FROM SYNDROMES TO NORMAL VARIATION:
A CANDIDATE GENE STUDY OF INTERORBITAL DISTANCES

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ABSTRACT

Hypertelorism and telecanthus are clinical phenotypes associated with many genetic syndromes. To date, research is limited regarding whether disease-causing genes are related to normal craniofacial development in unaffected individuals. The aim of this study is to determine whether common genetic variation in forty selected genes implicated in hypertelorism/telecanthus-related syndromes contribute to normal variation of intercanthal and outer-canthal distances of the orbits. Hypertelorism/telecanthus-related genes were selected based on significant prevalence of the phenotype in the described genetic syndrome. Using the 3D Facial Norms (TDFN) Repository, genomic and anthropometric data were utilized to test genetic association for common variants in two phenotypes: intercanthal and outer-canthal distances. Suggestive SNPs with evidence of association were annotated for relevant gene function related to craniofacial development. For the intercanthal distance measurement, one statistically significant SNP ($p<4.05 \times 10^{-6}$) in *LINC00482* and two suggestive SNPs ($p<10^{-4}$), one in *HMGCS2* and another within 200kB of *FAM58A,* were observed. For the outer-canthal distance measurement, five suggestive SNPs ($p<10^{-4}$) were observed near *ADAMTS18*, *GLI3*, *ACTG1*, *MEGF11*, and *SPECC1L*. We hypothesize that identified SNPs have regulatory effects on the expression of these genes and contribute to interorbital distances in unaffected individuals.

Identifying genetic determinants of craniofacial development in the normal population is important for the understanding of mechanisms underlying craniofacial dysmorphology. In addition, understanding the mechanisms that contribute to the transition from normal variation to a disease state in a population is important to public health because most genetic diseases exist on a spectrum. With better understanding the unaffected side of the spectrum allows us to better identify the disease side of the spectrum, allowing for better diagnosis and treatment for individuals with craniofacial anomalies. This study attempts to identify these risk loci and hypothesize what impact these loci might have on craniofacial development.

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PREFACE

I would like to take this opportunity to thank the individuals who have provided unwavering support throughout my time in graduate school. First, I would like to thank the members of my thesis committee for guiding me and providing constructive feedback throughout the writing process. Additionally, I would like to thank my thesis committee chair, Dr. Seth Weinberg, for encouraging me for this past year. He has been a great advisor and has taught me a great deal. I appreciate his dedication to our project!

I would like to thank Dr. Robin Grubs and Dr. Andrea Durst for their support throughout the Genetic Counseling Program. They have been a great source of encouragement since I began graduate school and it has helped immensely. I would like to thank my genetic counseling classmates for always being able to provide a smile, a silver lining, or a sweet treat when in need. Our unique bond can never be broken and I cannot wait to have such great colleagues in the genetic counseling field!

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1.0   INTRODUCTION

This study investigated the genetic basis of normal variation in interorbital distance measures in humans. Several genome-wide association studies (GWASs) have reported that common variants in or around genes that cause craniofacial syndromes may contribute to normal facial variation[1–3]. In a recent GWAS, Shaffer et *al*. (2016) reported associations at 1p13.3 and Xq13.2 for intercanthal width, a measure of the linear distance between the inner commissures of the eyes[3]. Several genes near these loci (e.g., *ALX3* and *HDAC8*) have been implicated in syndromes characterized by hypertelorism.

Several monogenic disorders have ocular hypertelorism and/or telecanthus as hallmark features. Ocular hypertelorism is defined as an increased distance between the bony orbits (eyes), while telecanthus is defined as an increased distance in intercanthal distance without increased lateralization of the orbital wall[4]. In this study, we hypothesized that common single nucleotide polymorphisms (SNPs) in 40 candidate genes implicated in hypertelorism and telecanthus syndromes are associated with measurements of orbital spacing in a cohort of healthy individuals. To test this hypothesis, we used an existing dataset comprised of 3D facial measurements and genome-wide markers obtained from the 3D Facial Norms dataset. The following specific aims of the study included:

- Identify a set of candidate genes linked to Mendelian syndromes where hypertelorism or telecanthus are cardinal features.

- Based on available imputed genotypes, extract SNPs within a 400kB interval of selected candidate genes.

- Perform an association test between extracted SNPs and measures designed to capture aspects of hypertelorism/telecanthus (intercanthal and outer-canthal width).

- Explore possible function of associated SNPs to develop hypotheses on their potential role in craniofacial development.

This project is innovative in that it applies a unique approach to underrepresented phenotypes in the literature. A well-described hypothesis has emphasized that normal variants in or around genes that cause Mendelian syndromes impact complex phenotypes[5], in which this project attempts to contribute. This project may provide insight on the possible role of genes in both normal and atypical facial morphogenesis. This information can have an impact on the evaluation of craniofacial dysmorphology and contribute to the identification of risk loci for hypertelorism.

The results of this study are relevant for clinical geneticists, genetic counselors and researchers. This information will be useful in the clinical genetics setting because it will better define variation in the normal face, which can aid the work of genetic specialists who attempt to identify genetic syndromes. Researchers can also utilize the results from this study to better understand the control group that has been created in the 3D Facial Norms Repository, a group utilizing 3D facial images to provide normative reference data on human facial morphology.

2.0   LITERATURE REVIEW

2.1   HYPERTELORISM AND TELECANTHUS PHENOTYPES

2.1.1   Definitions

Hypertelorism and telecanthus are two phenotypes that describe how distance between the eyes differ from the norm. Both features, however, are similar and frequently clinicians and researchers use one term in place of another, which can complicate how both characteristics are defined.

In the 1920s, D.M. Greig defined hypertelorism simply as wide-set eyes.[6] However, as technology advanced, the definition of hypertelorism was modified to an "increased lateralization of the entire orbital complex,"[7]. This observation implies that the orbital walls are shifted in a dorsolateral direction, causing an increased distance between the inside and outside corners of the eye (intercanthal and outer-canthal width, respectively). Hypertelorism can also be defined as an increased pupillary distance,[4] which attempts to identify the dorsolateral increase between the orbits. Hypertelorism can be mistaken in individuals with broadened or flat nasal bridges because it appears as if there is an increased space between the orbits, so proper measurement is essential to properly identify hypertelorism. In summary, hypertelorism is defined as a dorsolateral shift between the eyes, which results in an increased distance between the intercanthal distance and outer-canthal distance of the eyes.

Telecanthus is a phenotype similar to hypertelorism in that it appears to have an increased intercanthal distance, so the eyes appear wide set. However, unlike hypertelorism, telecanthus does

not show an increased outer-canthal distance. This is the most significant distinction from hypertelorism: there is no dorsolateral change in the orbital bones that result in increased inter- and outer-canthal distances in telecanthus[4]. Consequentially, there is not an increased interpupillary distance in telecanthus[8]. Increased intercanthal distance appears to be due to an increased amount of soft tissue between the innercanthi[8]. As such, telecanthus is often described in conjunction with other subtle ocular findings such as epicanthal folds, which are skin folds that run across the eyelid. Epicanthal folds appear to add to the distance between the eyes, creating a "wide set eye" appearance[8]. Most commonly, telecanthus is characterized as an increased distance between innercanthi, but not an increased pupillary distance.

There is a common misconception in the literature regarding the differences and similarities between hypertelorism and telecanthus. Recognizing the distinction between the two is essential in differentiating the genetic etiology of hypertelorism and telecanthus. Hypertelorism has become a synonym for telecanthus because of the increased intercanthal distances, but does not consider the outer-canthal distances that differentiate one phenotype from the other[9]. In 2009, human malformation terminology was standardized with the hope that there would be less subjectivity and more objectivity in classifying dysmorphology. This accurately differentiated hypertelorism from telecanthus by emphasizing that hypertelorism is represented as an increased pupillary distance and telecanthus is represented as an increased intercanthal distance[10]. These phenotypes are both considered as extreme phenotypes because they both need to be greater than two standard deviations of the mean, accounting for age, sex and ethnic differences[10]. This has allowed researchers and clinicians to better understand and distinguish these two characteristics from one another.

2.1.2   Measuring hypertelorism and telecanthus

There are a number of ways in which hypertelorism and telecanthus can be identified. Over time, these methods have changed due to advances in technology and imaging systems. Each of these methods has benefits and limitations, which may complicate which one to choose. These methods are useful in the clinical arena to better detect craniofacial dysmorphology, which can ultimately lead to a genetic diagnosis. These methods include anthropometry, radiography, and 3D photogrammetry.

2.1.2.1 Anthropometry

Classically, anthropometry is defined as the science of measuring physical characteristics of the entire body[11]. This includes measuring the limbs, trunk, and elements of the face with specific anthropometric instruments. These measurements are used to describe growth of an individual, determine surgical plans, and estimate possible surgical outcomes[12]. When taking measurements of the trunk and limbs, the landmarks, or spots on the body to start and stop measuring, are well defined. However, for craniofacial anthropometry, landmarks need to be explicitly defined to ensure accurate measurements. On occasion, palpation of the face needs to occur in order to pursue the proper landmark placement[13]. The landmarks of interest are intercanthal distance, which is described as the distance between the inner corners of the eye[14] and outer-canthal distance, which is the distance between the outside corners of the eye[14]. Interpupillary distance can also be measured to determine the presence of hypertelorism, but it can be difficult to measure on uncooperative individuals such as children or individuals who are incapable of keeping their eyes still[14].

This method is the classical way of taking physical measurements. Craniofacial anthropometry is beneficial because it is a low-cost method and there are standards for different ethnic and sex groups that allow for quick comparison. However, there is room for subjectivity based on the individual taking the measurements and inconsistent measurements due to uncooperative patients. This can result in approximations being made, which sacrifices the accuracy of the measurement. This method can also be considered an invasive approach, as calipers need to be in physical contact with the face throughout the measurement process[15]. Another limitation of using calipers is that calipers can measure straight lines and distances quite well, but cannot distinguish curvature or depth of a facial characteristic, which is now known to be influenced by specific genetic factors[3].

2.1.2.2 Radiography

Radiographs, including X-rays and computer tomography (CT) scans were the first method of visualizing whether the orbital bones are laterally displaced[14]. These images can provide a visual depiction of other internal structures that may contribute to interorbital distances such as cysts, soft tissue or extra bone structures that make the orbits seem to be further apart than they truly are[16]. This method can also be used to determine if hypertelorism is a secondary effect due to an enlargement of the ethmoid sinuses, which was previously thought to cause hypertelorism[17]. In this circumstance, one could measure the distance between the innermost point of the bony orbits and could determine whether an individual has secondary hypertelorism due to enlarged ethmoid sinuses or true hypertelorism[17]. With these images, interactions between the bony orbits, the cranial vault, and facial and temporal bones are visualized and can identify what may be contributing to a potential dysmorphic feature, such as hypertelorism or telecanthus[16].

A benefit of this method is its ability to visualize the internal structures of the face, which allows clinicians and investigators to determine the bony structure of the face. When looking at an individual, soft tissue can distort an interpretation, while radiographic images provide clear boundaries for taking proper measurements. This method also provides more precise innermost points of the bony orbits to provide an exact measurement. CTs and radiographs are less invasive. Images can be retained to re-measure in the future, if needed, however, the images can take several minutes to capture correctly and may be difficult to obtain with uncooperative patients. Another limitation of this method is the radiation that an individual may be exposed to during the time needed to capture the image. This brings up a potential ethical issue when a research team is attempting to attain measurements on healthy individuals.

2.1.2.3 3D Photogrammetry

3D photogrammetry is the newest approach to capture facial images in order to determine craniofacial dysmorphology. This method consists of a 3D-capable camera that can detect not only facial landmarks as in classical anthropometry, but can also detect facial depth and curvature of the face[18]. With this digital photography, landmarks can be identified and distances can be measured between the landmarks. There are several types of 3D photogrammetry cameras, all of which employ similar technologies. This technology allows for quick captures with landmarks that are automatically calibrated to particular facial features. This method is ideal for uncooperative individuals and can easily be redone if the quality of the capture was reduced[15]. These images can also be saved and accessed at later points in time[15]. Capturing landmarks of the ear is difficult because of hair or shadows interfering with the side of the head and therefore, is a limitation of the method[15,18].

2.2 SYNDROMES CHARACTERIZED BY HYPERTELORISM AND TELECANTHUS

Dysmorphic facial features are often the first sign of a potential genetic condition. Several genetic conditions have hypertelorism and telecanthus as a phenotypic feature. Genetic conditions that include hypertelorism and telecanthus are described below.

2.2.1 Hypertelorism-Related Genetic Conditions

Some of the more common syndromes that have hypertelorism as a main phenotypic feature are listed in Table 1.

Table 1. Genetic Conditions Involving Hypertelorism

Syndrome	Gene	Protein Function	Inheritance	Relevant Clinical Symptoms
Frontonasal Dysplasia 1	*ALX3*	Expressed in frontonasal mesenchyme, suspected to increase Shh activity[19]	AR	Ocular hypertelorism, broad nasal root, median cleft lip/palate, widow's peak, agenesis of corpus callosum[19]
Frontonasal Dysplasia 2	*ALX4*	Homeobox gene expressed in bone tissue and is imperative for cranial development and neural tube closure in addition to limb development, expressed in frontonasal mesenchyme[20]	AR	Coronal craniosynostosis, ocular hypertelorism, depressed nasal bridge and ridge, agenesis of corpus callosum, cryptorchidism, intellectual disability[21]
Waardenburg Syndrome Type 1	*PAX3*	Essential for melanocyte	AD	Sensorineural hearing loss, heterochromia,

Table 1 Continued

		development in early embryos[22]		white forelock, hypertelorism, and other ocular abnormalities[22]
Apert/Crouzon Syndrome	*FGFR2*	Tyrosine kinase receptor for fibroblast growth factor (FGF), involved in retina development throughout embryonic period[23]	AD	Craniosynostosis, midface hypoplasia, ocular hypertelorism, prognathism, high arched palate, choanal stenosis/atresia, sensorineural hearing loss, Chiari malformations[24]
Noonan Syndrome	*PTPN11 (50%), SOS1, BRAF, MAP2K1, RAF, RIT1, KRAS*	PTPN11: tyrosine protein phosphatase, acts as a signaling protein that is involved with cell proliferation and differentiation[25]	AD	Short stature, developmental delay, congenital heart defects and facial dysmorphisms including: downslanting palpebral fissures, deep philtrum, ocular hypertelorism, low posterior hairline[26]
Saethre-Chotzen Syndrome	*TWIST1*	Aids in regulation of FGFs and cytokine signaling in a negative feedback loop[27]	AD	Coronal synostosis, strabismus, ptosis, cleft palate, ocular hypertelorism, maxillary hypoplasia, congenital heart defects[28]
X-Linked Opitz G/BBB Syndrome	*MID1*	Involved in cell proliferation, associates with microtubules throughout mitosis and aids protection of microtubule polymerization[29]	XLR	Hypospadias, hypertelorism, cleft lip/palate, cardiac defects, imperforate anus[30]
Kleefstra Syndrome	*EHMT1*	Lysine methyltransferase that contributes to brown adipose tissue cell fate and overall brown	AD, only de novo reports	Intellectual disabilities, heart defects, hypotonia, epilepsy, synophrys, hypertelorism,

Table 1 Continued

		adipose tissue homeostasis[31]		midface hypoplasia, etc.[32]
Craniofrontonasal Syndrome/Dysplasia	*EFNB1*	Scaffold protein responsible for ensuring tight junctions between cells[33]	XLD	Females experience developmental delay, skeletal malformations, strabismus, nystagmus, exotropia, hypertelorism; males experience only hypertelorism[34]
Grieg Cephalopolysyndactyly Syndrome	*GLI3*	Zinc finger transcription factor that acts in the sonic hedgehog pathway, acts to activate PTCH expression, which is involved in the TGF-beta and Wnt pathways[35]	AD	Frontal bossing, craniosynostosis, hypertelorism, pre- or post- axial polydactyly[36]

2.2.2 Telecanthus-Related Genetic conditions

Some of the more common syndromes that include telecanthus as a main phenotypic feature are listed in Table 2.

Table 2. Genetic Conditions Involving Telecanthus

Syndrome	Gene	Protein Function	Inheritance	Relevant Clinical Symptoms
MMCAT Syndrome	*ADAMTS18*	Metalloproteinase anchored to extracellular matrix that plays a role in early eye development[37]	AR	Microcornea, myopic chorioretinal atrophy, telecanthus[37]

Table 2 Continued

Shprintzen-Goldberg Craniosynostosis Syndrome	*SKI*	Proto-oncogene protein involved in muscle differentiation and neural tube development[38]	AD, only de novo reports	Craniosynostosis, brain abnormalities (hydrocephalus, Chiari I malformation, lateral ventricle dilatation), cognitive impairments, cardiac anomalies (mitral valve prolapse, aortic root dilatation, aortic regurgitation) and characteristic facies including telecanthus, downslanted palpebral fissures, micrognathia or retrognathia[39]
Barber Say Syndrome	*TWIST2*	Negative regulator of transcription in skeletogenesis, regulate mesenchymal stem cell differentiation[40]	AD, only de novo reports	Macrostomia, hypertrichosis, atrophic skin, telecanthus, bulbous nasal tip, low frontal hairline[41]
STAR Syndrome	*FAM58A*	Unknown	XLD	Toe syndactyly, telecanthus, short stature, anal stenosis, external genitalia malformations[42]

2.3   DEVELOPMENTAL BASIS OF HYPERTELORISM AND TELECANTHUS

Craniofacial development is a tightly controlled process that requires synchronization of multiple proteins throughout embryonic development. This process involves interactions between multiple tissues; differentiation of tissues is based on induction from nearby tissues. The skull and face require so many different interactions throughout the entirety of development and consequently craniofacial formation is considered one of the most complex aspects of all embryonic development[1].

2.3.1   Establishment of Interorbital Distance During Development

Orbital development begins at the neural plate where cranial neural crest cells (CNCC's) are induced and transition from the epithelium to the mesenchyme[43]. From the mesenchyme, they migrate to their destined location, the periocular region. The frontonasal prominence (FNP) consists of the forehead and the periocular region, where the distance between the eyes is established[43]. By embryonic day 9, CNCC's are fated and in the necessary location to begin differentiation[43].

Once development of the eye begins, they are located on each dorsolateral side of the developing head. As development continues throughout gestation, the eyes converge towards the front of the face and towards each other[43]. The orbital bones ossify at approximately 6-7 months of gestation, but eye distance continues to change minimally throughout childhood and adolescence and finally stabilizes in adulthood[43]. Many proteins are involved during this course of development, as craniofacial development is a tightly regulated process. One of the most important proteins found in the FNP is sonic hedgehog (Shh), which controls craniofacial patterning and is also seen throughout the growing body in embryonic development.

Sonic hedgehog (Shh) is a protein coded by the *SHH* gene. Shh acts as a chemical signal that is critical for many aspects of embryonic development, but this section is focused on how Shh is significant in craniofacial development. It is first expressed in the forebrain and is patterned in a way that determines the majority of the bilateralism of facial morphology, including interorbital distances[44,45,46].

Mutations in *SHH* are responsible for craniofacial malformations such as holoprosencephaly (HPE), which represents a defect in mediolateral patterning[47,48]. HPE is defined as a condition where the brain fails to divide into two hemispheres, which then affects the

bilateralism of the rest of the face[49]. A main feature of HPE is cyclopia, where there is one unilateral eye that develops because of the failure of the orbits to properly divide. Mutations in genes that are related to (or a part of) the Shh signal transduction pathway also show signs of varying degrees of HPE. An example is mutations in the Shh receptor *PTCH1,* which result in decreased Shh signaling, which can cause hypotelorism, midface hypoplasia and upslanting palpebral fissures[50]. Disturbances of Shh have also been shown to be correlated with decreased cell proliferation[46] or premature cell death of CNCC's[51]. This explains the reason why there can be disturbances in mediolateral facial patterning.

It has been suggested that excess Shh expression can cause an increase in the mediolateral patterning of the face due to truncated cilia on CNCC's [52]. Primary cilia are responsible for mediating the cell's interactions with its environment[52]. Ciliopathies, or genetic diseases that result in abnormal cilia, are thought to contribute to craniofacial dysmorphisms because these cells cannot adequately respond to their environment. In the case of Shh signal transduction, when the cilia are truncated, studies have shown that this increases Shh expression in CNCC's[52]. Excess Shh in the facial ectoderm results in increased midline features such as hypertelorism[52]. These data suggest that Shh is critical for normal bilateral facial patterning in embryonic development.

2.3.2   Hypertelorism and Telecanthus Model Organisms

Model organisms are important for researchers to determine the potential impact a particular condition will have on a human. Several models have been used to identify the genetic factors that contribute to midline defects[45,53]. Of note, chick embryo models have been established to examine the significance of Shh in the frontonasal process of the developing face[45,53].

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To evaluate whether Shh was truly affecting craniofacial development, specifically, the frontonasal process (where orbital distances are measured), Hu and Helms[53] completed a gain of function study for Shh. Gain of function studies examine how an increase in gene expression can affect a phenotype. Hu and Helms determined that an increase in Shh concentration resulted in an increased intercanthal distance and an increased mediolateral distance, which was a length identified as the distance between the nose and ear[53]. This ectopic expression of Shh induced *BMP2*, *PTC*, and *GLI1* in the ectoderm [53]. These are receptors and transcription factors that are involved in cell proliferation throughout craniofacial development. This study, one of the first of its kind, provided evidence demonstrating Shh as being a main contributor for facial patterning.

Young et *al.*[45] took a similar approach and added SHH-soaked beads to fertilized chicken eggs, but did so in various concentrations to the anterior neural tube. They saw a positive correlation in the frontonasal process, orbital bone distance and progressive hypertelorism with an increase of Shh concentration. This team also performed this experiment with decreasing concentrations of Shh, where they found progressive hypotelorism with overall facial narrowing. This study was pivotal in that it described a gradient of facial structure that is dependent on Shh concentration[45].

2.4   GENETICS OF NORMAL VARIATION IN ORBITAL SPACING

2.4.1   Population Differences and Heritability

Interorbital distance is defined as the distance between the medial canthi of each eye, and it can be easily measured using standard calipers or even a tape measure. Interestingly enough,

these measures are known to differ between ethnic groups[54,55,56,57,58]. This is significant because it suggests that regardless of age and sex, individuals from different ethnic groups have different interorbital norms. This suggests that genetic factors may influence the difference between ethnic groups. Largely, the specific genetic differences are unknown, but it could be hypothesized that similar genetic factors that contribute to ethnic differences in skull and bone development could influence interorbital distance.

The appropriate use of norms is critical because it ensures that hypertelorism and telecanthus are classified properly. Since hypertelorism and telecanthus are measured based on a Z score, it is imperative that the proper standards are used for an individual's evaluation.

Studying twins is a common way to determine how environmental and genetic factors influence phenotypes. Monozygotic twins (MZ) have the same exact genetic composition, so phenotypic differences between them are often considered due to environmental factors. MZ twins are typically compared to dizygotic twins (DZ), who share 50% of their DNA. This comparison of MZ and DZ twins is used to identify heritability: how much a phenotype is dictated by genetic variation. Classically, twin studies of anthropometric measurements of the face estimate the heritability of interpupillary distances (intercanthal distance) to be up to 70%[59]. However, with new technology and better landmarking abilityof 3D stereophotogrammetry, more refined heritability estimates are possible. In a recent study, it was determined that heritability for interorbital distances was approximately 40%[60]. This significant change is possibly due to our advanced technologies available today. However, 40% heritability is significant and means that a moderate amount of genetic factors exist that contribute to interorbital distances.

2.4.2   Genetic Studies of Interorbital Distances

With the onset of more advanced genotyping methods, research has begun to identify genetic variants that may contribute to interorbital distances. In these studies, investigators often look at genetic variants that contribute to overall facial shape in a GWAS design. A GWAS examines the association between millions of SNPs spread across the genome and one or more phenotypes. GWAS are considered "hypothesis-free" because a subset of SNPs is not selected beforehand based on suspected function.

Paternoster et *al.*[2] published the first GWAS of normal human facial shape. Using 3D photogrammetry, this study of adolescents from the UK failed to find statistical evidence of an association with intercanthal or outer-canthal distance. They did, however, report an association between variants in the *PAX3* gene and the morphology of the nasal root, located in the region between the orbits. That same year, Liu et *al.*[1] performed a second GWAS for facial shape in a large sample of adult individuals of European descent. These researchers used 3D MRI-based phenotyping and used nine well- characterized landmarks involving the innercanthi and outer-canthi. They reported an association between *TP63* and horizontal distance between the left and right soft-tissue orbits. They also reported an association between *PAX3* and the distance between nasion (a midline point on the nasal root) and the orbits laterally.

A more recent GWAS by Shaffer et *al.* examined normal facial traits in approximately 3,000 individuals from the FaceBase Consortium's 3D Facial Norms dataset and identified two significant loci for intercanthal distance: one at 1p13.3 and another at Xq13.2 [3]. These loci were in a region that included genes that cause syndromes that include hypertelorism/telecanthus as a phenotypic feature. Their findings suggest that there is a genetic contribution to interorbital distances and the relevant genes may be involved in hypertelorism/telecanthus-related syndromes.

3.0   MANUSCRIPT

3.1.1   Background

Hypertelorism is defined as an "increased lateralization of the entire orbital complex,"[7]. This observation implies that the orbital walls are shifted in a dorsolateral direction, causing an increased distance between the inside and outside corners of the eye (intercanthal and outer-canthal distance, respectively). Telecanthus is a phenotype similar to hypertelorism in that it appears to have an increased intercanthal distance, so the eyes appear wide set. Increased intercanthal distance is due to what appears to be an increased amount of soft tissue between the innercanthi[8].

Many proteins are involved during craniofacial development, as it is a tightly regulated process. One of the most important proteins found in the frontonasal process, the area of the face that includes the forehead and the periocular region where the distance between the eyes is established[43], is Sonic hedgehog (Shh). Shh controls craniofacial patterning and is also seen throughout the growing body in embryonic development[45,46,53].

With Hu and Helms[53] chick model, they determined that an increase in Shh concentration resulted in an increased intercanthal distance and an increased mediolateral distance, which was a length identified as the distance between the nose and ear[53]. This study, one of the first of its kind, provided evidence demonstrating Shh as being a main contributor for facial patterning. Young et al.[45] took a similar approach and added SHH-soaked beads to fertilized chicken eggs, but did so in various concentrations to the anterior neural tube. They saw a positive correlation in the frontonasal process, orbital bone distance and progressive hypertelorism with an increase of Shh concentration. This team also performed this experiment with decreasing concentrations of Shh,

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where they found progressive hypotelorism with overall facial narrowing. This study was pivotal in that it described a gradient of facial structure that is dependent on Shh concentration[45]. These models have shown some genetic contribution to hypertelorism, but other genetic contributions are unknown.

Both hypertelorism and telecanthus are phenotypic features seen in many genetic syndromes. Some syndromes that have hypertelorism as a main phenotypic feature include Frontonasal Dysplasia, Apert/Crouzon Syndrome, and Grieg Cephalopolysyndactyly Syndrome. These genes are related to developmental processes in the face[19,20,23,35]. Syndromes that include telecanthus as a main phenotypic feature include: MMCAT Syndrome, and STAR Syndrome, whose genes are also related to craniofacial development in the eye[36].

Paternoster et al.[2] published the first GWAS of normal human facial shape. Using 3D photogrammetry, this study of adolescents from the UK failed to find statistical evidence of an association with intercanthal or outer-canthal distance. They did, however, report an association between variants in the *PAX3* gene and the morphology of the nasal root, located in the region between the orbits. That same year, Liu et al.[1] performed a second GWAS for facial shape in a large sample of adult individuals of European descent. These researchers used 3D MRI-based phenotyping and used nine well- characterized landmarks involving the innercanthi and outer-canthi. They reported an association between *TP63* and horizontal distance between the left and right soft-tissue orbits. They also reported an association between *PAX3* and the distance between nasion (a midline point on the nasal root) and the orbits laterally.

A more recent GWAS by Shaffer et al.[3] examined normal facial traits in approximately 3,000 individuals from the FaceBase Consortium's 3D Facial Norms dataset and identified two significant loci for intercanthal distance: one at 1p13.3 and another at Xq13.2[3]. These loci were in

a region that included genes that cause syndromes that include hypertelorism/telecanthus as a phenotypic feature. Their findings suggest that there is a genetic contribution to interorbital distances and the relevant genes may be involved in hypertelorism/telecanthus-related syndromes.

The aim of this study is to determine whether forty selected genes implicated in hypertelorism/telecanthus-related syndromes contribute to normal variation of intercanthal and outer-canthal distances. We hypothesize that variants found through this biological candidate gene approach will uncover the genetic contributions of interorbital distances.

3.1.2   Methods

3.1.2.1 Study Population

Our study cohort is comprised of 2,447 participants, ages 3-49 and self-reported as of European ancestry. The vast majority of these individuals were recruited through the 3D Facial Norms (TDFN) Project[61]. The project was initiated in 2009 is a craniofacial normative dataset that consists of 3D facial images and genomic data, all accessible as a web-based application[61]. These participants were recruited through several types of targeted advertising in Pittsburgh, Pennsylvania; Seattle, Washington; Houston, Texas; and Iowa City, Iowa. Informed consent was provided by all participants. A demographic questionnaire was administered to capture self-reported age, sex, height, weight, and ancestry. A saliva sample was obtained using Oragene collection kits (DNA Genotek Inc., Ontario, Canada).

Five craniofacial measures were obtained using spreading calipers. These measures included maximum cranial width, minimum cranial width, minimum frontal width, maximum facial width, mandibular width and maximum cranial length. Then, participant's 3D facial images were captured with a two-pod 3dMD surface imaging technology (Atlanta, GA). All participants

were asked to remove jewelry or accessories that would interfere with capturing the image. The participant's hair was pinned back when necessary to prevent interference with the land marking. Selected landmarks were labeled directly on the participant's face using skin-safe markers. Those landmarks included tragion, gnathion, and pronasale, which facilitated the rest of the landmark process. The participant was facing the system with his/her head tilted slightly back to ensure capture of the chin. Instructions given to participants included to keep eyes open and mouths closed with a relaxed face. Twenty-nine measurements were taken at the time of capture.

Trained raters evaluated each image for quality, standard age and sex Z-scores were compared to collected images and Z-scores of greater than 3 or less than -3 were flagged for review to evaluate potential errors in landmark placement.

3.1.2.2 Genotype Data

Participants in the 3DFN Database have been genotyped using a genome-wide association array consisting of 964,193 single-nucleotide polymorphisms (SNPs) (Illumina OmniExpress+Exome v1.2) plus an additional 4,322 custom SNPs chosen based on prior craniofacial genetic studies. The genetic dataset has been imputed using the 1000 Genomes reference panel (phase 3) and quality checked according to protocols developed at the University of Washington Center for Inherited Disease Research (CIDR) Genetics Coordinating Center[3].

3.1.2.3 Candidate Gene Selection

Forty genes were selected based on their role in syndromes with hypertelorism or telecanthus as a primary feature. The candidate genes were found by combining search terms "hypertelorism," "wide set eyes," "telecanthus," and "syndrome" in Online Mendelian Inheritance in Man (OMIM)[62]. Further, the terms "hypertelorism" and "telecanthus" were used as search terms

in the Hereditary Ocular Disease Database from the Univeristy of Arizona[63]. Syndromes were also considered from Smith's Recognizable Pattern of Human Malformation[64] where greater than 50% of individuals were found to have hypertelorism/telecanthus, or were considered a "characteristic feature" of the syndrome.

Exclusion criteria included syndromes that did not have a genetic etiology per OMIM, syndromes that had less than fifteen reported cases in the literature, and syndromes that include epicanthal folds in addition to telecanthus, because epicanthal folds can be a confounding factor when identifying telecanthus. Hypertelorism-related genes that have already been identified per Shaffer et *al.*[3] were not included in these candidate genes, as they have already been identified. Selected genes for each phenotype can be found in Table 3 and Table 4.

Table 3. Candidate Genes Selected for Hypertelorism

Gene	Syndrome
ACTB	Baraitser-Winter Syndrome 1
ACTG1	Baraitser-Winter Syndrome 2
ANKH	Craniometaphyseal dysplasia
COL11A1	Marshall Syndrome
COLEC11/MASP1	3MC syndrome
EFNB1	Craniofrontonasal Syndrome
EHMT1	Kleefstra syndrome
ESCO2	Roberts Syndrome
EZH2	Weaver Syndrome
FGD1	Aarskog Syndrome, X- Linked
FGFR2	Apert/ Crouzon Syndrome
FLNA	Otopalatodigital Spectrum Disorders
FREM1	Manitoba oculotrichoanal syndrome
GLI3	Grieg Cephalopolysyndactyly Syndrome
GPC3	Simpson-Golabi-Behmel syndrome
KIF7	Acrocallosal Syndrome

Table 3 Continued

LRP2	Donnai-Barrow Syndrome
MAP2K1/MAP2K2	Cardio-Facio-Cutaneous syndrome
MED12	Opitz Kaveggia Syndrome
MID1	X Linked Opitz G/BBB Syndrome
NOTCH2	Hajdu- Cheney Syndrome
PAX3	Craniofacial-deafness-hand syndrome/ Waardenburg 1
PEPD	Prolidase deficiency
PTPN11	Leopard Syndrome/Noonan
ROR2	ROR2- Related Robinow Syndrome
SETBP1	Schinzel-Giedion Syndrome
SPECC1L	Opitz Syndrome Type 2 (GBBB2)
TGFBR1	Loeys-Dietz Syndrome
TWIST1	Saethre- Chotzen Syndrome
TXNL4A	Burn- McKeown Syndrome
ZEB2	Mowat-Wilson Syndrome

Table 4. Candidate Genes Selected for Telecanthus

Gene	Syndrome
ADAMTS18	Microcornea, Myopia, Telecanthus and Posteriorly Rotated Ears
ANKRD11	KBG Syndrome
FAM58A	STAR Syndrome
FOXC1	Axenfield-Rieger type 3
RPS6KA3	Coffin-Lowry Syndrome
SKI	Shprintzen-Goldberg Craniosynostosis Syndrome
TWIST2	Barber-Say Syndrome

3.1.2.4 Statistical Analysis and Results Annotation

Each phenotype was adjusted for sex, age, age^2, height, weight, and facial size. 76,779 SNPs were included in this analysis, which represented each candidate gene in addition to a 200kB flanking region on either side of the gene. All selected candidate genes were tested for association with both phenotypes. Linear regression was used to test for association between each phenotype (intercanthal width and outer-canthal width) and each SNP using the additive genetic model, while adjusting for the first four principal components of ancestry. The associations were tested using the genetic software PLINK[65]. Filters for association included a minor allele frequency (MAF) of .00621 and Hardy-Weinberg Equilibrium test filter P-value less than .0001. Results were visualized by utilizing LocusZoom[66](hg19), where association signals for candidate genes were plotted with 200kB flanking regions.

The total number of independent tests was determined to be 12,351, per Li and Ji[67], which corresponds to a study-wide p-value threshold of $p=4.05 \times 10^{-6}$ (Bonferroni, .05/12351 SNPs). However, all suggestive SNPs ($p<10^{-4}$) found within the 200kB flanking region and the selected candidate gene were annotated. The browsers that were used to collect significant functional and regulatory information were: 1000 Genomes, ClinVar, Exome Variant Server (ESP), ExAc, HaploReg, UCSC Genome Browser, Variant Effect Predictor (VEP), and dbSNP. All SNPs in high LD ($r^2>.80$) were also annotated through HaploReg to investigate potential regulatory function for the candidate gene.

3.1.3 Results

The results of the statistical analysis and gene annotation are organized by phenotype. While the results are reported separately by phenotype, we suspect that the genetic associations

are not necessarily specific to only one measurement. Our goal was to identify statistically significant SNPs within candidate genes that play a role in syndromes related to hypertelorism and telecanthus. In addition, our goal was to hypothesize what potential role these variants have in the development of interorbital distances.

3.1.3.1 Intercanthal Width Candidate Gene Analysis Results

The plot of intercanthal distance results is shown in Figure 1. The horizontal line represents the Bonferroni correction p-value threshold of 4.05×10^{-6} and the dotted line represents the suggestive p-value ($p < 10^{-4}$).
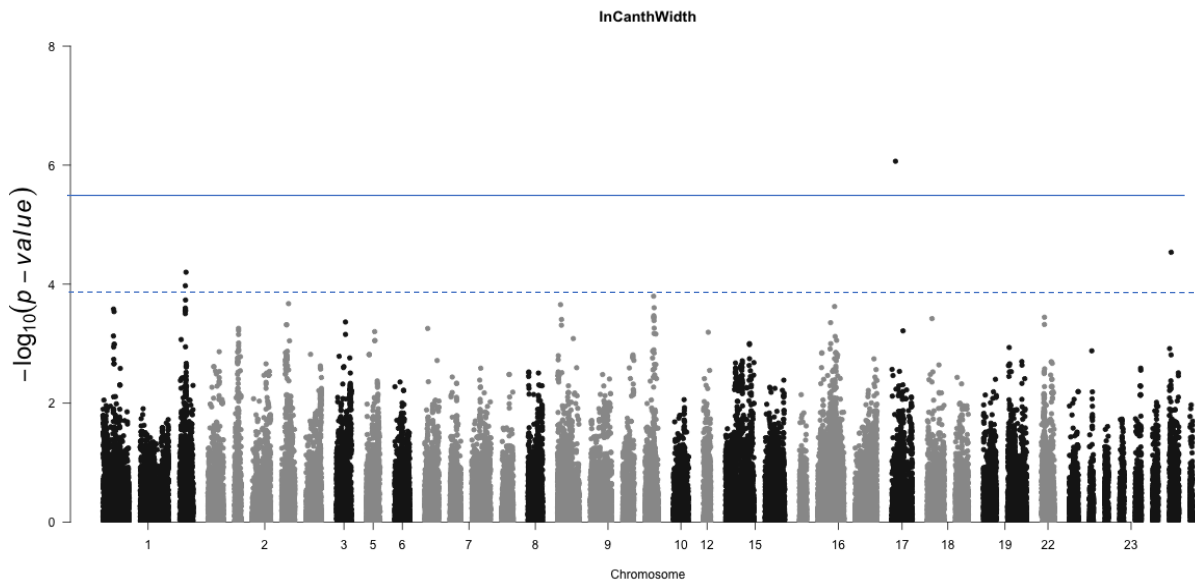

Figure 1: Intercanthal Distance Candidate Gene Analysis Plot

Table 5. Significant and Suggestive SNPs found Correlated to Intercanthal Distance

Candidate Gene	SNP	P	Chr	Distance from Candidate Gene
NOTCH2	rs200828254	6.29E-05	1:120291237	162 kB

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Table 5 Continued

ACTG1	rs116907632	8.59E-07	17:79281090	195 kB
FAM58A	rs9782761	2.91E-05	X:152660491	192 kB

The notable SNPs associated with intercanthal width are shown in Table 5. One of these SNPs, rs116907632, was statistically significant ($p=8.59 \times 10^{-7}$). This SNP was determined to be a single SNP with minimal information on its potential functionality in the candidate gene region. It was found to be within a long interspacing non-coding RNA segment, *LINC00482*. A suggestive SNP, rs9782761, was found to be over a recombination peak on chromosome X, which restricts the potential for annotating this result. However, the suggestive SNP found on chromosome 1 (rs200828254) is within *HMGSC2,* a gene upstream of the candidate gene, *NOTCH2*. No linkage disequilibrium or regulatory information is available to determine the involvement of this genetic region.

3.1.3.2 Outer-canthal Width Candidate Gene Analysis Results

The plot of outer-canthal distance results is shown in Figure 2. The horizontal line represents the Bonferroni correction p-value= 4.05×10^{-6} and the dotted line represents the suggestive p-value ($p < 10^{-4}$).
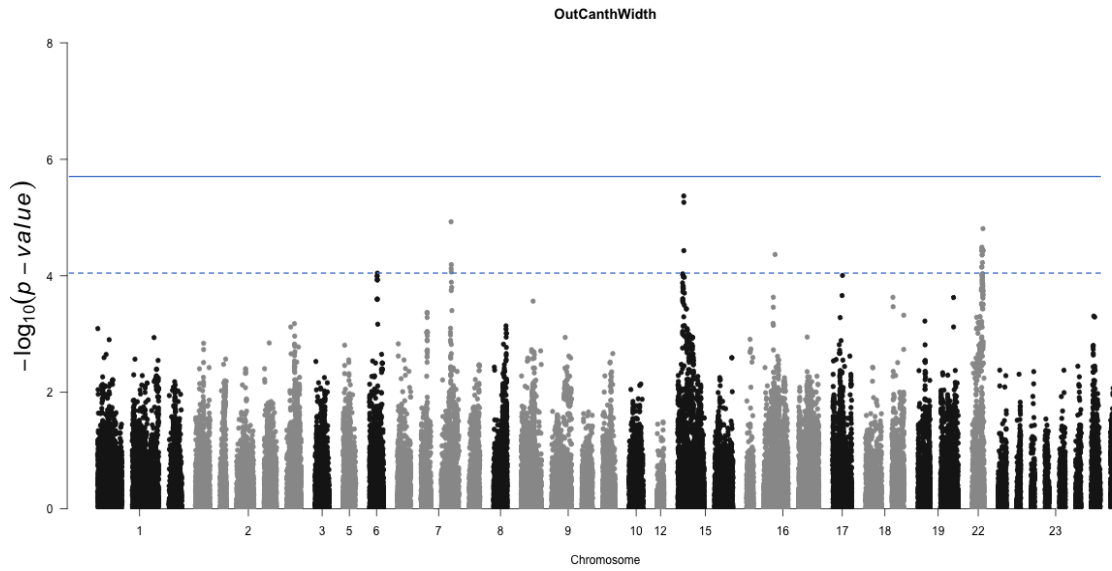
OutCanthWidth

Figure 2: Outer-canthal Candidate Gene Analysis Plot

Table 6. Suggestive SNPs Found Correlated to Outer-Canthal Distance

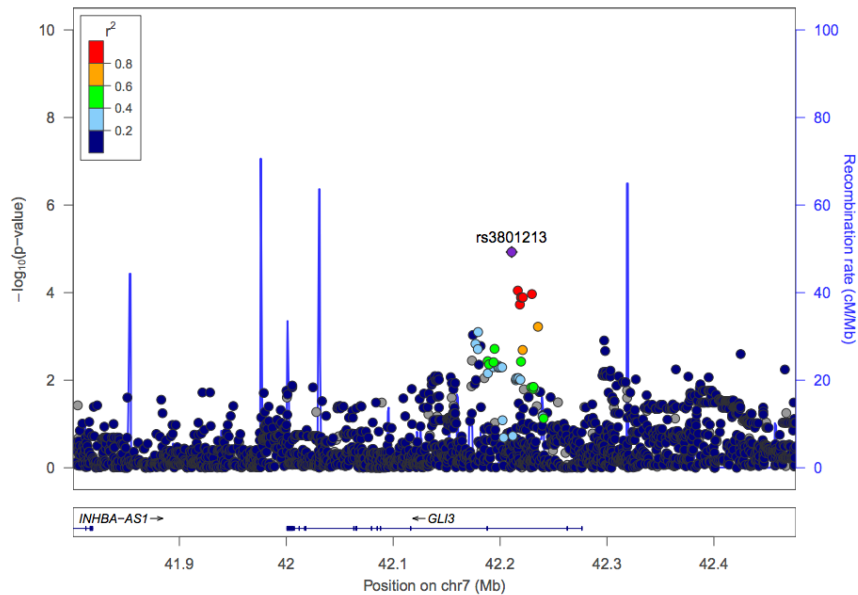| Candidate Gene	SNP	P-value	Chr	Distance from Candidate Gene
GLI3	rs3801213	1.18E-05	7:42210825	within gene
MAP2K1	rs16949689	4.26E-06	15:66496865	182 kB
ADAMTS18	rs76377892	2.54E-05	16:77365550	within gene
ACTG1	rs8064532	9.85E-05	17:79479469	within gene
SPECC1L	rs146084507	1.55E-05	22:24963351	149 kB

Figure 3: LocusZoom Plot for Significant SNP Associated with Outer-Canthal Distance

Figure 3 shows the suggestive SNP (p=1.18x10^{-5}) association with outer-canthal distance observed for rs3801213 within the *GLI3* gene. *GLI3* is a gene that functions in the sonic hedgehog (Shh) signal transduction pathway. The protein product is responsible for activating Ptc expression, a receptor for Shh[35]. Mutations in this gene cause Greig Cephalopolysyndactyly syndrome, a syndrome characterized by hypertelorism, craniosynostosis, postaxial polydactyly, syndactyly, and in some cases, agenesis of the corpus callosum[35]. A SNP in high LD with rs3801213 is rs3823731 (r^2=0.81), whose alternate allele increases Smad3 binding site score per HaploReg. Smad3 is a transcriptional regulator that is involved in responses to the TGF-β pathway. Mutations in *SMAD3* result in Loyes-Dietz Syndrome type 3, is a genetic connective tissue disorder with craniofacial abnormalities including hypertelorism and an abnormal uvula[68]. This suggests that *SMAD3* plays a role in craniofacial morphology and may contribute to facial patterning.
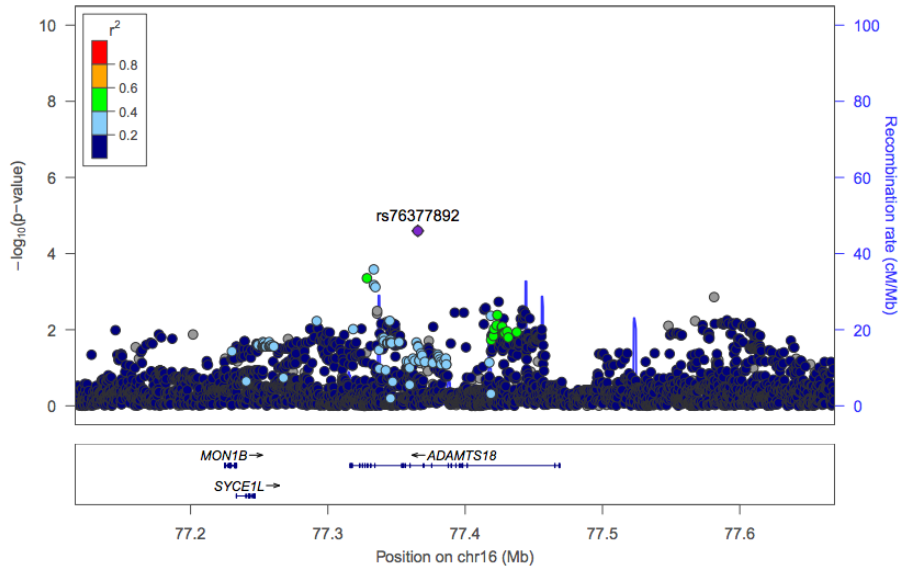
Figure 4: LocusZoom Plot for Significant SNP Associated with Outer-Canthal Distance

Figure 4 shows the suggestive SNP (p=2.54x10^{-5}) association with outer-canthal distance observed for rs76377892 within the *ADAMTS18* gene. This gene functions as a zinc-dependent protease that is anchored in the extracellular matrix and is important for a number of cellular functions[37]. Mutations in this gene cause MMCAT Syndrome, which is characterized by microcornea, myopic chorioretinal atrophy, and telecanthus[37]. This protein is actively expressed in multiple brain tissues and has been previously been reported in the lens of E12.5 mouse embryos[69].

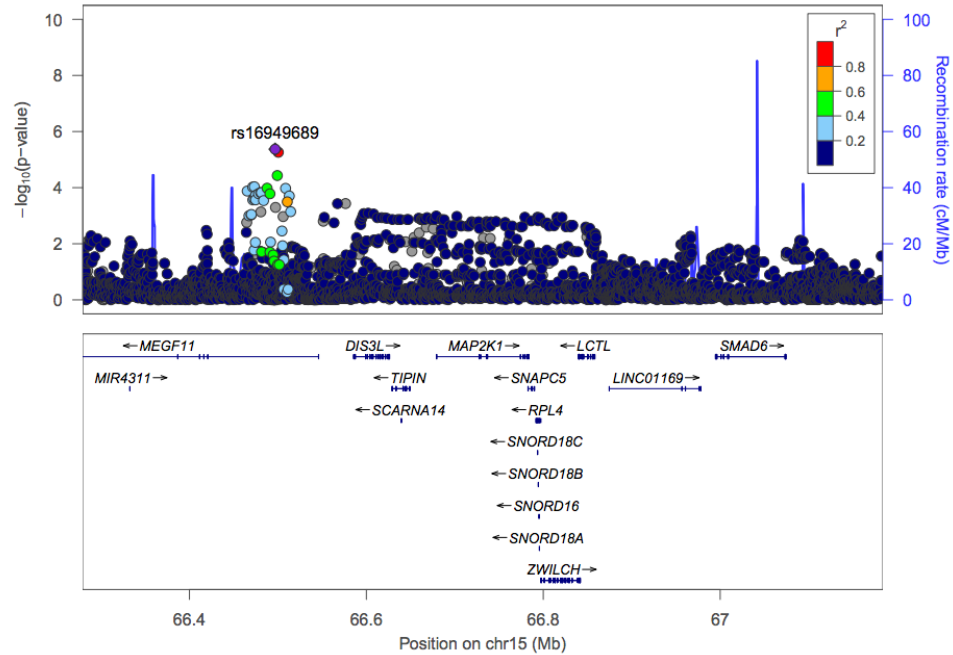**Figure 5: LocusZoom Plot for Significant SNP Associated with Outer-Canthal Distance**

Figure 5 shows the suggestive SNP (p= 4.26×10^{-6}) association with outer-canthal distance observed for rs16949689 within the *MEGF11* gene. This gene is upstream of the chosen candidate gene, *MAP2K1*. *MEGF11* is a gene involved in mosaic spacing of neuron subtypes in the retina during eye development[70].
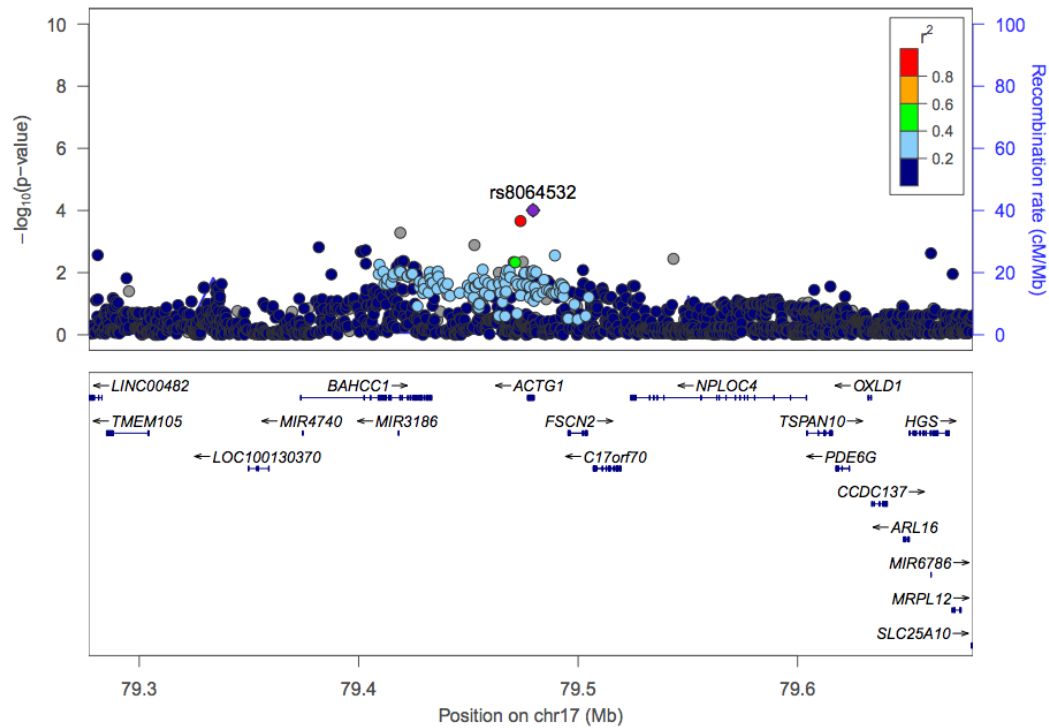
Figure 6: LocusZoom Plot for Significant SNP Associated with Outer-Canthal Distance

Figure 6 shows the suggestive SNP (p=9.85x10^{-5}) association with outer-canthal distance observed for rs8064532 within to the *ACTG1* gene. One transcription factor that binds to this region is SMARCA4. This transcription factor is thought to play a role in proliferation of neuronal stem cells by making stem cells unresponsive to differentiation per sonic hedgehog[71]. The exact mechanism in relation to Shh signaling is unknown, but it is possible that is has a relationship with facial patterning in development.

This SNP is found in a regulatory region that significantly increases the prediction score of HIC1, a regulatory motif related to craniofacial development. This regulatory motif is involved in outgrowth of peripheral nerves, but is noted mainly for its hypermethylated state in cancer[72].
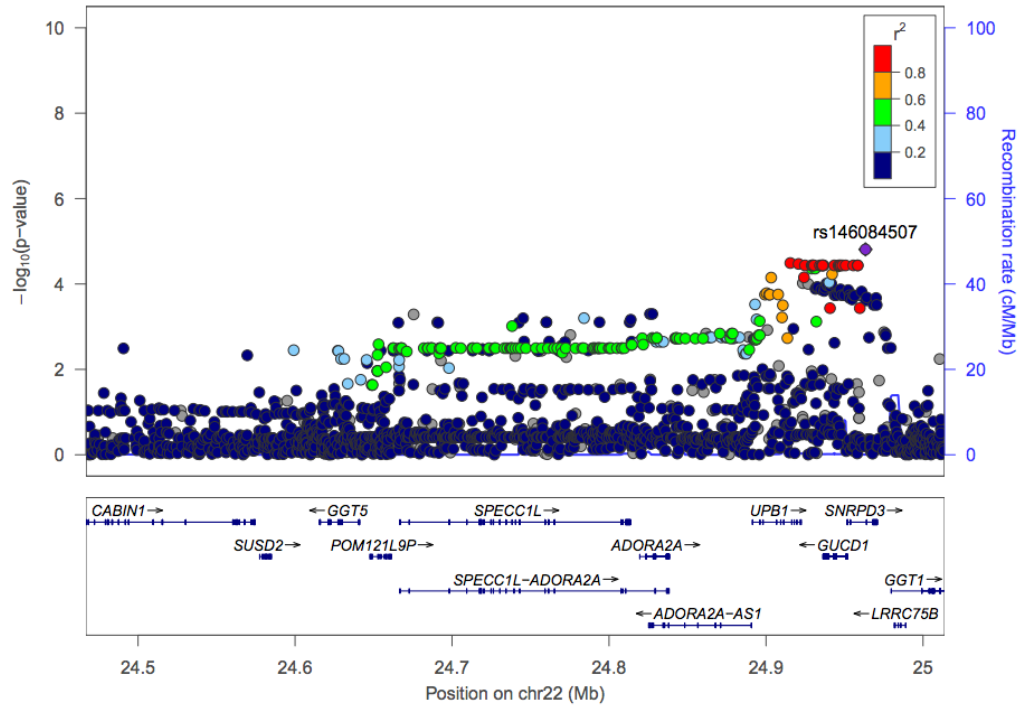
Figure 7: LocusZoom Plot for Significant SNP Associated with Outer-Canthal Distance

Figure 7 shows the suggestive SNP (p=1.55x10^{-5}) association with outer-canthal distance observed for rs146084507 within the *SNRPD3* gene. This gene is downstream of the chosen candidate gene, *SPECC1L*. SNRPD3 gene function is related to pre-mRNA splicing in a spliceosome complex[73].

A SNP in high LD with rs146084507 is rs75992726 (r^2=1.0), whose alternate allele increases the prediction score for the AP-1 motif per HaploReg. AP-1 activity is influenced by MAPK proteins, two of which, when mutated, are known to cause Cardio-facio-cutaneous syndrome[74]. Craniofacial malformations such as hypertelorism are implicated in this polygenic syndrome[74].

A second SNP in high LD with rs146084507 is rs190826971 (r^2=1.0), whose alternate allele increases the prediction score for the GLI motif. GLI is a transcription factor that is activated by Shh signal transduction cascade and regulates stem cell proliferation[44]. Knowing that this SNP

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impacts GLI function is suggestive that the region surrounding this SNP is important for interorbital distances.

3.1.4 Discussion

Hypertelorism and telecanthus are common features seen in many genetic syndromes. Though current research is focused on high impact sequence variants that cause extreme phenotypes along a defined spectrum of severity, little research exists regarding normal genetic variation of common facial features. Twin and family studies have established that there is a relatively high heritability for interorbital spacing[59,60], though the specific genetic factors are still poorly understood. In this study, we performed a candidate gene study to detect potential genetic variants associated with two phenotypes: intercanthal width and outer-canthal width. We successfully identified one statistically significant loci and seven suggestive variants within genes that are known to cause syndromes specifically related to hypertelorism and telecanthus. Out of the suggestive SNPs found, three are within their respective candidate gene. All other variants were found in the flanking regions surrounding the candidate gene. Although these SNPs do not impact protein structure, they may have a functional role impacting facial structure in subtle ways.

Interorbital distances are known to vary between ethnic groups[54,55,56,57,58]. This is significant because it suggests that regardless of age and sex, individuals from different ethnic groups have different interorbital norms. This suggests that genetic factors may influence the difference between ethnic groups. Largely, the specific genetic differences are unknown, but it could be hypothesized that similar genetic factors that contribute to ethnic differences in skull and bone development could influence interorbital distances. Twin studies using advanced technologies such as 3D stereophotogrammetry have estimated interorbital distances to be

approximately 40%[60], meaning a moderate number of genetic factors exist that contribute to interorbital distances.

To examine the specific genetic factors that contribute to normal facial variation, several published GWASs have uncovered loci that are implicated for these phenotypes. Paternoster et al.[2] published the first GWAS of normal human facial shape. Using 3D photogrammetry, this study of adolescents from the UK failed to find statistical evidence of an association with intercanthal or outer-canthal distance. They did, however, report an association between variants in the *PAX3* gene and the morphology of the nasal root, located in the region between the orbits. That same year, Liu et al.[1] performed a second GWAS for facial shape in a large sample of adult individuals of European descent. These researchers used 3D MRI-based phenotyping and used nine well- characterized landmarks involving the innercanthi and outer-canthi. They reported an association between *TP63* and horizontal distance between the left and right soft-tissue orbits. They also reported an association between *PAX3* and the distance between nasion (a midline point on the nasal root) and the orbits laterally. A GWAS for human facial variation in approximately 6,000 Latin American individuals identified a variant in *GLI3* that was statistically significant ($p=9x10^{-9}$) for nose bridge breadth, which is a similar measurement to intercanthal distance[75]. This shows that our discovery of a variant in *GLI3* coincides with recently published literature and that there seems to be a consistent correlation with *GLI3* variants and the frontonasal process. A more recent GWAS by Shaffer et al. examined normal facial traits in approximately 3,000 individuals from the FaceBase Consortium's 3D Facial Norms dataset and identified two significant loci for intercanthal distance: one at 1p13.3 and another at Xq13.2 [3]. These loci were in a region that included genes (*ALX3* and *HDAC8,* respectively*)* that cause syndromes that include hypertelorism/telecanthus as a phenotypic feature. This research team suggested that the functional

variants found in or around candidate genes would be related to regulatory elements that may impact candidate genes. In our research project, we selected the candidate genes to determine if significant or suggestive variants were indeed in the regulatory elements as hypothesized. This project adds to the previously published literature in that it agrees with the ongoing hypothesis Shaffer et *al.* suggested. With this being said, additional research needs to be done to determine if this is the case in other facial phenotypes.

A limitation of this study is understanding the SNPs that were identified in this study are not necessarily causal variants and may not be related to the candidate gene. The SNPs found in this study were found to be in LD with many other SNPs, some of which may have not been identified because of the small window flanking the selected candidate genes. Eventually, functional analysis of the gene and causal variant will be necessary to understand the specific biological mechanisms involved.

Another limitation of this study includes selection bias. We selected a small group of genes that have a known association with hypertelorism or telecanthus. Another approach to selecting candidate genes may be from a biological pathway perspective: selecting genes known to be involved in the sonic hedgehog pathway, for example. Our approach may have missed other underlying genetic factors that may not be directly linked to hypertelorism or telecanthus, but related to other clinical features of a selected syndrome.

Despite these limitations, this is one of few studies to report significant associations between common genetic variants and interorbital spacing in an unaffected population. This project emphasizes the polygenic nature underlying the complexities of craniofacial development. It is likely that many proteins contribute to interorbital distance and embryonic development is a

highly regulated process that involves many proteins. However, this analysis begins to uncover more about craniofacial development and normal facial morphogenesis.

3.1.5   Conclusion

In summary, this study aimed to identify genetic variants that contribute to normal variation in interorbital spacing in an unaffected cohort. Our hypothesis was that variants in the regulatory regions of candidate genes related to hypertelorism and telecanthus contribute to the interorbital distances in unaffected individuals. To identify these variants, forty candidate genes were selected based on their association with syndromes that include hypertelorism and telecanthus as a phenotypic feature. Association tests were conducted comparing SNPs to two phenotypes: intercanthal distance and outer-canthal distance. One statistically significant locus ($p<4.05\mathrm{x}10^{-6}$) and seven suggestive loci ($p<10^{-4}$) were identified in total. Associated loci included several genes with plausible roles related to interorbital distances, such as *ADAMTS18*, *GLI3*, *ACTG1*, *MEGF11*, and *SPECC1L*. Implicated genes may have significant roles throughout craniofacial development. Identifying genetic contributors for interorbital distances for a normal population may make it easier to identify individuals with more severe phenotypes. This study allowed us to better understand the more normative side of the disease spectrum and to begin to characterize what genes are at play throughout normal craniofacial development.

4.0   RESEARCH SIGNIFICANCE TO GENETIC COUNSELING AND PUBLIC HEALTH

This research study provides information that has the potential to be applied to the clinical genetics arena. For example, the type of data generated by this research can lead to information that would allow better characterization of the genetic contributions of facial structure in affected and unaffected individuals. The results of this study help us identify the genetic contributions of variation in the face. This can serve two purposes: first, it begins to allow for the identification of the normal side of phenotypic variation, which ultimately, can be compared to the disease spectrum, and second, it will aid in the detection of the differences between individuals diagnosed with disease.

In general, there is often an overall gestalt for a particular genetic syndrome, because the same genetic defect is responsible for the syndrome. However, there is commonly clinical variability in individuals who have the same genetic diagnosis. We usually attribute this to other genetic factors that are not as well understood. For intercanthal and outer-canthal distances, our research has begun to uncover potential genetic contributions that exist in the population. It is possible that our study will lead to identifying genetic differences that make two individuals with the same genetic disorder different from one another in that some individuals may have these common variants in addition to a monogenic genetic condition. This can begin to uncover what makes two individuals with the same genetic condition appear different from one another.

In addition, this study better characterizes the genetic contributions of interorbital distances in unaffected individuals, which may help clinical geneticists and dysmorphologists better understand how interorbital distances vary in unaffected people. With whole exome and genome

sequencing becoming rapidly incorporated into the clinical setting, this research study could provide information when interpreting a genetic test report and provide for a better identification of individuals who may not be clinically affected with hypertelorism or telecanthus, but have eyes that are wider than expected. Ultimately, this may aid in more precise clinical diagnoses.

Genetic counselors strive to provide genetic information to their patients in a way that is understandable. However, when complexities that influence the risks for a craniofacial deformity are brought into the conversation, it can complicate the conversation. For example, parents who have a child with a cleft lip and/or palate have a tendency to be mildly hyperteloric[76]. However, emphasizing that there are many genetic factors that contribute to how the face develops is important to convey. An explanation would include the challenges that genetics professionals face when trying to understand factors that can influence recurrence risk, but could also address the recognition of the relationship between parents having hypertelorism and the possible increased risk of having a child with a cleft. However, more research would need to confirm these findings and to translate them into the clinical setting. Additional research that identifies genetic contributions to facial structure could eventually lead to more tailored quantitative risks that a genetic counselor could provide to his/her patients.

Polygenic inheritance should be explained in a manner that reflects the complexities of craniofacial development in addition to the caveats of recent research findings. Concepts that are also highlighted by these results are variable expressivity and reduced penetrance, both of which address how individuals exhibit disease in different ways.

In summary, interorbital distances can provide clues in diagnosing genetic syndromes and the results of the study are significant for identifying genetic variants that may contribute to these distances. As this research continues, the results may create a better definition of interorbital

distances that are considered abnormal and associated with a syndromic presentation. As genetic factors that contribute to hypertelorism continue to be discovered, interorbital distances may be useful in providing more tailored risk estimates for having a child with a midline defect such as a cleft lip and/or palate.

This study uncovers significant public health implications in genetics moving forward. Public health interventions attempt to address three core functions: assessment, policy development and assurance. Assessment is the act of monitoring and diagnosing health concerns. In public health genetics, the target is diagnosing and managing genetic disorders. Policy development is focused around creating policy that attempt to address a created public health intervention based on a need. Often, policy development in public health genetics is focused around genetic testing to better diagnose individuals with genetic conditions. The last core function of public health is assurance, which addresses whether the public health intervention or developed policy is functioning as it should. This function of public health in genetics is associated with ensuring that there are enough genetic healthcare professionals that are accessible to the public, creating resources for individuals diagnosed with genetic conditions, and ensuring the policy created is fair, standardized and equally accessible to the public.

The results from this project mainly apply to the first core function of public health: assessment. These results apply to better diagnosing individuals with a genetic condition. As mentioned above, clinical exome and genome sequencing are quickly being incorporated into clinical genetics. Sometimes, these genetic test results are of unknown clinical significance, which hinders clinical correlation to patients and families. This research project has increased the quality of our control group, which allows us to better determine pathogenic variants from benign variants. This is of utmost importance when diagnosing individuals with a genetic disorder and provides

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more clarity to the patients that are seen in a clinical setting. The results of this project are not readily applicable to the other two core functions of public health, policy development and assurance. With more research over time that can more readily explain causal variants that contribute to normal craniofacial morphology, policy could be developed to ensure that all individuals are protected from or have access to genetic tests that may provide information on the genetic contributors of the face. Potentially, this may apply to genetic-related vanity traits, which may be associated with public health in the future. However, more research would need to emphasize the true genetic contribution to craniofacial morphology, which is why assessment is of utmost importance in relation to this project. The more information confirmed and replicated over time, the better we can create policy and public health interventions that can improve the overall health of the population.

APPENDIX: 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.

https://www.osiris.pitt.edu/osiris/Doc/0/66SJIPT8380KLBANU03BGIJ969/fromString.html   9/15/2016

University of Pittsburgh
Institutional Review Board

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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.

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

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