CHARACTERIZATION OF DELETIONS IN A COHORT WITH CLEFT LIP AND PALATE

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ABSTRACT

Orofacial clefts are relatively common birth defects with an estimated incidence of 1 in 700 live births that carry a significant public health burden. The causal factors of the development of orofacial clefts are complex; monogenic, multifactorial, non-syndromic and syndromic forms are all described in the literature. In almost all cases, genetics are thought to play a role in the etiology. In this study, we used an international cohort of 2,141 orofacial cleft patients and their families to find individuals with microdeletions, utilizing genome wide SNP chips for genetic analyses. We identified 94 individuals with deletions greater than 750 kb and compared them to registries with detailed phenotypic features and medical and family histories. We divided the cohort into distinct groupings: (1) individuals with a highly–penetrant orofacial cleft-associated deletion syndrome, (2) individuals with a lower-penetrant orofacial cleft microdeletion, (3) individuals with a large deletion encompassing likely contributory genes, and (4) individuals with a deletion of unknown significance. This cohort helps to support previous literature describing patients with orofacial clefts and microdeletions, along with presenting rarer associations, including an individual with a 12q21.1 deletion and cardiofaciocutaneous-like phenotype, an individual with a 7q36.3 deletion within the SHH regulator region with an absent nasal bone and cartilage, and an individual with a 3p26.3 deletion with a family history of polydactyly and

intellectual disability. With our cohort, we help to define the range of associated features in individuals with microdeletions and orofacial clefts.

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1.0   INTRODUCTION

Orofacial clefts are relatively common birth defects with an estimated incidence of 1 in 700 live births.[1] The impact of clefts is significant: cost of treatment averages $200,000 across the lifespan, and can affect feeding, speech, hearing, dentition, and mental health in affected individuals.[2] The causal factors of the development of orofacial clefts are complex; monogenic, multifactorial, and syndromic forms are all described in the literature. Monogenic inheritance is seen in conditions such as Stickler syndrome and Van der Woude syndrome, and come with health concerns other than orofacial clefts such as learning disabilities and vision loss.[3] Non-syndromic orofacial clefts are generally seen at a higher frequency, with approximately 70% of individuals with cleft lip and palate, and 50% of individuals with cleft palate only, considered to be non-syndromic.[4] Even in these cases, genetics are thought to play a role in the development of clefts. Concordance between monozygotic twins has been reported as high as 40-60%, and is about 5% for dizygotic twins, showing that non-syndromic cleft lip and palate are quite heritable, with an environmental influence playing a part in the presentation as well.[5] However, despite the discovery of many genes associated with the development of clefts, there is still a gap in knowledge in terms of unexplained clefting presentations.[6]

Much research has been done to better understand the genetic etiology of orofacial clefting, including linkage association studies, candidate gene studies, and genome-wide association studies.[2,3,7] While previous studies have provided a wealth of new information, there has been recent investigation into the effects of copy number variants on the development of orofacial clefts. The study of microdeletions has been particularly fruitful in other areas of medicine, such as the discovery of a causative gene for CHARGE association.[8] Traditionally, copy number variants have been detected in patients with syndromic

orofacial clefts, such as those with DiGeorge syndrome. However, recent research has looked at copy number variants in candidate genes and smaller family studies in individuals with non-syndromic clefts, and found associations between clefts and certain deletions in affected individuals.[1,6,8]

To address these gaps in knowledge concerning microdeletions as potential causes of orofacial clefts, this study aims to:

- Identify microdeletions in a cohort of cases and families with orofacial clefts

- Characterize and review reported phenotypic features in individuals identified with microdeletions. This group will include 94 individuals from multiple countries with a microdeletion who have a cleft lip and/or palate, or have a close family member with the condition.

- For microdeletions with no previous connection to orofacial clefts, explore possible role of deleted genes in craniofacial development.

The results of this study will provide information on syndromic and non-syndromic causes of orofacial clefting within an ethnically and geographically diverse group. This study will hopefully present a collection of data on microdeletions found in individuals with orofacial clefts, and serve as a foundation for a variety of other research projects on the molecular basis of cleft lip and palate. After more clinical research has been pursued, this information could be used so that individuals with orofacial clefts may be screened for these deletions to help predict an individual's medical prognosis and outcome.

2.0   LITERATURE REVIEW

2.1   OVERVIEW AND EPIDEMIOLOGY OF OROFACIAL CLEFTS

Orofacial clefts are some of the most common congenital malformations worldwide. Orofacial clefting refers to a group of related malformations, primarily affecting the upper lip and palate. The three most common types of orofacial clefts fall into three categories: cleft lip, cleft palate, and cleft lip with cleft palate. Traditionally, cleft lip and cleft lip and palate have been combined as one disorder: cleft lip with or without cleft palate (CL/P).[9] The international prevalence of cleft lip with or without cleft palate is approximately 9.92 per 10,000 births.[10] Worldwide, cases of cleft palate only have been found to have a birth prevalence of 6.39 per 10,000 births, and cleft lip a prevalence of 3.28 per 10,000 births.[11] Orofacial clefts also vary among ethnic populations. In the United States, Caucasian populations have the highest recorded prevalence of orofacial clefts (1.8 per 1000 births), with individuals of Asian and Hispanic descent following closely (1.04 per 1000 births). African Americans consistently have the lowest prevalence of cleft lip and palate in comparison to other racial and ethnic populations (0.67-0.61 per 1000 births).[12] Outside of the US, orofacial clefting tends to be more common in Eastern Asia, India, and South America, whereas clefting is less common in Africa and Southern Europe.[13] There is also a difference of prevalence in the sexes, with cleft lip only and cleft lip and palate more common in males (1.23:1 and 1.56:1 respectively) and cleft palate only more common in females (1.77:1).[14]

The high prevalence of orofacial clefts is significant because of the substantial burden these malformations have on affected individuals and their families as well as the health care system.

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Cleft lip and palate increases an individual's mortality risk, with a standardized mortality ratio of 1.4 for males and 1.8 for females.[15] Management of cleft lip and palate patients requires a team of specialists including dentists, radiologists, surgeons, geneticists, speech therapists, feeding specialists, and otolaryngologists to provide standard of care. Surgeries are quite common in these patients, and they often consist of multiple oral reconstruction procedures. Treatment and management of clefts is not a luxury that merely corrects cosmetic differences.[16] Surgical repair of orofacial clefts is required because infants suffer from feeding difficulties that result in low weights and sometimes requiring specialized feeding appliances or tools. As children age, an orofacial cleft can also negatively affect speech ability. Difficulties relating to the palate can lead to problems with the middle ear, resulting in an excess of otitis media and even hearing loss.[17] On average, the average annual cost of medical care of children with an orofacial cleft was $13,405 more than those without an orofacial cleft in the United States. Children with a cleft and another major defect had costs that were 25 times higher than an unaffected infant. Lifetime costs for medical treatment have been reported to be over $100,000.[18]

Medical care of orofacial clefts is not limited to infants or childhood due to additional comorbidities. International cleft lip and palate registries show that 29% of affected individuals had an additional defect, and could be considered syndromic. These most commonly included polydactyly, limb abnormalities, and cardiac defects.[19] Even individuals with apparently isolated orofacial clefts have other medical complications, which may not be visible. For example, individuals with isolated orofacial clefts have been found to have abnormal brain structure and development in comparison to those without clefts.[20] Long term, affected individuals and their unaffected family members have an increased risk for cancer, particularly breast cancer, more so than the unaffected population.[321]

Having an orofacial cleft has been shown to have a significant psychosocial effect from early life throughout adulthood. Although overall functioning tends to be adequate,[22] many children and adolescents have reported that they are teased more than their peers at school, an experience that often negatively effects an individual psychosocially. Most of the bullying was due to the child's facial or speech differences. Many also have noted an unhappiness with an aspect of their face related to their orofacial cleft.[23] Parents of children with cleft lip and palate also reported that their children tended to have more anxiety and depression than their unaffected peers, along with more behavioral problems.[24]

Orofacial development is complex, as are the factors that contribute to orofacial clefts, which include genetic and environmental exposures.[4] The negative impact of smoking during pregnancy has been widely documented, and orofacial clefting is one outcome that has been recorded. Women who smoked during their pregnancy or were exposed to cigarette smoke (such as when living with a smoker) were found to be at an increased risk for isolated cleft lip and palate.[25] Maternal alcohol use has been looked at for possible connections between cleft lip and palate due to the range of other congenital problems that can occur, such as fetal alcohol syndrome. Results have been mixed, ranging from conclusions stating that even low levels of alcohol consumption can lead to an increased risk for orofacial clefts, to others reporting that there is no significant link between the proposed exposure and outcome.[26][27][28] Similarly, there is mixed evidence that maternal nutritional supplements reduce the risk of orofacial clefts. Some studies suggest that supplementation of folic acid, a compound that has been linked to the fusion of palate shelves, can help significantly reduce the risk of orofacial clefts, among other malformations, up to 50%.[29] Similar results are found when B12 and zinc are supplemented as well.[30] This has been supported by lower rates of cleft lip and palate in children of women who took a prenatal vitamin.[31]

Maternal health has also been suggested to play a role in orofacial cleft risk. For example, women with diabetes are more likely to have children with a congenital anomaly, including cleft lip and palate.[32] Multiple studies have supported a correlation between maternal obesity and an increased risk to have a child with an orofacial cleft.[33,34] Finally, men older than 40 are 58% more likely to father children with a cleft lip or palate than their younger (20-39 year-old) male peers.[35]

2.2   EMBRYOLOGY OF THE DEVELOPMENT OF THE LIP AND PALATE

The development of the face is a relatively long, complex, and multi-stepped process. For the face to grow properly, five facial primeval parts must fuse to together. These include the midline frontonasal prominence, two maxillary prominences, and two mandibular prominences.[36] These five structures can be seen around the 4th week of development, and are formed from cranial neural crest cells, which in themselves originate from the ectodermal cells of the neural tube.[37] Through multiple stages, including growth and migration, the frontonasal prominences divide in to two nasal processes: the medial and the lateral. From the 6th and 7th week of growth, the nasal processes along with both maxillary processes merge together to form the beginnings of the primary palate and upper lip. This is a sensitive time in the development of the face, and errors can lead to orofacial clefts in the fetus.[38] In a similar series of events, beginning in the 7th week, the maxillary prominences begin to form the palate shelves, which in turn becomes the rest of the adult hard and soft palate. During the period, the palatal shelves grow vertically along the tongue.[39] After, the shelves of the palate fuse and elevate to a horizontal position above the tongue. This sets the stage for the differentiation of muscle and bone, creating the soft and hard palates. As this occurs, the palates fuse with the nasal septum, effectively separating the oral and nasal cavities.[40]

2.3   PHENOTYPIC SPECTRUM AND CLASSIFICATION OF OROFACIAL CLEFTS

There are three general orofacial subgroups that are typically used: cleft lip only (CL), cleft lip and palate (CLP) and cleft palate only (CP). Traditionally, cleft lip and cleft lip and palate have been lumped together in the cleft lip with or without cleft palate subgroup (CL/P). This is because development of a severe cleft lip in a fetus can lead to a cleft in the hard or secondary palate. However, based on data describing the severity, gender, consanguinity, genetics, and other factors that increase the likelihood of CP versus CLP, there is more data supporting the idea of separating the two subgroups.[41,42]

Orofacial clefts vary widely among individuals in severity. Clefts of the lip can occur unilaterally or bilaterally. Among unilateral clefts, although both are prevalent, left-sided orofacial clefts are more common than right-sided.[43] Historically, laterality was one of the only descriptors used for orofacial clefts. However, clefts also vary in completeness (i.e., how severely the structure is affected). The LAHSHAL coding system was invented to help easily describe orofacial clefts and their severity by noting the anatomy that the cleft has affected. The acronym addresses clefts that affect the lip (L), the alveolus(A), hard (H), and soft (S) palates, as you look at the affected individual. Thus, the left side of the acronym describes the right side of the face, and the right side of the acronym the left side of the face.[36] More recent literature has shown that in addition to the type of cleft present (CP, CLP, and CL), there are also subclinical features that may contribute to the entire clinical picture. These features include submucosal cleft palates, bifid uvulas, and microform clefts (a notch composed of a fibrous tissue across the lip).[44,45] These phenotypes can be found in individuals previously thought to be purely unaffected, and further research will hopefully help to predict risks for typically affected children.[44] Much attention has been given to subepithelial defects of the orbicularis oris muscle (OOM defects) and their link to orofacial clefts.

OOM defects have been detected in previously described unaffected relatives of patients with orofacial clefts at a higher frequency than in control populations.[45,44] This has led some researchers to believe that this feature may be a mild form of orofacial cleft. Dental anomalies are also commonly seen in affected individuals and their relatives. In one study focusing on unaffected parents of children with orofacial clefts, 51% had one or more dental anomalies. Defects of this nature that have been noted include microdontia, tooth displacement, congenital absence of teeth, supernumerary teeth, and enamel defects.[46,47] In unilaterally affected individuals, agenesis of the lateral incisor on the side unaffected by clefting is the most common dental anomaly, and may potentially be a sign of a reduced penetrance bilateral cleft lip and palate.[48] Differences in overall face shape between unaffected relatives and control populations have been recorded in the literature by using 3-D image capturing to provide a closer look at the details of the face. These changes found in parents of children with orofacial clefting include a flat profile, excess intraorbital and nasal cavity widths, and increased lower and reduced upper facial heights.[49,50] Finally, data has been found tying a pattern of lower lip "whorls" and facial clefts. These unique prints resemble the lower lip pits found in the Mendelian orofacial cleft disorder known as Van der Woude syndrome, and are found in non-syndromic cases of cleft lip and palate as well as their family members.[51,52]

2.4 SYNDROMIC CLEFTS

Although most CL/P cases are isolated, approximately 30% of individuals have other congenital defects in addition to an orofacial cleft.[19] Patients with multiple anomalies may be considered syndromic. For example, aneuploidy conditions can affect multiple organ systems, and

orofacial development is no exception. Almost 20% of all patients with trisomy 13 have cleft lip, and more than 4% of patients with trisomy 18.[53] Women with Turner syndrome have also been reported to have orofacial abnormalities, particularly cleft palate.[54,55] Currently, over 500 distinct genetic syndromes, including single gene Mendelian disorders, have listed cleft lip and or palate among their described features.[56] Much progress has been made in determining the genetic causation of these syndromes, helping researchers to glean insight into the molecular pathways of cleft lip and palate.

Over 200 mutations in the interferon regulatory factor 6 (IRF6) gene located on 1q32.2 have been linked to orofacial development and two different syndromic orofacial clefting syndromes: Van der Woude syndrome and popliteal pterygium syndrome.[575859] As mentioned previously, Van der Woude syndrome is a mendelian orofacial clefting disorder. Although only affecting 1 in 35,000 individuals, it accounts for approximately 2% of all orofacial clefts in humans.[60] An autosomal dominant condition, Van der Woude syndrome (VWS) can present with lower lip fistulae, or lip pits, hypodontia, and CL, CP, or CLP.[61] Although VWS is highly penetrant at about 97%, the features present in affected individuals vary, with lip pits being the most common phenotypic characteristic described.[62] Popliteal pterygium syndrome (PPS) is the second syndromic orofacial clefting disorder associated with *IRF6*. It is quite rare, occurring in 1 in every 300,000 live births. Like VWS, PPS symptoms include lower lip fistulae and CL, CP, or CLP. Additional phenotypic features are frequently found in PPS patients, such as abnormal genitalia, webbing of the skin from the ischial tuberosities (also known as the "sit bones") to the heels, syndactyly of fingers and toes, and dermatological abnormalities of the skin around the nails of the hands and feet.[63]

Collagen plays an important role in the development of the palatal shelves, so unsurprisingly mutations in collagen producing genes can lead to syndromic orofacial clefts.[64] Stickler syndrome is a highly penetrant autosomal dominant condition affecting 1 in 7,500 live births with 90% of patients with myopia, 60% with retinal detachment, 70% with hearing loss, 90% with joint problems including hypermobility and early onset arthritis, and 84% with craniofacial anomalies including cleft palate and midface retrusion.[65] Approximately 85% of causative pathogenic mutations for Stickler syndrome are found in the *COL2A1* gene and about 10% in the *COL11A1* and *COL11A2* genes. [66]

2.5   NON-SYNDROMIC CLEFTS

The majority (approximately 70%) of cleft lip and palate patients present with no additional features and have a non-syndromic form of clefting. Despite a complex array of potential causes, there is substantial evidence supporting a genetic etiology for non-syndromic orofacial clefts.[676869] Relative recurrence risk for a CL/P in a first degree relative is 32, indicating a strong genetic component to non-syndromic orofacial clefts.[70] Twin studies have also provided evidence for a significant genetic component; dizygotic twins have a lower concordance rate of 1-5% in comparison to that of monozygotic twins with 35-60% concordance rate.[4471]

Multiple approaches have been used to identify genetic risk factors for orofacial clefts. Linkage studies, which look at segregation of alleles through families, have produced mixed results. Although many different research studies have found loci thought to contribute to orofacial development, few have been successfully replicated.[67] One significant locus found via a meta-analysis of 388 multiplex families is 9q21, which encompasses the suspected causative *FOXE1*

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gene.[72] The association between this region and cleft lip and palate has been replicated in several further candidate gene studies.[73,74] Additional linkage findings within this study include loci 2q32-35 and 16q24.

Studies of candidate genes like those of *FOXE1* have added to the genetic orofacial cleft literature. In a 2004 study, point mutations were found in *FOXE1*, along with *GLI2*, *MSX2*, *SKI*, *SATB2* and *SPRY2* in a single individual with non-syndromic cleft lip and palate, and in none of the 186 controls sequenced. These results lead researchers to believe that the candidate genes listed have a cumulative effect.[75] Candidate gene *MSX1* was previously found in mice with cleft palate.[76] Follow up human genetic studies have found that *MSX1* mutations can be identified in approximately 2% of non-syndromic orofacial clefting.[77] Candidate gene selection can be performed by choosing syndrome-causing genes associated with clefting. *IRF6*, the causative gene for Van der Woude syndrome, is also known to be associated with non-syndromic orofacial clefts.[42] *TP63*, associated with ectrodactyly-ectodermal dysplasia, and *MID1*, a causative gene for X-linked Opitz G/BBB syndrome, have been linked to non-syndromic clefting as well.[78]

Historically, genome wide association studies (GWAS) have been the most successful approach for identifying genetic risk factors for orofacial clefts. A 2010 GWAS with 401 individuals with non-syndromic cleft lip/palate and 1323 controls showed associations between clefting and the loci 17q22 and 10q25.3.[79] A separate GWAS that was performed within the same year confirmed the significance of *IRF6*, and also noted links between loci *MAFB*, *ABCA4*, and 8q24.[80] Two years later, new susceptibility regions were discovered, including 1p36, 2p21, 3p11.1, 8q21.3, 13q31.1, and 15q22.[81] Most recently, in 2016, 19q13 and 2p24 were new loci reaching genome-wide significance in relation to orofacial clefting, in addition to confirming associations in 1p36, 1p22, 1q32, 8q24, and 17p13.[82] However, despite these successes, GWAS-

identified loci only account for up to 25% of the heritability of orofacial clefts, indicating that the remaining genetic risk could reside in other types of variation such as rare mutations or copy-number/structural variants.[82]

2.6 MICRODELETIONS

Up to 6% of malformations (including orofacial clefts) are caused by a chromosomal abnormality.[83] As mentioned previously, aneuploidies like trisomy 13 and monosomy X contribute to this figure. Smaller losses within the chromosomes can also lead to malformations like cleft lip or palate. These microdeletions are commonly associated with syndromic clefting, and diagnostic testing like microarray is a useful clinical tool to identify such microdeletions, and to take precautionary care for other associated symptoms, such as surveillance for other described affected organs of the body.

22q11.2 deletion syndrome, also known as DiGeorge or Velocardiofacial syndrome, is the most well-known microdeletion clefting syndrome. The gene *TBX1* is thought to be responsible for most of the features associated with this microdeletion, which include cleft palate, developmental delay, intellectual disability, heart defects, autoimmune disorders, hearing loss, kidney abnormalities, and psychiatric problems.[84][85] In children with cleft palate, microarray testing to rule out a 22q11.2 deletion is standard of care.[86] Other microdeletion syndromes with cleft lip and/or palate include 1p36 deletion syndrome,[87] Wolf-Hirschhorn syndrome (4p deletion),[88] and Smith-Magenis syndrome (17p11.2 deletion).[89]

Recently, microdeletions are being found in non-syndromic clefting. Microarray testing has revealed new candidate genes for cleft lip and/or palate in individuals with isolated orofacial

clefts. A 2008 study discovered that chromosomal regions 6q25.1-25.2 and 10q26.11-26.13 were deleted in patients with cleft lip. Genes *ESR1* and *FGFR2*, located within those areas, are currently suspected to play a role in the clefting seen in these patients.[8] Shi et. al (2007) took a similar approach, but used genome-wide association studies to search for microdeletions within non-syndromic orofacial cleft patients. This data revealed deletions in *CYP1B1*, *FGF10*, *SP*8, *SUMO1* and *TFAP2A* in their cohort. Genes such as *CYP1B1* play a role in metabolizing toxins, and may provide a link between smoking and orofacial clefts if that ability is reduced by a deletion. *TFAP2A* is a gene that is associated with facial development.[6] Another study looking at *de novo* microdeletions in individuals with non-syndromic orofacial clefts identified a deletion in the 7p14.1 region, which has been previously described as causing malformations of the face.[90] One microdeletion study took a different approach, and looked at inherited deletions in non-syndromic clefting participants. This study revealed two significant deleted regions on chromosomes 7 and 8: 7q34 and 8q24.[91] Further research into the possible significance of the genes involved in rare or common microdeletions could help enhance our understanding of the etiology of non-syndromic clefting.

3.0   MANUSCRIPT

3.1   BACKGROUND

Orofacial clefts (OFCs) are common birth defects with an estimated incidence of 1 in 700 live births.[1] All three categories of orofacial clefts, cleft lip (CL), cleft palate (CP), and cleft lip with cleft palate (CLP), are linked with many environmental and genetic factors, and can present with a variety of other structural anomalies (syndromic) or can be isolated (non-syndromic).[3] Approximately 50% of CP cases and 30% of CL or CLP cases occur as part of syndromes.[44] In the past, genome wide association studies have been successful at identifying genetic risk factors for non-syndromic OFCs. These studies discovered new loci for non-syndromic OFCs (e.g., 19q13 and 1p22) as well as identified associations between non-syndromic OFCs and genes typically associated with OFC syndromes (e.g., *IRF6*, *GRHL3*, and *TP63*). [79,81,82,92] Despite this success, genome wide association studies identified only 25% of heritability of non-syndromic OFCs.[82] The remaining heritability may be accounted for by rare variants, gene-gene or gene-environment interactions, or by structural variants.[69]

Chromosomal abnormalities, including microdeletions, cause up to 6% of malformations.[83] These small losses in chromosome material can lead to cleft lip and palate when the genetic material missing includes genes or regulatory elements that contribute to craniofacial development. Historically, chromosomal aberrations have been detected using many different methods. Large abnormalities such as translocations or aneuploidies can be detected using a karyotype, including significantly sized deletions of at least 4-6 Mb.[93] This can help determine the etiology for a number of patients with cleft, such as those with Turner syndrome.[55]

Fluorescence in situ hybridization (FISH) can pick up known smaller duplications or deletions, like those in DiGeorge syndrome or Wolf-Hirschhorn syndrome, but due to its use of a probe, FISH is limited to detecting specific, targeted regions within a chromosome, and may miss other relevant aberrations within the genome.[94][86]   To detect unknown copy number variants, array comparative genome hybridization (CGH) is used, which can pick up chromosomal losses or gains within the genome by comparing the sample to a control.[95]   *De novo* and rare inherited microdeletions have been tied to non-syndromic OFCs, such as in the *ESR1* and *FGFR2* genes, and have been identified in affected individuals using this method in a study performed by Oesagawa et al..[89][1]

More recently, SNP arrays have been utilized in clinical and research settings to detect copy number variants. Although SNP arrays are limited by SNP distribution and are not as high density compared to CGH testing, they have been used successfully in detection of microdeletions, particularly for those with non-syndromic OFCs.[96][95] In 2009, Shi et al. conducted a study using GWAS and SNP array to detect microdeletions in a clefting cohort, revealing deletions in candidate genes such as *CYP1B1*, *FGF10*, *SP8*, *SUMO1* and *TFAP2A*.[6] Younkin et al. similarly used SNP array in individuals with *de novo* deletions and non-syndromic CL/P, and found a deletion along 7p14.1 more commonly observed in cleft trios.[90] To follow up, in 2015 Younkin and his colleagues conducted the study with inherited deletions within non-syndromic clefting individuals, and detected deletions within the *MGAM*, *ADAM3A*, and *ADAM5* genes.[91] In 2016, Fu et al. looked at the exome rather than the genome within multiplex OFC families, and utilized SNP array to detect microdeletions. With the use of this technology, 88 hemizygous potentially causative deletions were detected from 56 families.[97]

Our international study aims to further understand the role of microdeletions as part of the genetic architecture of OFCs. Using a genome wide SNP array, we have detected 94 microdeletions larger than 0.75 Mb and have divided them into distinct groups based on phenotype, family history, deletion size, and deletion characterization, effectively adding to the literature of microdeletions in an OFC cohort.

3.2   METHODS

3.2.1 Study Sample

Our international cohort was recruited from 18 sites in 13 countries within North America, South America, Asia, Europe, and Africa as previously described.[82] Recruitment and data collection were done at orofacial cleft treatment centers in respective sites as part of ongoing studies by the University of Pittsburgh Center for Craniofacial and Dental Genetics and the University of Iowa. Institutional Review Board approval was obtained for each site; informed consent was obtained for all participants, including consent for large genomic studies with data sharing. The full cohort of 11,727 samples were comprised of individual OFC cases, their unaffected family members, and unrelated controls.

3.2.2   Genotyping

Samples were genotyped for 580,000 single nucleotide polymorphisms (SNPs) on the Illumina Human Core+Exome array at the Center for Inherited Disease Research at Johns Hopkins University.

3.2.3   Identification of Deletions

Microdeletions were identified from SNP intensity data using the Bioconductor package "GWASTools". The deletions were detected from the "Log R Ration" (LRR), a measure of the relative signal intensity, and "B Allele Frequency" (BAF), an estimate for the B allele frequency of a SNP in the population of cells the DNA originated from. GWASTools uses circular binary segmentation to identify change points in BAF and loss of heterozygosity by change in LRR. In a region with normal copy number, we would expect to see a B allele frequency of 0 (AA), 0.5 (AB) or 1 (BB) with an LRR of 0. Heterozygous deletions were identified based on a loss of the intermediate BAF band and corresponding decrease in LRR. For more information, please refer to the Quality Control Report issued by the University of Washington GCC here: (http://www.ccdg.pitt.edu/docs/Marazita_ofc_QC_report_feb2015.pdf)

We restricted our analyses to large deletions (greater than 750 kb) found in OFC cases to enrich for likely pathogenic variants. LRR and BAF for each deletion were plotted for the case and both parents (if available). Plots were manually inspected to determine if deletions were inherited or occurred *de novo*. Poor quality deletions were removed as likely artifacts. Out of our original sample of 2,989 individuals with OFCS, we detected 94 individuals with CL, CP, or CLP with such deletions.

3.2.4 Annotation

Demographic and medical history data, pedigrees, and photos and videos were reviewed for each individual. Patient presentations and deletions were then cross-referenced with the literature to determine whether the deletion, or the region in which the deletion was located, had previously been described as OFC-associated. Based on this, our data was organized into 4 groups: (1) individuals with a highly–penetrant orofacial cleft-associated deletion syndrome (e.g. DiGeorge syndrome or Smith Magenis syndrome), (2) individuals with a lower penetrant orofacial cleft microdeletion (e.g. 1p36 and 1q21 microdeletions), (3) individuals with a large deletion encompassing likely contributory genes (e.g. *COL11A*), and (4) individuals with a deletion of unknown significance (<1 Mb). Only one individual had a causative non-syndromic OFC gene deleted (*FGFR2*) that had been previously published by Osoegawa et al., and was excluded from our sample.[8]
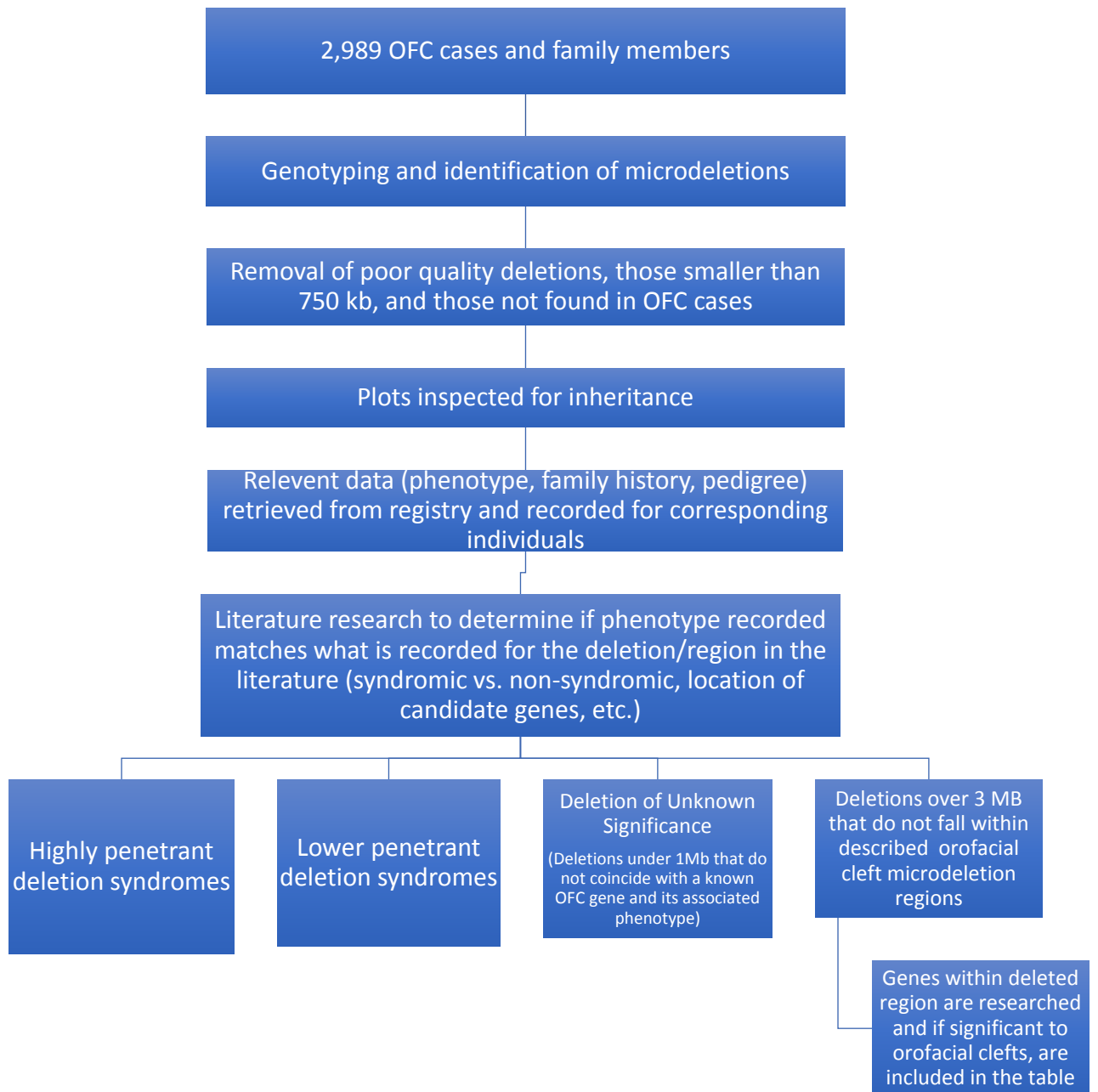
Figure 1. Methods Flowchart

3.3   RESULTS

We identified a number of deletions that varied by size, inheritance, phenotypic spectrum, rarity, and previous report in the literature. Descriptions of our subgroups are below.

Table 1. Highly Penetrant Deletion Syndromes

DELETION	Mb	INHERITANCE	PHENOTYPE	FAMILY HISOTRY	SYNDROME
4p16.3-p16.2	4.6	unknown, not maternal	BCL, bifid uvula, pulmonary stenosis, seizures, hypoplastic kidney, triangular face, micrognathia, down slanting palpebral fissures, hypertelorism, simple ears, intellectual disability, developmental and speech delay	sister with speech and developmental delay, synophrys, upward slanting palpebral fissures	Wolf-Hirschhorn syndrome[88]
17p11.2	3.6	de novo	CP, micrognathia, chronic ear infections, and mild to moderate intellectual disability	father with bifid uvula; brother with convergent strabismus	Smith Magenis syndrome[89]
22q11.21	2.6	unknown, not maternal	LCLP, speech delay, type 1 diabetes, protruding ears with bumps, macroglossia, and micrognathia	NA	DiGeorge syndrome[85]
22q11.21	2.6	de novo	SMCP, dysmorphic features, double hernias, malrotation of organs, difficulty swallowing, and large tongue	mother with SMCP; brother with functional heart murmur, frontal bossing, prominent/protruding ears, single palmar creases	DiGeorge syndrome[85]
22q11.21	2.1	de novo	RCLP	NA	DiGeorge syndrome[85]
22q11.21	2.6	de novo	LCLP	NA	DiGeorge syndrome[85]
22q11.21	1.4	unknown, not maternal	CP	maternal 5th degree relative with CP	DiGeorge syndrome[85]
22q11.21	2.4	de novo	BCLP	NA	DiGeorge syndrome[85]
22q11.21	2.6	unknown	LCLP	NA	DiGeorge syndrome[85]
22q11.21	2.8	unknown, not maternal	CP	NA	DiGeorge syndrome[85]
22q11.21	2.6	unknown, not maternal	SMCP, chronic ear infections, VSD, hoarseness, loss of voice	NA	DiGeorge syndrome[85]
22q11.21-q11.23	2.2	de novo	BCLP	NA	DiGeorge syndrome[85]
22q11.22-q11.23	2.0	de novo	CLP	NA	DiGeorge syndrome[85]
22q11.21-q11.22	2.9	de novo	SMCP, speech delay, learning disability, fine motor skill delay, ADHD, anxiety, and hearing loss in left ear	father with speech delay; mother with SMCP, speech delay, nerodermatitis; brother with speech delay	DiGeorge syndrome[85]
whole X	100	unknown	CP	NA	Turner syndrome[55]
whole X	90	unknown	BCLP	NA	Turner syndrome[55]

CL – cleft lip; RCL – right cleft lip; LCL – left cleft lip; BCL – bilateral cleft lip; CP – cleft palate; RCP – right cleft palate; LCP – left cleft palate; SMCP – submucosal cleft palate; CLP – cleft lip and palate; LCLP – left cleft lip and palate; RCLP – right cleft lip and palate; BCLP – bilateral cleft lip and palate; ADHD – attention deficit hyperactive disorder; VSD- ventricular septal defect

3.3.1 Highly Penetrant Deletion Syndromes

As shown in Table 1, 22q11.2 deletions, the most common cause of cleft palate syndromes, make up more than 75% of our highly penetrant deletions group. These deletions were on average around the standard 3Mb and encompassed the regions typical of DiGeorge syndrome. Most were *de novo*, and if not listed as such, were described as having "unknown inheritance" because a DNA sample was unavailable from one or both parents. Some of our registry data entries were more descriptive than others, including characteristic DiGeorge features such as developmental and speech delay, dysmorphic facial features, hoarse voice, and ventricular septal defect. Others were sparse in information outside of clefting type. Two individuals within this subgroup were found to have monosomy X, or Turner syndrome, but had no phenotypic information detailed other than cleft type.

Wolf-Hirschhorn syndrome and Smith Magenis syndrome were represented in our cohort with one individual respectively. Both patients presented with typical characteristics of their respective conditions, such as intellectual disability, dysmorphic facies, and seizures. The individual with Smith Magenis syndrome had a 3 Mb *de novo* 17p11.2 deletion. DNA was unavailable from the father of the patient with Wolf-Hirschhorn syndrome, so we could not determine if the deletion was inherited or *de novo*. Interestingly, the proband's sister also had delays and dysmorphic facies (although much milder than our patient), but no deletion was detected.

Table 2. Lower Penetrant Deletion Syndromes

DELETION	Mb	INHERITANCE	PHENOTYPE	FAMILY HISTORY	POSSIBLE SYNDROME	RELEVANT GENES
1p36.21	2.6	de novo	RCL	NA	1p36 microdeletion syndrome	PDPN - usually results in heart defects, not CL/P[98]
1p36.33-p36.23	6.9	de novo	BCLP, seizures, hearing loss, hypotonia, hydronephrosis	NA	1p36 microdeletion syndrome	SKI; PRMD16 cleft in mice[98]
1q21.1-q21.2	1.5	maternal	BCLP, learning and speech delays, clogged tear ducts, kidney reflux, hypertelorism, smooth philtrum	maternal half-brother with supernumerary teeth, fetal pads, kidney reflux, learning disability in reading, tracheomalacia, ADD	1q21.1 microdeletion syndrome	GJA5, GJA8, CDH1L, GPR89B, NBFL11, BCL9 - usually low penetrance[99,100]
1q21.1-q21.2	1.5	paternal	BCLP	paternal uncle with seizures	1q21.1 microdeletion syndrome	GJA5, GJA8, CDH1L, GPR89B, NBFL12, BCL9 - usually low penetrance[99,100]
3p26.3	.86	paternal	BCLP	paternal aunt with intellectual disability; paternal aunt with absent fingers; brother with polydactyly	CNTN6 deletion	CNTN6[101]
3q29	1.6	paternal	BCLP	brother with LCLP	3q29 microdeletion syndrome	DLG1, PAK2[102]
3q29	.92	maternal	BCL, leukemia	sister with hypertelorism, nystagmus, hypermobile joints, single palmar; paternal half-brother with severe acne, frontal bossing, right epicanthal folds, bilateral ptosis, anteverted nares, tapering fingers	3q29 microdeletion syndrome	DLG1[102]
3q29	1.9	de novo	RCLP	NA	3q29 microdeletion syndrome	DLG1, PAK2[102]
3q29	1.7	de novo	RCLP and was born at 37 weeks and weighed 4 lbs. 8 oz.	NA	3q29 microdeletion syndrome	DLG1, PAK2[102]
6p25.3-p24.3	9.1	de novo	RCLP, hypertelorism, Dandy Walker malformation	NA	6p25 deletion syndrome	FOXC1, F13A1, BMP1, DSP[103,104,105]
6p25.1-p24.3	2.9	maternal	LCLP	sister with BCLP, mother (LCL) and aunt (BCLP) with microdeletion	6p25 deletion syndrome	BMP1, DSP[103,104,105]
7q36.3	5.9	Unknown	CLP	NA	7q36 deletion syndrome	SHH[106]
7q36.3	2.7	de novo	RCL, autism, strabismus, slanting palpebral fissures,	NA	7q36.3 deletion syndrome	LMBR1[107,108]

Table 2 Continued

			epicanthal folds, missing nasal bone and cartilage			
12q14.3-q21.1	7.1	unknown	BCLP, bifid uvula, 0.1 percentile for weight and BMI, 20th percentile for height, small facial features	NA	12q14 microdeletion syndrome	GRIP1, HMGA2
12q21.1	2.1	de novo	SMCP, pyloric stenosis, low set ears, dysmorphic facies, curly hair, speech delays	sister with LCLP	Cardiofaciocutaneous-like syndrome[109110111]	TRHDE and ATXN7L3B
14q32.32-q32.33	4.3	de novo	LCLP, right inguinal hernia, developmental delay, hearing impairment	NA	14q32	JAG2[112], XRCC3, ZFYVE21[113]
15q11.2	.85	paternal	BCLP, speech delays	2nd cousin with speech delay, LCLP; 2nd cousin with learning disability	15q11.2 microdeletion syndrome	NIPA1, NIPA2, CYFIP1[114]
15q14	3.5	maternal	BCLP	maternal aunt with deletion and LCLP	15q14 deletion syndrome	does not include candidate cleft gene MEIS2[115]

CL – cleft lip; RCL – right cleft lip; LCL – left cleft lip; BCL – bilateral cleft lip; CP – cleft palate; RCP – right cleft palate; LCP – left cleft palate; SMCP – submucosal cleft palate; CLP – cleft lip and palate; LCLP – left cleft lip and palate; RCLP – right cleft lip and palate; BCLP – bilateral cleft lip and palate; ADD – attention deficit disorder

3.3.2   Lower Penetrant Deletion Syndromes

18 microdeletions were placed into our lower penetrant deletion syndrome subgroup as shown in Table 2. These are deletions that are found in the region of known deletion syndromes, but are often smaller than those described in the literature. Moreover, our sample clearly showed a range of penetrance and severity. For example, we identified two deletions each on 1q21 and 1p36; in both cases, one individual presented with only an OFC while the other had a syndromic presentation, showing symptoms such as seizures, hearing loss, and global delays. In the case of 1p36, the syndromic features were present in the individual with a large 6.9Mb deletion. The apparently isolated OFC individual had a much smaller 2.6Mb deletion.

Some deletions had less of a recorded phenotypic impact than predicted based on the literature. An almost 6 Mb 7q36.3 deletion, which included the *SHH* gene, had no recorded effects other than CLP, however photographs were unavailable to assess minor features of holoprosencephaly. Other phenotypes more clearly matched their genotypes; for example, an individual with a de novo 6p25 microdeletion had Dandy Walker malformation along with RCLP, consistent with the brain anomalies often seen with the syndrome.[116] Similarly, a large 14q32 terminal deletion patient had OFC (LCLP), developmental delay, and inguinal hernia which are often seen in terminal 14q32 microdeletion syndrome.[1123]

The most represented deleted region in this subgroup was 3q29, with four individuals carrying a deletion. The deletions averaged 1.5 Mb, the typical size of the deletion and in the same critical region. Unlike what has been described in the literature (affected individuals with delays, kidney anomalies, microcephaly, and skin conditions), few features other than OFCs were recorded. Two individuals with inherited microdeletions, one with a small 15q11.2 deletion and another with a large 14q14 deletion, also had limited phenotypic descriptions other than OFC, and few affected relatives. This seemed to be consistent with the literature: 15q11.2 deletions are commonly cited as low penetrance, and the 15q114 deletion did not have many of the causative genes typically seen within the syndrome, such as *MEIS2*.

Interestingly, we saw a family with a mid-sized 2.9Mb 6p25 microdeletion showing clear autosomal dominant inheritance and penetrance: an affected individual with LCLP had an affected mother with LCL and maternal aunt with BCLP, both who had the recorded deletion. Although the region did not contain some of the genes more commonly encompassed within 6p25 microdeletion syndrome, such as *FOXC1*, it seems possible that the deletion may be causative of the OFC phenotype within the family.

3.3.3 Rare Syndromic Deletions of Interest

Within our "Lower penetrant deletion syndromes" subgroup, four individuals stood out: one with a 3p26.3 deletion with a family history of polydactyly, a patient with a 12q14.3-q21.1 deletion and 12q14 deletion syndrome phenotype, another individual on the holoprosencephaly spectrum with a 7q36.3 deletion not including *SHH*, and one with a 12q21.1 deletion and a cardiofaciocutaneous syndrome-like phenotype.

Table 3. Large Deletions

DELETION	Mb	INHERITANCE	PHENOTYPE	FAMILY HISTORY	RELEVANT GENES
1p21.1-p13.3	4.1	de novo	LCLP	brother with LCLP	COL11A1[17,72]
1q31.1-q31.3; 1p31.3; 1p22.3; 1p22.1	7.6	unknown	LCLP	NA	LHX8[75,118]
4p15.1-p14	6.3	unknown	BCLP	NA	NA
4q35.2	3.1	Paternal	LCLP	NA	FRG1[119]
10p13-p12.31	3.9	unknown, not maternal	RCLP, intellectual disability, developmental delay, ADD	brother with developmental delay, learning disability	NA
11p15.1-p14.3	3.7	maternal	BCLP	NA	NA
12q14-q15	4.8	de novo	CLP	NA	GRIP1[120]
18q22.1-q23	14	de novo	LCL	maternal aunt with LCL	TXNL4A[121]
20p12.3-p12.1	7.7	de novo	CP	NA	BMP2[122], JAG1[123]

CL – cleft lip; RCL – right cleft lip; LCL – left cleft lip; BCL – bilateral cleft lip; CP – cleft palate; RCP – right cleft palate; LCP – left cleft palate; SMCP – submucosal cleft palate; CLP – cleft lip and palate; LCLP – left cleft lip and palate; RCLP – right cleft lip and palate; BCLP – bilateral cleft lip and palate; ADD – attention deficit disorder

3.3.4 Large Deletions

Table 3 included microdeletions larger than 3 Mb that did not fall into our high or low penetrant deletion syndrome categories because there were no associated syndromes. However, given their size, they may contribute to the pathogenesis of OFCs. We reviewed each deletion for potentially causative genes and the following were found: *COL11A1*, *LHX8*, *FRG1*, *GRIP1*, *TXNL4A*, *BMP2*, and *JAG1*. Other deletions, including a paternally inherited 6.3 Mb 4p14.1-p14 deletion, a maternally inherited 3.7 MB 11p15.1-p14.3 deletion, and a 3.9 Mb 10p14-p12.31

deletion, had no genes within the deleted regions that were suspected to be causative and could be considered variants of unknown significance.

3.3.5   Deletions of Unknown Significance

Table IV (Appendix A) includes deletions that do not have clear connections to OFC syndromes or OFC-associated genes. In our cohort, 54% of the deletions fall into this category. These deletions should be further inspected in other OFC cohorts and in controls to determine if they may be associated with OFCs. The genes within them should also be considered for expression analyses or animal model studies.

3.4   DISCUSSION

The goal of this study was to describe the deletions and characteristics of an international orofacial clefting cohort with microdeletions. We found that in our 94-patient sample, most of the microdeletions within our cohort were associated with a syndromic presentation, particularly DiGeorge syndrome. Based on the empirical literate, we expect that these deletions are causal alone. A large subset was also composed of lower penetrance microdeletions, such as individuals with 3q29 microdeletion syndrome.   These deletions are most likely contributing to the multifactorial inheritance of OFCs, and together with environmental and other genetic factors, produced an OFC phenotype. Nine individuals had substantially sized deletions, most containing OFC-contributing genes such as *JAG1* and *COL11A*. Fifty-one deletions were of uncertain significance, or came with limited phenotypic data. Interestingly, only one non-syndromic OFC-

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associated deleted region was found within our sample, despite non-syndromic OFCs traditionally being cited as more common than syndromic OFCs.[2] Finally, four deletions of interest were noted in our sample, and their potential significance along with suggestions for future review are described in the following section:

3.4.1   3p26.3

3p26 deletions, particularly terminal 3p26.1 deletions, have been associated with a wide variety of symptoms, including developmental delay, intellectual disability, polydactyly, renal anomalies, heart defects, ptosis, and sacral dimples.[124125] Although some research has suggested that smaller 3p deletions, such as 3p26.3 deletions which encompass the *CNTN6* gene, may cause developmental delay, learning disabilities, or milder intellectual disability, the broader range of symptoms seen with larger deletions has not been recorded.[101] An individual with a paternally inherited 0.9 Mb 3p26.3 microdeletion has a bilateral cleft palate, along with a brother with polydactyly and a paternal aunt with intellectual disability. Although these phenotypic findings may be associated with other genetic or environmental factors, they may also be suggestive of 3p26.3 and *CNTN6* microdeletions having a larger range of associated phenotypes than previously suggested. Genetic testing of the possibly affected family members would be helpful in determining the significance of the deletion.

3.4.2   7q36.3

The *SHH* gene is well known for playing a role in embryonic development, taking part in craniofacial, limb, and spinal cord organization and growth, with a particular influence on the

midline of the body.[126] The cis-regulators that help moderate *SHH* are located within *SHH*, in the gene desert adjacent to *SHH*, and the genes following it, including *LMBR1*, in which the *ZRS* regulator falls. Mutations within *LMBR1* can affect *ZRS*, which impacts the development of the limbs, causing symptoms such as post-axial polydactyly.[107,108] Our cohort contains an individual with a 7q36.3 deletion that does not include *SHH*, but does include *LMBR1*. Although our individual does not have any recorded limb abnormalities, they do have an absent nasal bone and cartilage, a phenotype seen in individuals with midline defects tied to the *SHH* gene. This opens the possibility of the presence of other regulatory factors, perhaps within *LMBR1*, that can affect SHH and its role in the development of the midline.

3.4.3   12q14.3-q21.1

12q14 microdeletion syndrome, a condition often compared to Russel-Silver syndrome, encompasses a spectrum of characteristics including dwarfism, slow growth, small facial features, developmental delay, and osteopoikilosis (dense, hyperstatic areas of the bone).[127] In the literature, loss of the gene *HMGA2* within this region is thought to be the cause of the growth problems seen within the disorder. We identified one deletion of 12q14 that included *HMGA2* with concomitant features, such as being in the 0.1 percentile for BMI and 20th percentile for height, and having small facial features. A second, smaller deletion did not include *HMGA2*, leading to the possibility that another gene may be contributing to the clefting phenotype shared between these individuals. Both deletions included *GRIP1*, a gene also associated with 12q14 microdeletion syndrome, but not tied directly to problems of size and growth.[128]

3.4.4 12q21.1

Cardiofaciocutaneous syndrome (CFC) is a genetic syndrome with features including heart defects, dysmorphic facial features, skin and hair abnormalities, and intellectual disability.[129] Most commonly, mutations are found in the *BRAF* gene on chromosome 7. However, in recent years, multiple case reports have been published describing individuals with a CFC-like phenotype and 12q21 deletions, despite some controversy concerning the fit of the diagnosis.[130] The three patients described have some shared features with CFC including ptosis, low set ears, micrognathia, sparse, coarse hair, cardiac defects, developmental delay, generalized follicular hyperkeratotic papular eruption, and pyloric stenosis.[109–111] Within our cohort, we have also found an individual with a 2 MB *de novo* 12q21.1 deletion presenting with submucosal cleft palate, pyloric stenosis, speech delays, low set ears, curly hair, dysmorphic facies, and a rash on the skin like that seen in children with generalized follicular hyperkeratotic papular eruption (Figure 2). These phenotypic findings may represent a candidate gene location for CFC or new condition with similar symptoms.

Figure 2. Patient with 12q21 Deletion and CFC-like Features

A limitation of our study was that the data was collected in a research setting, and not a clinical one. Patients were assessed by people without medical training, and not by clinical geneticists with expertise in dysmorphology; photographs were only taken sporadically. Most of the subjects were recruited as part of the Pittsburgh Orofacial Cleft Study (POFC), focused on identifying subclinical phenotypes of OFCs. The protocols for this study are extensive compared to other recruitment strategies, but still rely on self-reported demographic and medical histories. POFC involved multiple international recruitment sites, each with variations in the amount of phenotypic data recorded. When available, the 3D photographs and videos were useful in identifying dysmorphic features, however not all relevant features are visible in photographs or known to the families. This is especially true when the children were recruited during infancy or as toddlers, who may not have had prominent dysmorphic facies or who had not yet failed to meet

developmental milestones. Even for a well-described microdeletion syndrome like DiGeorge syndrome, a heart defect may have previously gone undetected, due to a lack of prior concern for a chromosomal disorder.

Another consideration is that the standard of care continues to change. At present, infants with multiple congenital anomalies typically receive a clinical microarray to identify or rule out microdeletion syndromes; others receive mutation screening or clinical whole exome sequencing. The POFC sample was recruited over the last twenty years, prior to widespread use of clinical microarrays. In addition, many of the subjects were recruited from international sites, including on cleft repair missions to the Philippines and Guatemala, where diagnosing syndromes is not the highest priority. It is also important to note that diagnosis of syndromes is often easier after the identification of the deletion, as was the case in this study.

3.5 CONCLUSION

The international data we have collected and analyzed adds to the literature of deletions associated with OFCs. Unlike previous research that examined microdeletions in OFCs, our data showed almost no deletions in previously described non-syndromic regions.[8,80,131] Most of our deletions were within described syndromic regions previously associated with OFCs, with many that have a wide variety of other associated symptoms. These additional deletions will be helpful in delineating critical regions for craniofacial and OFC phenotypes. Our cohort, by showing the range of body systems affected by these deletions, help to demonstrate that careful monitoring along with proactive care are important for clinical management when one of the deletions is detected. Finally, the syndromic deletions of interest that we have noted could also open avenues

of new and further research into their significance, and could potentially enter the clinical realm

in the future.

4.0 RESEARCH SIGNFICANCE TO GENETIC COUNSELING AND PUBLIC HEALTH

Orofacial clefts (OFCs) pose a significant public health burden. OFCs are common malformations, occurring in 1 in every 700 births worldwide. The health care cost of these malformations has been well documented; the average cost over the lifetime for cleft lip and palate (CLP) treatment is over $200,000. Monetary consequences are not the only ones that exist; OFCs can cause a range of health concerns including dental, feeding, hearing, speech, and mental health problems.[2] Because of this, research to determine the etiology of OFCs has been extensive to help identify possible targets for treatment development to relieve the economic and medical burden that present with OFCs. Although the literature on the genetics of OFCs is extensive, only 25% of the heredity of non-syndromic OFCs has been explained, with 75% still unexplained.

The present study looks at only one kind of causative genetic abnormality, microdeletions. Despite this narrow scope, the information gleaned from our research adds to the growing literature of the genetics of OFCs. The data also contributes meaningful information when considering the public health significance of these malformations. In terms of the first core function of public health, assessment, our research has successfully "collect(ed) and analyz(ed) information about health problems." Within our cohort, we have identified and described new microdeletion syndromes, particularly in the case of the individual with a de novo 12q21.1 deletion and a cardiofaciocutaneous-like presentation. Additionally, in terms of previously described microdeletions, we have found several features that have extended our understanding about potential phenotypes, such as the individual with 12q14 deletion syndrome who had bilateral cleft lip and palate, which has not been described in the literature. With the recognition of these rare

syndromes, we have collected information to provide to medical professionals, including genetic counselors that will aid in the diagnosis and management of individuals with OFCs. As more is studied and understood, we can give this information to families to provide accurate descriptions of phenotypic presentations, disease course, and recurrence risk, along with using it for shaping management and surveillance standards.

Another essential public health core function that our research addresses is assurance, particularly in terms of "assur(ing) a competent public health and personal healthcare workforce." As mentioned previously, many of the individuals in our cohort would benefit from surveillance to determine whether other previously recorded characteristics are present. However, many of the individuals within our cohort were not recognized as having a syndromic presentation prior to genetic testing, despite a wide range of other symptoms being listed. These missed diagnoses may be indicative of a lack of proper genetics education among national and international medical professionals. Additionally, microarray, a genetic test used to detect microdeletions, is a front-line test now offered to individuals with a congenital anomaly.[1] Many of these individuals, particularly with common microdeletions such as 22q11.2, the causative deletion for DiGeorge syndrome, could have been diagnosed much earlier if they had been given this testing upon detection of an OFC and other symptoms. This is particularly relevant because other internal anomalies associated with the condition could be recognized and treated early, such as heart defects, and supports like early intervention services could be offered to individuals at risk for developmental delay. Again, our research illustrates the differences in standards of care and the importance of educating clinicians on the relevance of genetic testing for their patients. Genetic counselors are uniquely qualified for this type of role, and perhaps in the future could develop educational programs and/or resources targeted in areas known for having a lower genetic literacy.

This research provides information that could impact genetic counseling practice. The etiology of CL, CP, and CLP is complex; as described previously, environmental multifactorial, and Mendelian causes all exist. This can make genetic counseling for orofacial clefts challenging. Typically, recurrence risks are cited as 3-5% when a previous child or other close relative has non-syndromic CL/P (the typical recurrence risk quoted for most congenital anomalies). This is based on the thought that most non-syndromic OFCs are due to multifactorial inheritance.[69] Although the discovery of new genetic regions tied to OFC help elucidate possible causes, it remains unclear about how much (or little) they contribute to the phenotype. Deletions within the 1q21 region, for example, though associated with a wide array of symptoms including OFC, have a low penetrance, and characteristics in individuals with the deletion can vary significantly even within a family.[100] Because of this level of uncertainty, more research will need to be conducted on non-syndromic OFC related regions to better counsel patients on risk. Once this is done, we will be able to quantify and quote more accurate risk values to patients and their families. This study has outlined many low penetrance deletions, their inheritance patterns, and phenotypic characteristics. The information we found can be used clinically to help inform patients of the spectrum their child may fall along. As similar studies are performed in the future and more data on these specific deletions are collected, we may be able to better determine the significance of the deletions and how they interact with the genome and the environment.

APPENDIX A: SUPPLEMENTARY TABLE

Table 4. Deletions of Unknown Significance

DELETION	Mb	INHERITANCE	PHENOTYPE	FAMILY HISTORY
1p22.1	1.0	de novo	SMCP	NA
1p22.1	1.0	de novo	LCLP	father with LCLP
1p22.1	1.4	de novo	RCLP	NA
1p22.1	.84	both (homozygosity)	LCL, minor epicanthal folds	NA
1p22.1	.89	unknown	CLP	brother with LCL; brother with bifid uvula
1p22.1	.89	unknown, not maternal	microform LCL	NA
1p35.3	.88	unknown, not paternal	BCLP	brother with LCLP; brother with CLP; brother with RCL; paternal half-brother with CLP
2p25.3	1.5	paternal	RCLP	NA
2q11.2	1.4	unknown	LCL	NA
2q12.3	1.3	unknown	LCLP	mother with SCP, bifid uvula
2q14.2	1.2	maternal	CP	NA
2q33.2-q33.3	1.1	de novo	RCLP	father with CP; first cousin once removed with RCLP
2q33.2-q33.3	.77	unknown	LCLP	father with LCLP
2q33.2-q33.3	.77	unknown	BCLP	NA
2q33.2-q33.3	.77	maternal	CLP, heart murmur	mother with learning disability; sister with heart murmur
3q26.1	1.4	de novo	RCLP, chronic ear infections, blocked left tear duct	maternal grandmother with intellectual disability, paternal grandmother with extra adult teeth, father with heart murmur, mother with bipolar disorder
3q28	1.9	unknown, not maternal	RCLP	great nephew with speech and developmental delay
4q35.2	.95	maternal	BCLP	NA
5q13.1-q13.2	1.6	unknown	BCLP	NA
5q21.2	.90	maternal	RCLP	NA
6p22.1	1.3	paternal	incomplete LCL	NA
6q22.32-q22.33	.94	unknown	BCLP	NA
7p22.1	1.9	unknown, not maternal	BCLP	5th degree paternal relative with RCLP
7p22.3-p22.1	2.9	de novo	LCL	NA
7q11.23	1.4	unknown, not maternal	LCLP	NA
8q23.2-q23.3	3.8	unknown, not maternal	RCL	NA
10q25.3-q26.11	.78	unknown, not from mother	incomplete RCL	maternal grandmother LCLP
10q25.3-q26.11	.79	unknown	LCL	NA
10q25.3-q26.15	.79	unknown, not maternal	BCLP, speech and behavioral disorder, low set ears	NA
11p11.12-p11.1	1.0	paternal	BCLP	NA
11p15.4	1.1	unknown	RCL	brother with CL; sister with CL; father with CLP; first maternal cousin with LCLP
13q12.12-q12.3	1.4	unknown	BCLP	NA
13q31.3	.79	maternal	CP	brother with CL
13q33.2-q34	8.9	unknown, not maternal	BCLP	NA
14q11.2	.94	unknown	RCLP	NA
14q12	.90	unknown	BCLP	NA

Table 4 Continued

15q11.1-q11.2	3.1	maternal	LCL	NA
15q11.2	.77	maternal	LCLP	NA
15q11.2	.85	paternal	RCLP	NA
15q13.3	.87	paternal	LCLP, speech delays, learning disability, small	father with hemihypertrophy
15q13.3-q14	1.9	maternal	BCLP	NA
15q14	.90	unknown	BCLP and speech delays	father with behavioral problems, learning disability, undescended left testicle
17p12	1.3	de novo	LCL	NA
17q11.2	1.2	de novo	BCLP and bifid uvula	NA
17q21.33	1.6	unknown	BCLP, speech delay	brother with CL, and 4th degree paternal relative with CL
17q22-q23.2	2.0	de novo	BCLP	NA
17q25.3	.77	maternal	BCLP	maternal half-brother with CL; maternal half-brother with CLP; maternal 5th degree relative with CP; maternal 5th degree relative with CL
18q11.2-q12.1	1.3	paternal	LCLP	NA
19q13.12	.85	unknown, not from mother	SMCP, bifid uvula, learning disability, difficulty running, hearing loss	father with CP; twin sister with bifid uvula
19q13.12-q13.2	.98	de novo	CP, speech delay, behavioral problems, learning disability, failure to thrive, heart murmur, ODD and ADHD	4th and 5th degree maternal relatives with CP

CL – cleft lip; RCL – right cleft lip; LCL – left cleft lip; BCL – bilateral cleft lip; CP – cleft palate; RCP – right cleft palate; LCP – left cleft palate; SMCP – submucosal cleft palate; CLP – cleft lip and palate; LCLP – left cleft lip and palate; RCLP – right cleft lip and palate; BCLP – bilateral cleft lip and palate

APPENDIX B: INSTITUTIONAL REVIEW BOARD APPROVAL

University of Pittsburgh
Institutional Review Board

3500 Fifth Avenue
Pittsburgh, PA 15213
(412) 383-1480
(412) 383-1508 (fax)
http://www.irb.pitt.edu

Memorandum

To: Mary Marazita , PhD
From: IRB Office
Date: 8/29/2016
IRB#:   REN16080177 / IRB0405013
Subject: University of Pittsburgh: Coordinating Center for Oral-Facial Cleft Families: Phenotype and Genetics

Your renewal for the above referenced research study has received expedited review and approval from the Institutional Review Board under:

45 CFR 46.110.(7)

Please note the following information:

Approval Date: 8/29/2016
Expiration Date: 9/27/2017

Please note that it is the investigator's responsibility to report to the IRB any unanticipated problems involving risks to subjects or others [see 45 CFR 46.103(b)(5) and 21 CFR 56.108(b)]. Refer to the IRB Policy and Procedure Manual regarding the reporting requirements for unanticipated problems which include, but are not limited to, adverse events. If you have any questions about this process, please contact the Adverse Events Coordinator at 412-383-1480.

The protocol and consent forms, along with a brief progress report must be resubmitted at least **one month** prior to the renewal date noted above as required by FWA00006790 (University of Pittsburgh), FWA00006735 (University of Pittsburgh Medical Center), FWA00000600 (Children's Hospital of Pittsburgh), FWA00003567 (Magee-Womens Health Corporation), FWA00003338 (University of Pittsburgh Medical Center Cancer Institute).

Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.

University of Pittsburgh
Institutional Review Board

3500 Fifth Avenue
Pittsburgh, PA 15213
(412) 383‑1480
(412) 383‑1508 (fax)
http://www.irb.pitt.edu

Memorandum

To: Mary Marazita, PhD
From: IRB Office
Date: 6/9/2016
IRB#:   REN16060012 / IRB0607057
Subject: Oral-Facial Cleft Families: Phenotype and Genetics: (Pittsburgh and Guatemala Sites)

Your renewal for the above referenced research study has received expedited review and approval from the Institutional Review Board under:

45 CFR 46.110.(9)

Please note the following information:

Approval Date: 6/9/2016
Expiration Date: 7/8/2017

Please note that it is the investigator's responsibility to report to the IRB any unanticipated problems involving risks to subjects or others [see 45 CFR 46.103(b)(5) and 21 CFR 56.108(b)]. Refer to the IRB Policy and Procedure Manual regarding the reporting requirements for unanticipated problems which include, but are not limited to, adverse events. If you have any questions about this process, please contact the Adverse Events Coordinator at 412-383-1480.

The protocol and consent forms, along with a brief progress report must be resubmitted at least **one month** prior to the renewal date noted above as required by FWA00006790 (University of Pittsburgh), FWA00006735 (University of Pittsburgh Medical Center), FWA00000600 (Children's Hospital of

https://www.osiris.pitt.edu/osiris/Doc/0/1UJ78VK432RKH49CCKDLTF2B04/fromString....   6/27/2016

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