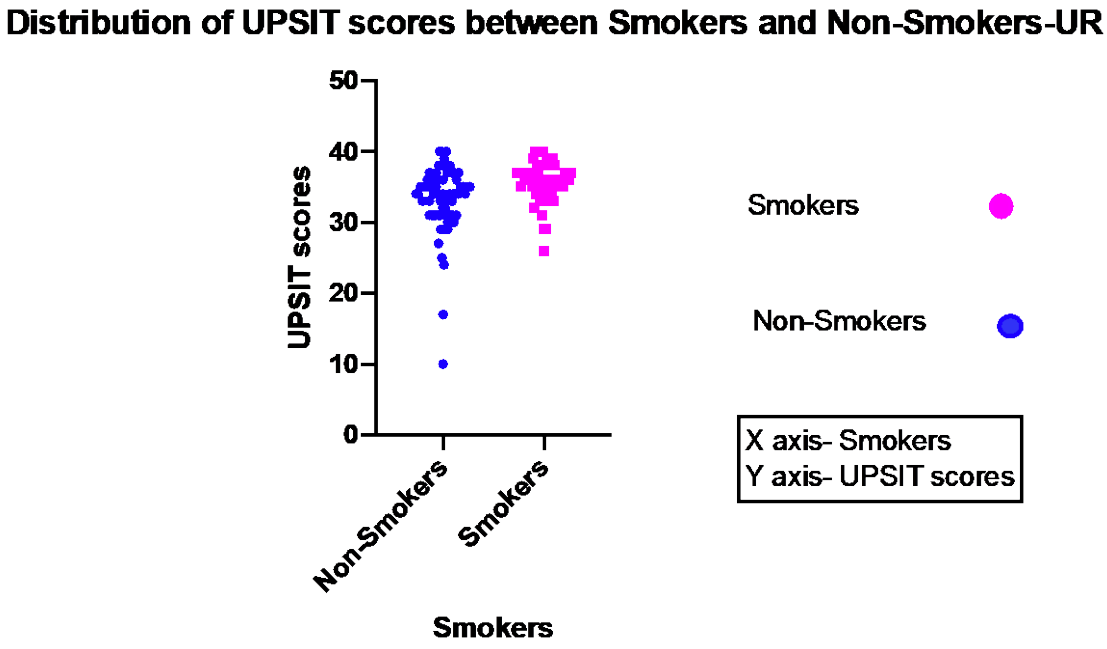


Figure 13 Distribution of UPSIT scores in UR between Smokers and non-smokers

In figure 14 it could be observed that 199 of the 447 controls were smokers. Their UPSIT scores were between 16 and 40.

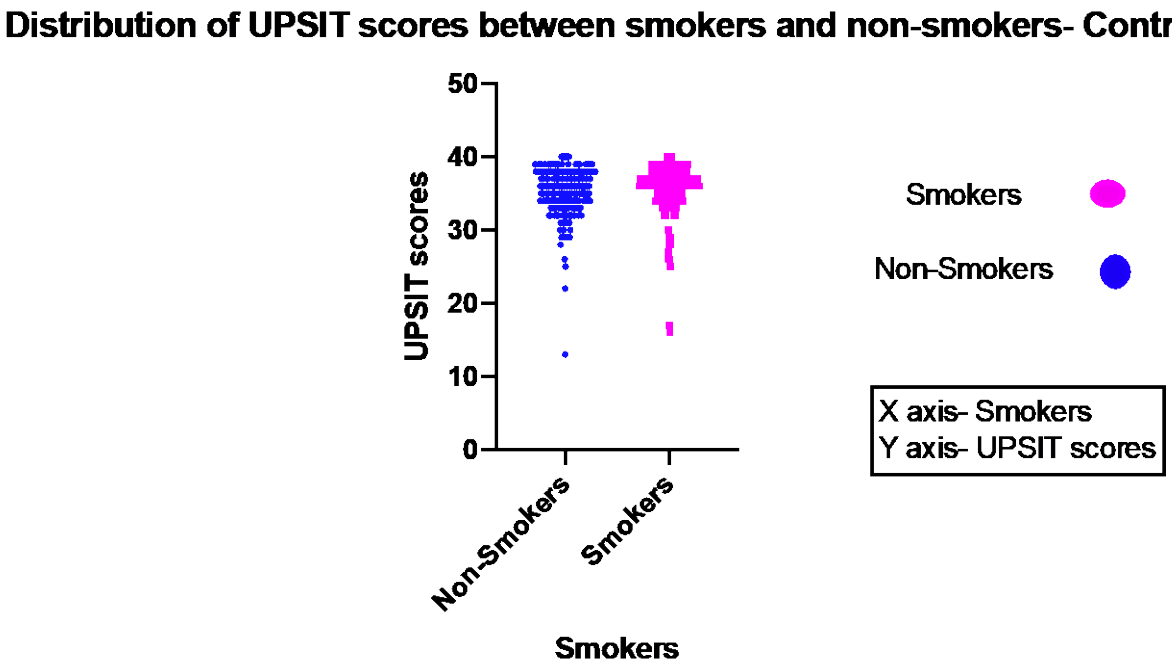


Figure 14 Distribution of UPSIT scores in Controls between smokers and non-smokers

4.1 Results for specific aim 1

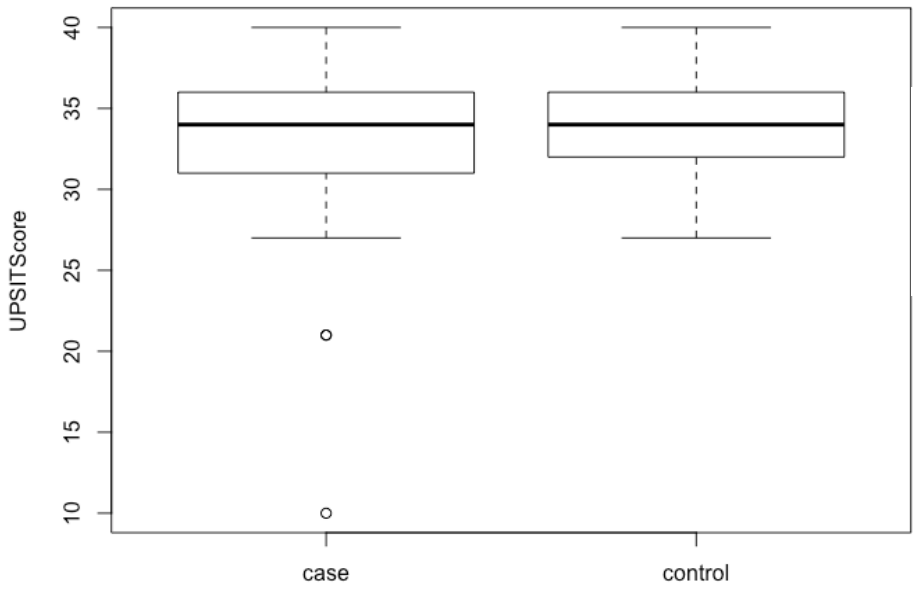
For specific aim 1, olfactory deficits in cases were compared to controls. The comparisons were based on "smell deficit" categories and "UPSIT scores". It could be seen from Table 4 that there is a significant difference when comparing cases vs controls based on smell deficits. Although mean UPSIT scores were higher in age and sex matched controls (as predicted), the difference was not statistically significant (Table 5). Figure 15 shows the distribution of scores between cases and controls.

Table 4 All Cases vs Controls based on smell deficits

	Cases n=32	**Controls n=64**
Smell deficit	17	114
No deficit	15	333
p-value	0.00004	

Table 5 All Cases vs Controls based on UPSIT scores

	Case n=32	**Controls n=64**
UPSIT Average	32.7	33.9
p-value	0.37	



X axis: cases and Controls

Y axis: UPSIT scores

Figure 15 Graph describing the UPSIT scores for all cases vs controls

4.2 Results for Specific Aim 2

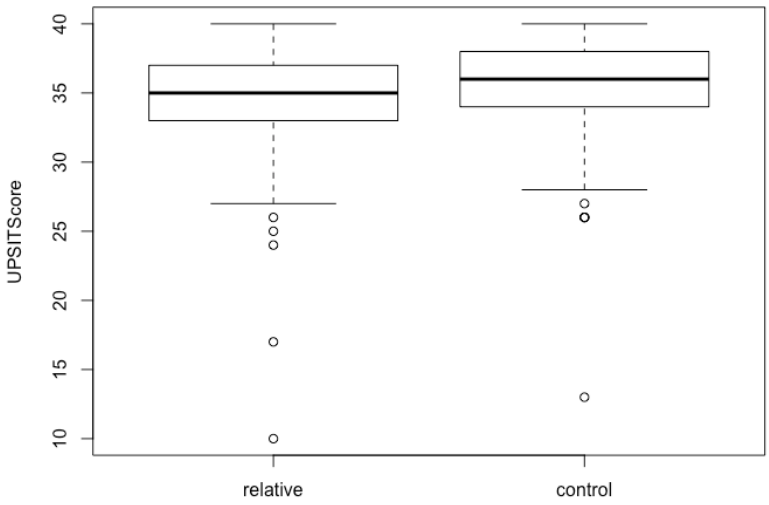
For specific aim 2, olfactory deficits in UR were compared to controls. The comparisons were based on "smell deficit" categories and "UPSIT scores". It could be seen from Table 6 and 7 that UR showed both significantly higher rates of olfactory deficit and lower mean UPSIT scores compared with controls. Figure 16 shows the distribution of scores between UR and controls.

Table 6 All UR vs Controls based on smell deficits

	Unaffected relatives	**Controls**
Smell deficit	44	114
No deficit	78	333
p-value	0.06	

Table 7 All UR vs Controls based on UPSIT scores

	Unaffected relatives	**Controls**
UPSIT average	34.06	35.22
p-value	0.0035	



X axis: UR and Control
Y axis: UPSIT Scores

Figure 16 Graph describing the UPSIT score for All UR vs Controls

4.3 Specific Aim 3

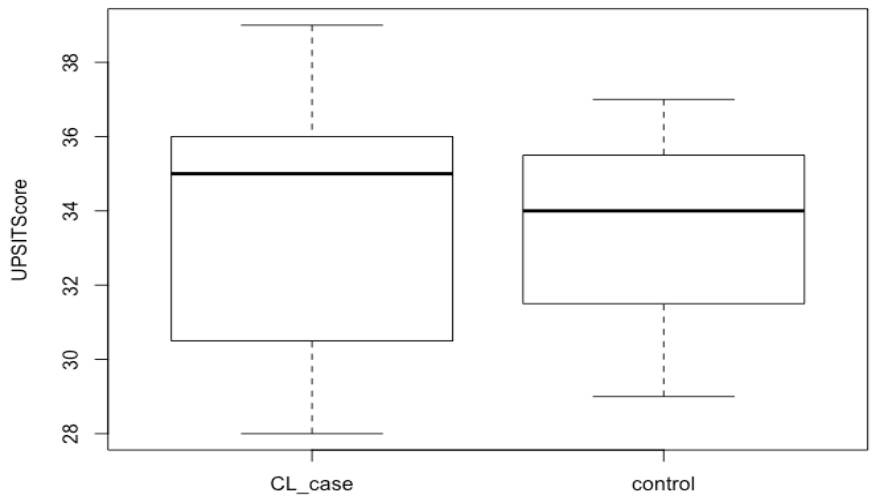
For the first part of specific aim 3, the affected cases were divided by cleft type and each compared with controls. Based on smell deficit categories, all three cleft types showed significantly higher rates of deficit compared with controls (Table 8). Although UPSIT the mean scores were lower in CLP and CP cases vs age and sex matched controls, these results were not statistically significant (Table 9 and Figures 17 and 18). In contrast, in CL cases the mean score was higher than controls, but this was also not significant (Table 9 and Figure 19).

Table 8 Cleft phenotypes of cases vs controls based on smell deficits

	CL	CLP	CP	Controls
Smell Deficits	4	9	4	114
No Deficits	4	6	5	333
P value	0.0002	4.41E-07	0.002	

Table 9 Cleft phenotypes of cases vs controls based on UPSIT scores

	CL	Controls	CLP	Controls	CP	Controls
UPSIT average	33.75	33.25	31.2	33.8	34.22	34.78
p-value	0.32		0.28		0.43	



X axis: CL cases And Control
Y axis: UPSIT Scores

Figure 17 Graph describing UPSIT score of CL vs Control

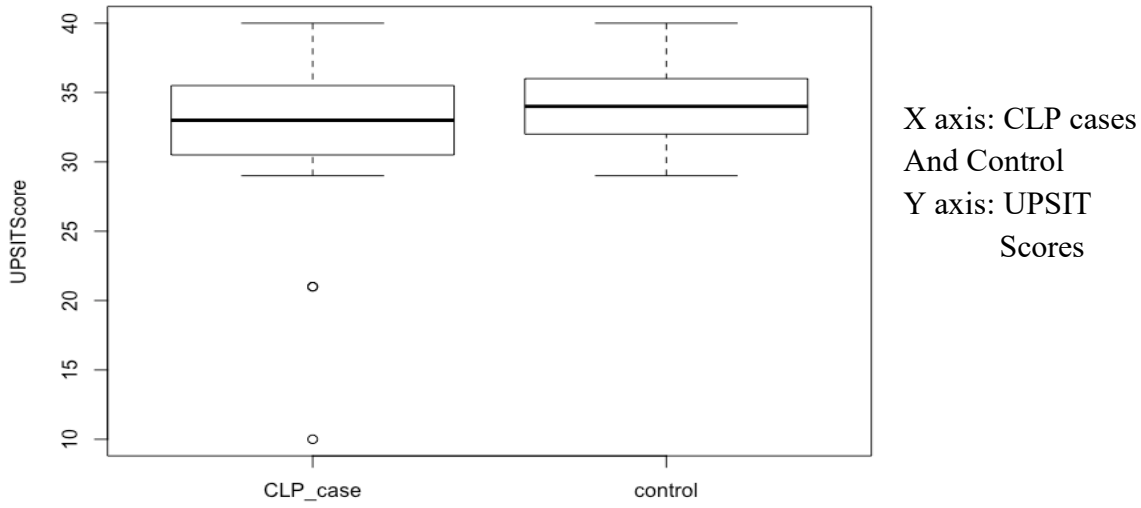


Figure 18 Graph describing UPSIT score of CLP vs controls

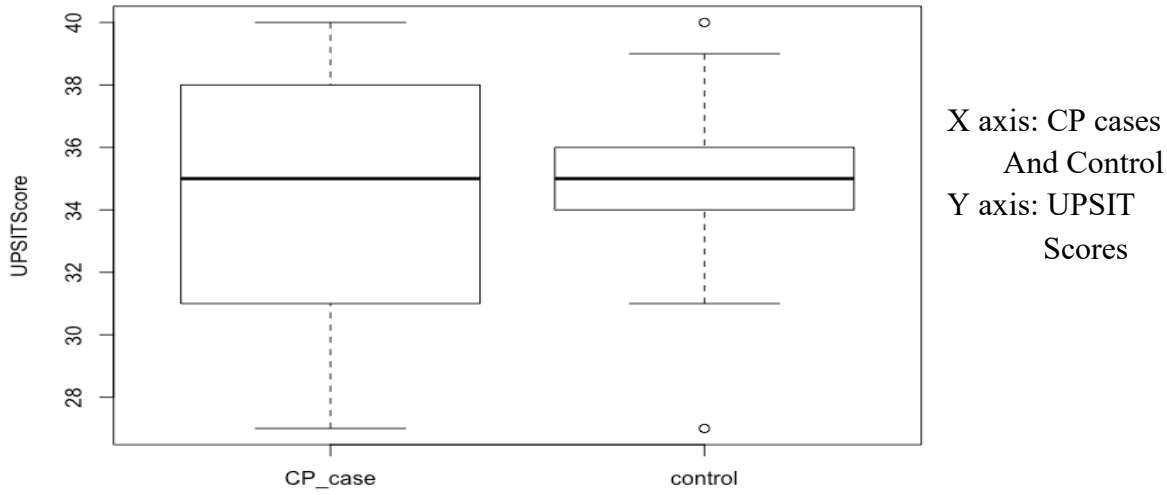


Figure 19 Graph describing UPSIT score of CP vs Controls

For the second part of specific aim 3, URs were divided by cleft family type and compared with controls. Based on smell deficit categories, URs from CL/P family types showed significantly

higher rates of deficit compared with controls (Table 10). URs from CL and CP family types also showed an increased deficit rate, but these differences were not statistically significant. All three UR groups showed lower mean UPSIT scores compared with age and sex matched controls, but this difference was only significant in CL/P and CP cases (Table 11 and Figures 20-22).

Table 10 Cleft family type of uR vs Controls based on smell deficits

	CL UR	**CL/P UR**	**CP UR**	**Controls**
Smell Deficits	5	26	6	114
No Deficits	9	41	14	333
p-value	0.06	0.03	0.26	

Table 11 Cleft family type of UR vs Controls based on UPSIT scores

	CL UR	**Controls**	**CL/P UR**	**Controls**	**CP UR**	**Controls**
UPSIT average	34.6	35.1	33.57	34.99	35.25	36.1
p-value	0.76		0.03		0.048	

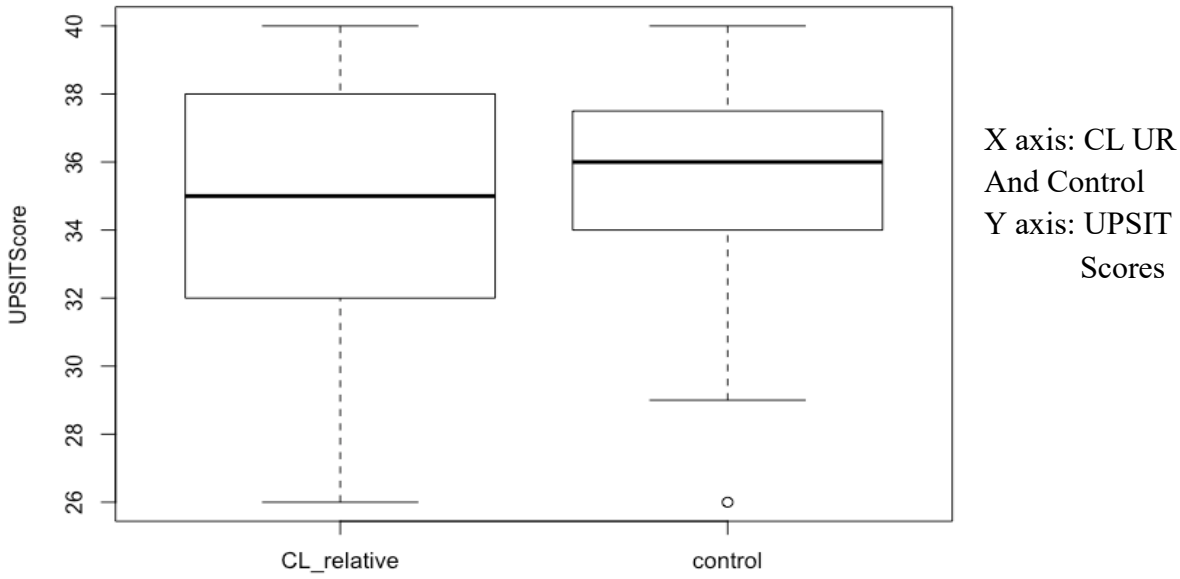


Figure 20 Graph describing UPSIT score of CL relative vs Control

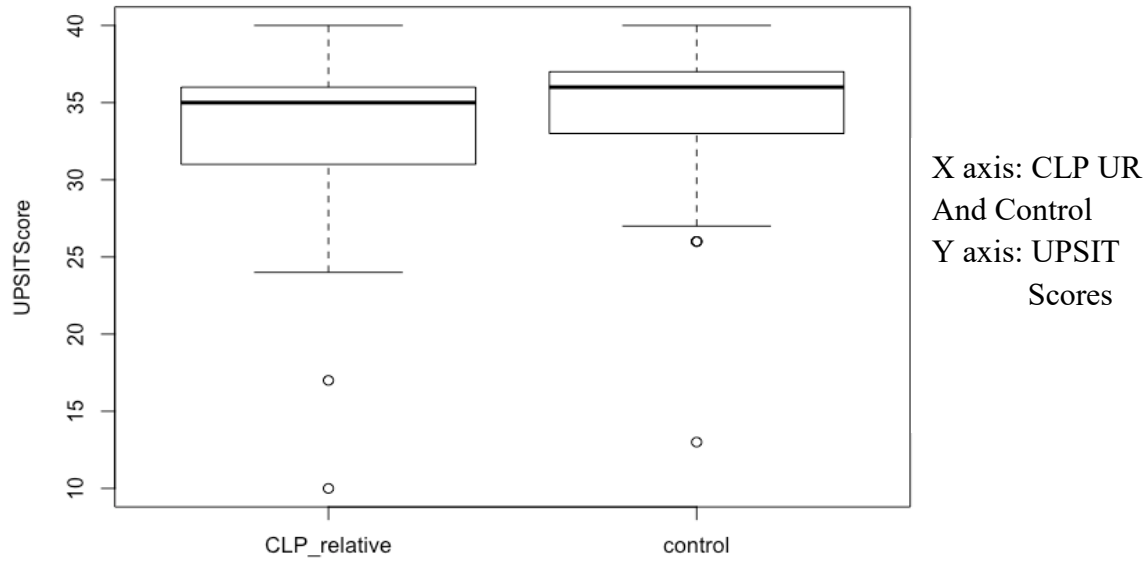
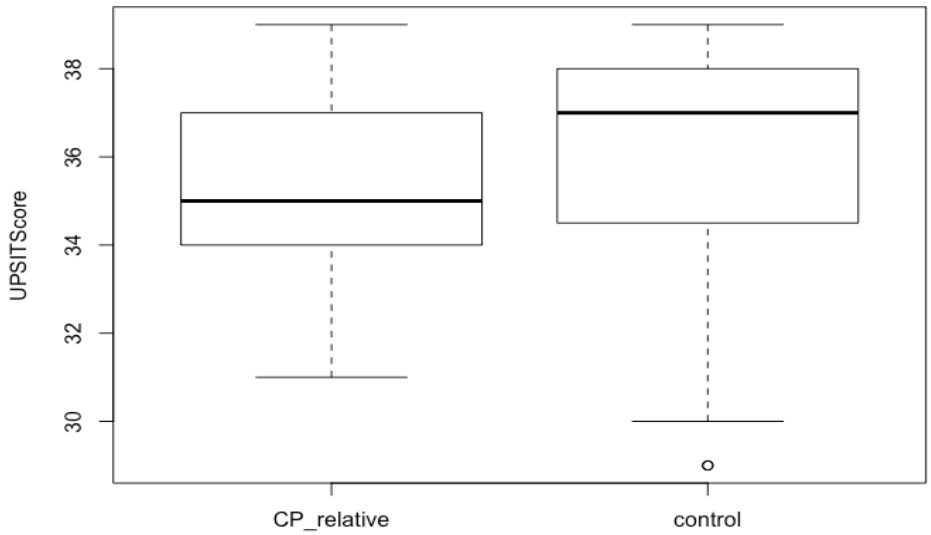


Figure 21 Graph describing UPSIT score of CLP relative vs Controls



X axis: CP UR
And Control
Y axis: UPSIT
Scores

Figure 22 Graph describing UPSIT score of CP relative vs Control

5.0 Discussion

It has been observed previously that individuals affected with OFC show decreased olfactory ability compared with the general population (Richman *et al*., 1988; Grossman *et al*., 2005; May *et al*., 2015; Roosenboom *et al*., 2015; 2017). Our results lend strong support to these earlier reports. 53.1% of our OFC cases had some evidence of a smell deficit compared to 24.4% of controls. For reports that focused only on affected cases (Richman *et al*., 1988; Grossman *et al*., 2005) there was no way of distinguishing whether the high rate of olfactory deficits was simply a secondary byproduct of the surgical repair or was an intrinsic part of the OFC phenotype. Looking at the rate of olfactory deficit in unaffected family members is one way to approach this problem, since these individuals have the genetic risk factors for OFC but have not had a surgical repair. Several prior reports have also shown reduced olfaction in relatives (May *et al*., 2015; Roosenboom *et al*., 2015; Roosenboom *et al*., 2017). Our results support this claim, with 36% of our URs showing olfactory deficits compared to 24.4% of controls. The prevalence of olfactory deficits in URs suggests that olfactory deficits may be inherent to OFC may be considered part of the OFC phenotypic spectrum. It is notable that these replications were observed despite differences in the olfactory testing protocols among studies.

One advance of the current study is that we were able to test and compare olfactory ability across different cleft subtypes. In an earlier report, Roosenboom *et al*., (2017) concluded that there were no significant differences in olfactory ability between the cleft subtypes. While our study also found that CL, CLP and CP cases all showed reduced olfactory ability compared to controls, we were able to extend some of these findings to URs. When looking at smell deficit as a categorical trait, URs from all three family types (CL, CL/P, and CP) all showed evidence of

reduced olfactory ability, but this difference was only significant in URs from CL/P families. The CL/P group was also the largest of the three, so this may be a sample size effect. When the raw UPSI scores were compared, again all three UR groups showed evidence of worse olfactory performance, but the difference was only significant in URs from CL/P and CP families. This study is the first to compare the difference in olfactory deficits among the UR family types of OFC. The results overall provide only weak evidence that clefts involving the secondary palate (CL/P and CP) show greater olfactory deficits compared to clefts involving only the primary palate. In fact, the rate of smell deficit observed in both cases and URs was actually higher in CL compared to CP. To confirm this, larger sample sizes for each subtype would be needed.

Olfactory dysfunction may be due to pathology in the normal anatomy such as damage to the olfactory filaments due to a trauma. Viruses may cause an inflammation that may result in olfactory dysfunction (Hummel *et al.,* 2012). Kallman syndrome and CHARGE syndrome suggests that there might be an involvement of genetic components. A 16-year-old female who presented with Kallman syndrome had anosmia and CLP. The R609X nonsense mutation was found in the *FGFR1* gene of this female. Her father who presented with isolated CLP also had the same R609X mutation (Riley *et al*., 2007). Zenaty *et al*., (2006) had reported three cases with Kallmann syndrome. Of the three cases two had unusual phenotype feature associated with it such as ear anomalies. Kallmann syndrome features such as CP, anosmia and dental anomalies were present. They identified de novo mutations Cys178Ser and Arg622Gly in two cases and the third case inherited Arg622Gln mutation with intrafamilial variable phenotype. These cases suggest the non- penetrance effect that makes studying isolated clefts difficult. FGFR1 has a role in clefting, 16 mutations have been identified in the FGFR1 coding region of patients with Kallmann syndrome (Dode *et al*.,2003; Dode *et al*., 2007; Zenaty *et al*., 2006; Pitteloud *et al*., 2006; Pitteloud

et al., 2006; Trarbach *et al*., 2006). Testing of FGFR1 mutations in families with olfactory deficits may identify genetic subtypes of clefting. It was seen that in a group of children affected with 22q11 deletion syndrome, olfactory dysfunction is a result of surgery and insertion of nasogastric tubes (Sobin *et al*, 2009). The prevalence of olfactory deficits in the UR in our study and previous studies suggest that olfactory dysfunction might not be a consequence of external physical factors such as surgical repair. Grossmann *et al*., (2005) reported that there was a decreased nasal airflow in UCLP and BCLP subjects. But there was no relationship between cleft type, nasal airflow and smell threshold. If changes in anatomy is responsible for changes in nasal airflow that may affect odor discrimination ability, then one can expect that similar anatomical difference in family member may cause olfactory dysfunction.

Another possibility is that the root cause of the deficit is in the parts of the brain where smell is processed. One of the limitations for our study was we were not able to study function in relation to the anatomy of olfactory system components in the brain. Roosenboom *et al*., (2017) showed that there was reduced olfactory sulcus depth in URs with smell deficits. Her previous study also showed that there was a reduced upper nasal region in URs (Roosenboom *et al*., 2015). During facial development the olfactory placode, central upper lip and primary palate share the same facial prominence. One could expect that olfactory dysfunction and CL/P may share the same pathogenesis. In the future, it would be informative to study olfactory bulb and other olfaction-related brain centers in these URs and cases compared to controls.

The study of olfaction is relevant to public health. Impaired olfactory ability has been associated with a decreased quality of life (Frasnelli and Hummel, 2005). One reason for this is that olfaction is an important factor in a person's emotional and social behavior (Sarafoleanu *et al* 2009; Seo *et al*., 2013). Sometimes reduced olfaction can also be life threatening when one is not

able to detect a gas leak (Miwa *et al.*, 2001) or spoiled food (Stevenson, 2010). Thus, reduced olfaction should be considered seriously as it can negatively impact one's life. Assessing olfactory deficits in the OFC population may be an important factor for improving quality of life and could lead to earlier interventions. Identifying a genetic basis of the olfactory deficit in this population may provide new insights into the etiology of the condition and enable screening of individuals likely to exhibit olfactory problems.

Bibliography

Ayari B, Soussi-Yanicostas N (2007). "*FGFR1* and anosmin-1 underlying genetically distinct forms of Kallman syndrome are co-expressed and interact in olfactory bulbs".Dev Genes Evol. 217(2):169-75.

Beaty TH, Maestri NE, Hetmanski JB et al. (1997). "Testing for interaction between maternal smoking and TGFA genotype among oral cleft cases born in Maryland 1992-1996". Cleft Palate Craniofac J. 34:447-454

Beaty TH, Murray JC, Marazita ML, Munger RG, Ruczinski I, Hetmanski JB et al. (2010). "A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near *MAFB* and *ABCA4*". Nat. Genet. 42: 24-26.

Bhattacharyya N (2005). "A comparison of symptom scores and Radiographic Staging systems in Chronic Rhinisinusitis". Am J Rhinol. 19(2):175-9.

Birnbaum S, Ludwig KU, Reutter H, Herms S, Steffans M et al. (2009). "Key susceptibility locus for non-syndromic cleft lip with or without cleft palateon chromosomes 8q24". Nat Genet.41: 473-477

Cantor RM (2013). "Analysis of Genetic Linkage ". Rimoin D, Pyeritz R. Korf B. "Emery and Rimoin's Principles and Practice of Medical genetics 6th Edition". Philadelphia, PA. Elsevier.

Carlson BM (2009). "Human Embryology and Developmental Biology 4th Edition". Philadelphia, PA. Elsevier.

CHARGE SYNDROME (Date accessed on 25th February 2019) https://www.omim.org/entry/214800?search=charge%20syndrome&highlight=syndromic%20charge%20%22charge%20%28syndromic%7Csyndrome%29%22%20syndrome

Chung KC, Kowalski CP, Kim HM, Buchman SR. (2000). "Maternal cigarette smoking during pregnancy and the risk of having a child with cleft lip/palate". Plast Reconstr.Surg. 105:485-491.

Cowart BJ, Flynn-Rodden K, Mc Geady SJ, Lowry LD. (1993). "Hyposmia in allergic rhinitis". J Allergy Clin Immunol. 91(3):747-51.

Dixon MJ, Marazita ML, Beaty Th, Murray JC. (2011). "Cleft lip and palate: synthesizing genetic and environmental influences". Nat Rev Genet. 12(3):167-178.

Dode C, Levilliers J, Dupont JM, De Paepe A et al. (2003). "Loss of function mutations in FGFR1 cause autosomal dominant Kallmann syndrome". Nat Genet. 33(4): 463-5

Dode C, Fouveaut C, Mortier G, Janssens S et al. (2007). "Novel FGFR1 sequence variants in Kallmann syndrome, and genetic evidence that the FGFR1c isoform is required in olfactory bulb and palate morphogenesis". Hum Mutat. 28(1):97-8.

Doty RL, Shaman P, Dann M. (1984). "Development of the University of Pennsylvania Smell identification Test: A standardized Microencapsulated test of Olfactory Function". Physiol Behav 32: 489-502.

Doty RL, Shaman P, Applebaum SL, Gibberson R, Siksorski L, Rosenberg and L (1984a). "Smell Identification ability: changes with age". Science 226: 1441-1443.

Doty RL, Applebaum S, Zusho H and Settle RG (1985a). "Sex differences in odor identification ability: a cross cultural analysis". Neuropsychologia. 23:6 67-672.

Doty RL, Frye RE, Agarwal U. (1989a). "Internal consistency reliability of the fractionated and whole University of Pennsylvania Smell Identification Test". Percept Psychophys. 45: 381-384.

Doty RL (2008). "The smell Identification Test Administration Manual: 3rd Edition Philadelphia, Sensonics, Inc.

Eerens K, Vlientinck R, Heidbuchel K et al. (2001). "Hypodontia and tooth formation in groups of children with cleft, siblings without cleft, and non related controls". Cleft Palate Craniofac J. 38:374-378.

Frasnelli J, Hummel T (2005). "Olfactory disorder in children with 22q11 deletion syndrome". Pediatrics 118(3): e697-703.

Frye RE, Schwartz BS, Doty RL. (1990). "Dose-related effects of cigarette smoking on olfactory function". J Am Med Assoc. 263:1233-1236.

Gildestad T, Bjørge T, Vollset SE et al. (2015). "Folic acid supplements and supplements and risk for oral clefts in the newborn: a population-based study". Br J Nutr. 114(9):1456-63.

Grossmann N, Brin I, Aizenbud D, Sichel JY et al. (2005). "Nasal Airflow and Olfactory function after the repair of cleft palate (with and without cleft lip)". Oral Surg Oral Med Oral Path Oral Radiol Endod. 100(5): 539-44.

Hardelin JP, Dode C. (2008). "The complex Genetics of Kallmann syndrome: KAL1, FGFR1, FGF8, PROKR2, PROK2, et al.". Sex Dev. 2(4-5):181-93.

Hoffman HJ, Cruickshanks KJ, Davis B (2009). "Perspectives on population- based epidemiological studies of olfactory and taste impairment". Ann N Y Acad Sci, 1170:514-530.

Honrado CP, Bradley TD et al., (2018). "Facial Embryology". In:Azzizadeh B, Murphy MR, Johnson CM Jr, Massry GG, Fitzgerald R. "Master Techniques in Facial Rejuvenation". Philadelphia, PA. Elsevier.

Hummel T, Landis BN, Hüttenbrick KB (2011). "Smell and Taste disorders ". GMS Curr Top Otorhinolarygol Head Neck Surg. 10:DOC 04.

Hypogonadotropic Hypogonadism 1 with or without anosmia; HH1 (Date accessed on 25th February2019). https://www.omim.org/entry/308700?search=kallmann%20syndrome&highlight=syndromic%20kallmann%20%22kallmann%20%28syndromic%7Csyndrome%29%22%20syndrome

Jugessur A, Murray JC (2005). "Orofacial clefting: Recent Insights into a complex trait". Curr Opin Genet Dev. 15(3):270-8.

Keteyian AJ, Mishina Y (2017). "A review of Orofacial clefting and Current Genetic Mouse Models. Almasri MA (Editor). "Designing strategies for Cleft lip and Palate care". London, UK. Intech Open.

Koh KS, Kim Do Y, Oh TS (2016). "Clinical Features and Management of a Median Cleft lip". Arch Plast Surg. 43(3):242-7.

Kohli P, Soler ZM, Nguyen SA, Muus JS, Schlosser RJ (2016). "The association between Olfaction and Depression: A systematic Review". Chem Senses. 41(6):479-86.

Kondo S, Schutte BC, Richardson RJ et al. (2002). "Mutations in IRF6 cause Van Der Woude and Popliteal Pterygium Syndromes". Nat Genet. 32(2):285-9.

Leslie E, Marazita ML (2013). "Genetics of cleft lip and cleft palate". Am J Med Genet C Semin Med Genet. 163c(4):246-58

Lidral AC, Moreno LM, Bullard SA (2008). "Genetic Factors and Orofacial Clefting". Semin Orthod. 14(2): 103-114.

Lötsch J, Knothe C, Lippmann C, Ultsch A, Hummel T, Walter C (2015). "Olfactory drug effects approached from human-derived data". Drug Discov Today. 20(11):1398-406.

Mangold E, Ludwig KU, Birnbaum S, Balurado C, Ferrian M, Herms S, et al. (2010). "Genome-wide association study identifies two susceptibility loci for non syndromic cleft lip with or without cleft palate". Nat Genet. 42: 24-26.

May MA, Sanchez CA, Deleyiannis FWB, Marazita ML, Weinberg SM (2015). "Evidence of Olfactory Deficits as part of the Phenotypic Spectrum of Nonsyndromic Orofacial Clefting". J Craniofac Surg. 26(1):84-86.

Modesto A, Moreno LM, Krahn K, King S, Lidral AC (2006). "MSX1 and orofacial clefting with and without tooth agenesis". J Dent Res. 85(6):542-546.

Mossey P, Little J, Munger RG, Dixon MJ, Shaw WC (2009). "Cleft Lip and Palate". Lancet. 374: 1773-1785.

Mitchell B and Sharma R (2011). “Development of the Head and Neck, the Eye and Ear”. In: Horne T. “Embryology 2nd Edition”. London, UK Elsevier Health sciences.

Miwa T, Furukawa M, Tsukatani et al. (2001). “Impact of Olfactory Impairment on Quality of Life and Disability”. Arch.Otolaryngol.Head and Neck Surg. 127:497-503.

Mueller CA, Hummel T (2009). “Recovery of Olfactory function after nine years of post-traumatic anosmia: a case report”. J Med Case. 3:9283.

Munger RG (2002). “Maternal nutrition and oral clefts”. In:Wyszynski DF,ed. “Cleft lip and palate: from origin to treatment”. Oxford: Oxford University Press. 170-92.

Murray JC, Schutte BC (2004). “Cleft palate: players, pathways and pursuits”. J Clin Invest. 113(12):1676-1678.

Neiswanger K, Cooper ME, Weinberg SM et al (2002). “Cleft lip with or without palate and dermatoglyphic asymmetry: evaluation of Chinese population”. Orthod Craniofacial Res. 5:140-146.

Neiswanger K, Cooper ME, Ludwig KU et al (2005). “Assessment of bilateral symmetry in Chinese families with cleft lip with or without cleft palate”.Cleft Palate Craniofac J. 42:192-196.

Neiswanger K, Weinberg SM, Rogers CR et al. (2007). “Orbicularis oris muscle defects as an expanded phenotypic feature in non-syndromic cleft lip with or without cleft palate. Am J Med genet A. 143A(11):1143-9.

Norrgard K (2008). “Genetic variation and Disease: GWAS”. Nature Education 1(1):87

Parker SE, Mai CT, Canfield MA et al. (2010). “Updated National birth prevalence estimator for selected birth defects in the United States, 2004-2006. Birth Defects Part A: Clinical and Molecular Teratology”. 88:1008-1016.

Pansky B (1982).” Development of the Palate”. “Review of Medical embryology”. Alameda, CA. Embryonic Sciences.

Pansky B (1982).” Congenital malformations of the Lip and Palate”. “Review of Medical embryology”. Alameda, CA. Embryonic Sciences.

Perikomaki MR, Yoon KJ, Tallents RH et al. (2003). “Association of distinct craniofacial features in non-syndromic cleft lip and palate family members”. Cleft Palate Craniofacial J. 40: 397-402.

Pitteloud N, Acierno JS, Meysing A et al (2006). “Mutations in fibroblast growth factor receptor 1 cause both Kallmann syndrome and normosomic idiopathic hypogonadotropic hypogonadism”. Proc Natl Acad Sci USA. 103(16): 6281-6286.

Pitteloud N, Meysing A, Quinton R et al. (2006). “Mutations in fibroblast growth factor receptor 1 cause Kallmann syndrome with a wide spectrum of reproductive phenotypes”. Mol Cell Endocrinol. 254-255:60-9.

Pulst SM (1999). “Genetic Linkage Analysis”. Arch Neurol. 56(6):667-672.

Raghavan R, Sidhu SS, Kharbanda OP (1994). “Craniofacial pattern of parents of children having cleft lip and/or palate anomaly”. Angle Orthodont. 64:137-144.

Rice R, Spencer-Dene B, Connor EC et al. (2004). “Disruption of fgf10/Fgf2b- coordinated epithelial-mesenchymal interactions cause cleft palate”. J Clin Invest. 113(12): 1692-700.

Richman RA, P R Shehee, et al. (1988). “Olfactory deficits in boys with cleft palate”. Pediatrics 82(6):840-4.

Riley BM, Mansilla MA, Ma J et al. (2007). “Impaired FGF signaling contributes to cleft lip and palate”. Proc Natl Acad Sci USA. 104(11):4512-4517.

Romiti PA, Sun L, Honein MA et al. (2007). “Maternal Periconceptional alcohol consumption and risk of orofacial clefts”. Am J Epidemiol. 166(7):775-85.

Roosenboom J, Saey I, Peeters H et al. (2015). “Facial Characteristics and Olfactory Dysfunction: Two Endophenotypes Related to Nonsyndromic cleft lip and/or palate”. BioMed Research International 2015:8

Roosenboom J, Hermans R, Lammens F et al. (2018). “Olfactory function in patients with Nonsyndromic orofacial clefts and their unaffected relatives”. AmJMedGen. 1-7

Sarafoleanu C, Mella C et al. (2009). “The Importance of Olfactory sense in the human behavior and Evolution”. J Med Life. 2(2):196-8.

Seo HS, Lee S, Cho S (2013). “Relationship between personality traits and attitudes toward the sense of smell”. Frontiers in psychology.4

Schutte Bc, Murray JC (1999). “The Many faces and factors of orofacial clefts”. Hum Mol Genet. 8(10):1853-9.

Scott NM, Weinberg SM, Neiswanger K et al. (2004). “Hair whorls and handedness: informative phenotypic markers in non-syndromic cleft lip with or without cleft palate (NSCL/P) cases and their unaffected relatives”, Am J Hum genet 75(suppl):164.

Scott NM, Weinberg SM, Neiswanger K et al. (2005). “Dermatoglyphic fingerprint heterogeneity among individuals with Nonsyndromic cleft lip with or without cleft palate and their unaffected relatives in China and Philippines”. Hum Biol 77(2):257-66.

Shaw GM, Lammer EJ, Wasserman CR et al. (1995). “Risks of Orofacial clefts in children born to women using multivitamins containing folic acid periconceptionally”. 346(8972):393-6.

Som PM and Naidich TP (2013). “Illustrated review of the embryology, and development of the facial region, part 1: Early face and lateral nasal cavities”. American Journal of Neuroradiology. 35(1):10-18

Som PM and Naidich TP (2014). “Illustrated review of the embryology, and development of the facial region, part 2: Late Development of the fetal face and changes in the face from the newborn to adulthood”. American Journal of Neuroradiology. 35(1):10-18

Sobin CK, Kiley-Brabeck et al. (2006). “Olfactory disorder in children with 22q11 deletion syndrome”. Pediatrics 118(3): e697-703.

Stevenson RJ (2010) “An Initial Evaluation of the function of Human Olfaction”. Chem. Senses 35:3-20.

Tepper OM (2010) “Craniofacial Embryology”. In: Weinzweig J. “Plastic Surgery secrets plus 2nd edition”. Philadelphia, PA: Elsevier.

Trarbach EB, Costa EM et al. (2006). “Novel fibroblast growth factor receptor 1 mutations in patients with congenital hypogonadotropic hypogonadism with and without anosmia”. J Clin Endocrinal Metab. 91(10):4006-12.

Thisse B and Thisse C (2005).” Functions and Regulations of Fibroblast Growth factor signaling during embryonic development”. Dev Biol.287:390-402.

Van Der Woude A. (1954). “Fistula labii Inferioris Congenita and Its association with cleft lip and palate”. Am J Hum Genet. 6(2):244-56.

Warbrick JG (1960) “The Early development of the nasal cavity and upper lip in thr human embryo”. J Anat. 94(Pt 3):351-362.

Weinberg SM, Neiswanger K et al. (2006). “The Pittsburgh Oral-facial cleft study: expanding the cleft phenotype. Background and justification”. Cleft Palate Craniofac J.43(1):7-20.

World Health Organization. (2001). “Global registry and Database on Craniofacial anomalies: Report of a WHO registry meeting on Craniofacial anomalies”. Bauru, Brazil.

Zenety D, Bretones P et al. (2006). “Pediatric phenotype of Kallmann syndrome due to mutations of fibroblast growth factor receptor 1 (FGFR1)”. Mol Cell Endocrinol. 254-255():78-83

Zucchero TM, Cooper ME et al (2004). “Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate”. N Engl J Med. 351(8):769-780.