

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

by

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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 *SPECCIL*. 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

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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 variation¹⁻³. 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 eyes³. 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 wall⁴. 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 phenotypes⁵, 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 telecanthus⁴. Consequentially, there is not an increased interpupillary distance in telecanthus⁸. Increased intercanthal distance appears to be due to an increased amount of soft tissue between the innercanthi⁸. 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” appearance⁸. 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 other⁹. 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 distance¹⁰. 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 differences¹⁰. 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 body¹¹. 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 outcomes¹². 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 placement¹³. The landmarks of interest are intercanthal distance, which is described as the distance between the inner corners of the eye¹⁴ and outer-canthal distance, which is the distance between the outside corners of the eye¹⁴. 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 still¹⁴.

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 process¹⁵. 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 factors³.

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 displaced¹⁴. 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 are¹⁶. 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 hypertelorism¹⁷. 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 hypertelorism¹⁷. 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 telecanthus¹⁶.

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 face¹⁸. 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 reduced¹⁵. These images can also be saved and accessed at later points in time¹⁵. 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	<i>ALX3</i>	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	<i>ALX4</i>	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	<i>PAX3</i>	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	<i>FGFR2</i>	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	<i>PTPN11</i> (50%), <i>SOS1</i> , <i>BRAF</i> , <i>MAP2K1</i> , <i>RAF</i> , <i>RIT1</i> , <i>KRAS</i>	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	<i>TWIST1</i>	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	<i>MID1</i>	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	<i>EHMT1</i>	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	<i>EFNB1</i>	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	<i>GLI3</i>	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	<i>ADAMTS18</i>	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	<i>SKI</i>	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	<i>TWIST2</i>	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	<i>FAM58A</i>	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 development¹.

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 mesenchyme⁴³. 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 established⁴³. By embryonic day 9, CNCC's are fated and in the necessary location to begin differentiation⁴³.

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 other⁴³. 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 adulthood⁴³. 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 face⁴⁹. 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 fissures⁵⁰. Disturbances of Shh have also been shown to be correlated with decreased cell proliferation⁴⁶ or premature cell death of CNCC's⁵¹. 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's⁵². Primary cilia are responsible for mediating the cell's interactions with its environment⁵². 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's⁵². Excess Shh in the facial ectoderm results in increased midline features such as hypertelorism⁵². 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}.

To evaluate whether Shh was truly affecting craniofacial development, specifically, the frontonasal process (where orbital distances are measured), Hu and Helms⁵³ 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 ear⁵³. This ectopic expression of Shh induced *BMP2*, *PTC*, and *GLII* in the ectoderm⁵³. 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 concentration⁴⁵.

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 ability of 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 Xq13.2³. 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 innercanthi⁸.

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 established⁴³, 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 Helms⁵³ 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 ear⁵³. 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 concentration⁴⁵. 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 eye³⁶.

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 Xq13.2³. 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) Project⁶¹. 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 application⁶¹. 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 landmarking. Selected landmarks were labeled directly on the participant's face using skin-safe markers. Those landmarks included trignon, 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 Center³.

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 University of Arizona⁶³. Syndromes were also considered from Smith’s Recognizable Pattern of Human Malformation⁶⁴ 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
<i>ACTB</i>	Baraitser-Winter Syndrome 1
<i>ACTG1</i>	Baraitser-Winter Syndrome 2
<i>ANKH</i>	Craniometaphyseal dysplasia
<i>COL11A1</i>	Marshall Syndrome
<i>COLEC11/MASP1</i>	3MC syndrome
<i>EFNB1</i>	Craniofrontonasal Syndrome
<i>EHMT1</i>	Kleefstra syndrome
<i>ESCO2</i>	Roberts Syndrome
<i>EZH2</i>	Weaver Syndrome
<i>FGD1</i>	Aarskog Syndrome, X- Linked
<i>FGFR2</i>	Apert/ Crouzon Syndrome
<i>FLNA</i>	Otopalatodigital Spectrum Disorders
<i>FREMI</i>	Manitoba oculotrichoanal syndrome
<i>GLI3</i>	Grieg Cephalopolysyndactyly Syndrome
<i>GPC3</i>	Simpson-Golabi-Behmel syndrome
<i>KIF7</i>	Acrocallosal Syndrome

Table 3 Continued

<i>LRP2</i>	Donnai-Barrow Syndrome
<i>MAP2K1/MAP2K2</i>	Cardio-Facio-Cutaneous syndrome
<i>MED12</i>	Opitz Kaveggia Syndrome
<i>MIDI1</i>	X Linked Opitz G/BBB Syndrome
<i>NOTCH2</i>	Hajdu- Cheney Syndrome
<i>PAX3</i>	Craniofacial-deafness-hand syndrome/ Waardenburg 1
<i>PEPD</i>	Prolidase deficiency
<i>PTPN11</i>	Leopard Syndrome/Noonan
<i>ROR2</i>	ROR2- Related Robinow Syndrome
<i>SETBP1</i>	Schinzal-Giedion Syndrome
<i>SPECC1L</i>	Opitz Syndrome Type 2 (G BBB2)
<i>TGFBR1</i>	Loeys-Dietz Syndrome
<i>TWIST1</i>	Saethre- Chotzen Syndrome
<i>TXNL4A</i>	Burn- McKeown Syndrome
<i>ZEB2</i>	Mowat-Wilson Syndrome

Table 4. Candidate Genes Selected for Telecanthus

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

3.1.2.4 Statistical Analysis and Results Annotation

Each phenotype was adjusted for sex, age, age², 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 PLINK⁶⁵. 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 LocusZoom⁶⁶(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 Ji⁶⁷, 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 ($p < 10^{-4}$).

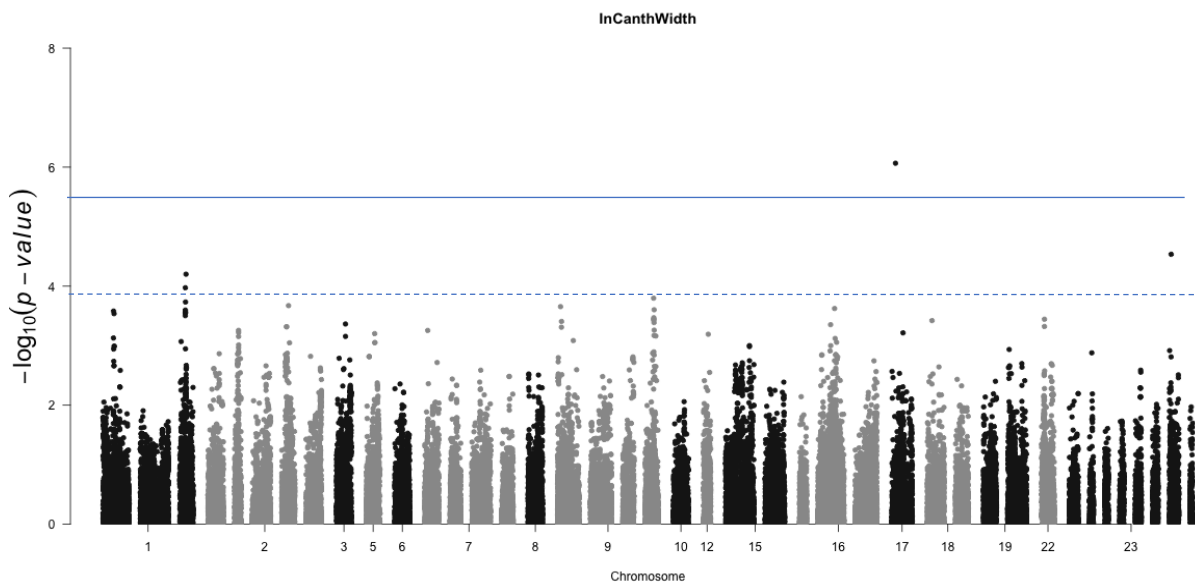


Figure 1: Intercanthal Distance Candidate Gene Analysis Plot

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

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

Table 5 Continued

<i>ACTG1</i>	rs116907632	8.59E-07	17:79281090	195 kB
<i>FAM58A</i>	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 ($p < 10^{-4}$).

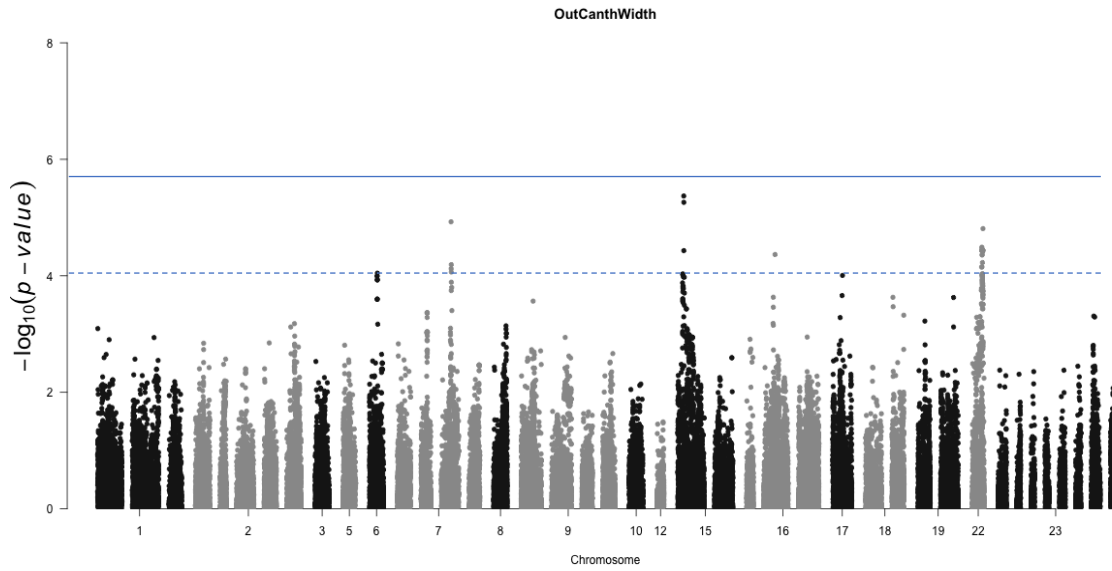


Figure 2: Outer-canthal Candidate Gene Analysis Plot

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

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

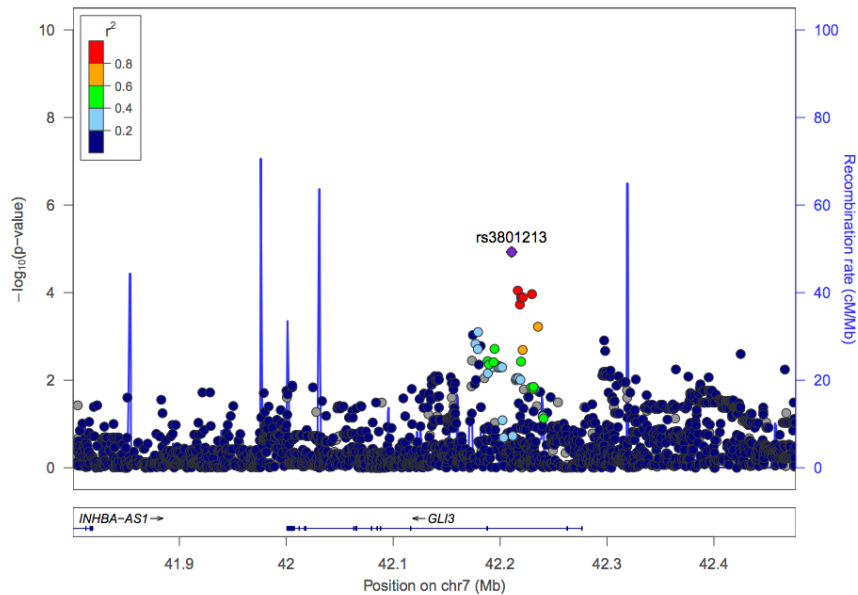


Figure 3: LocusZoom Plot for Significant SNP Associated with Outer-Canthal Distance

Figure 3 shows the suggestive SNP ($p=1.18 \times 10^{-5}$) 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 Shh³⁵. 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 callosum³⁵. 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 uvula⁶⁸. This suggests that *SMAD3* plays a role in craniofacial morphology and may contribute to facial patterning.

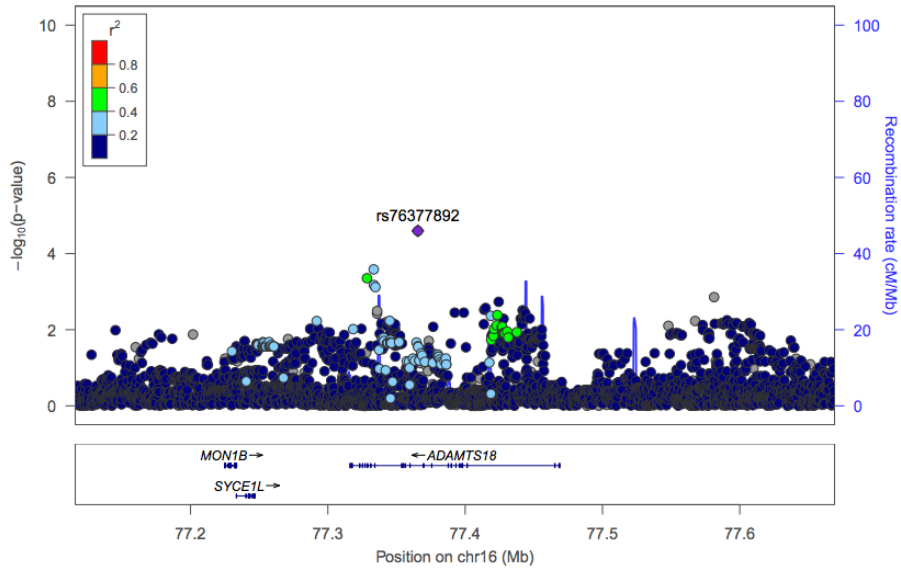


Figure 4: LocusZoom Plot for Significant SNP Associated with Outer-Canthal Distance

Figure 4 shows the suggestive SNP ($p=2.54 \times 10^{-5}$) 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 functions³⁷. Mutations in this gene cause MMCAT Syndrome, which is characterized by microcornea, myopic chorioretinal atrophy, and telecanthus³⁷. This protein is actively expressed in multiple brain tissues and has been previously been reported in the lens of E12.5 mouse embryos⁶⁹.

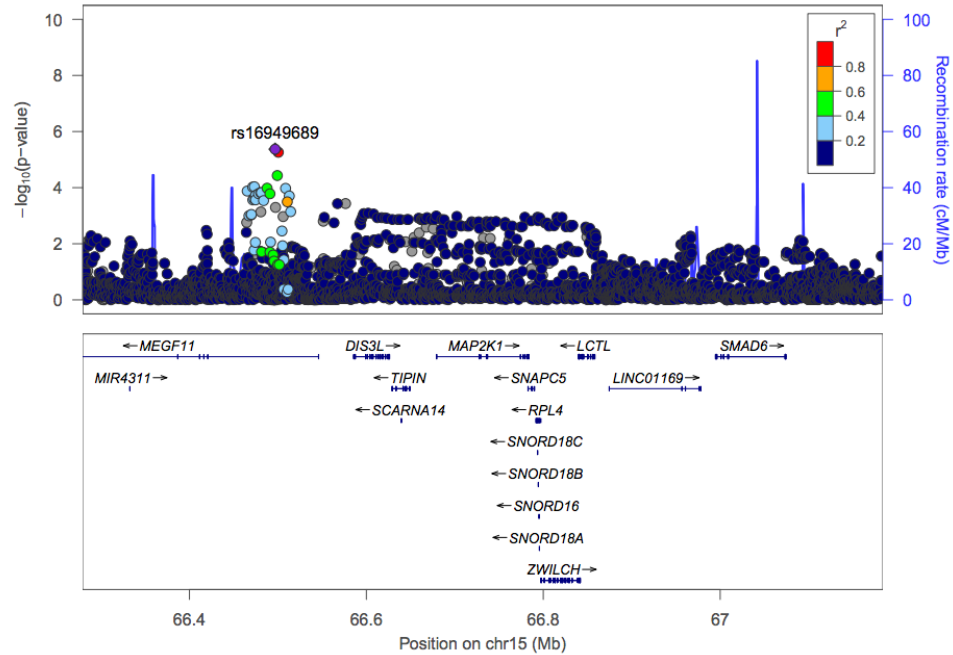


Figure 5: LocusZoom Plot for Significant SNP Associated with Outer-Canthal Distance

Figure 5 shows the suggestive SNP ($p=4.26 \times 10^{-6}$) 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 development⁷⁰.

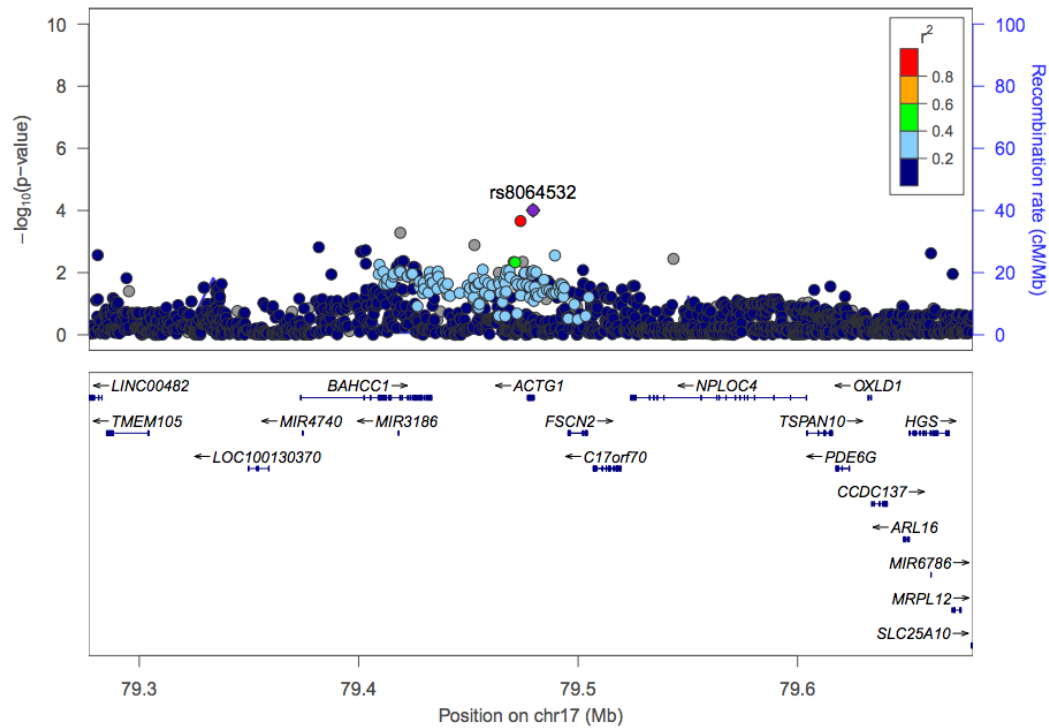


Figure 6: LocusZoom Plot for Significant SNP Associated with Outer-Canthal Distance

Figure 6 shows the suggestive SNP ($p=9.85 \times 10^{-5}$) 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 hedgehog⁷¹. The exact mechanism in relation to Shh signaling is unknown, but it is possible that it 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 cancer⁷².

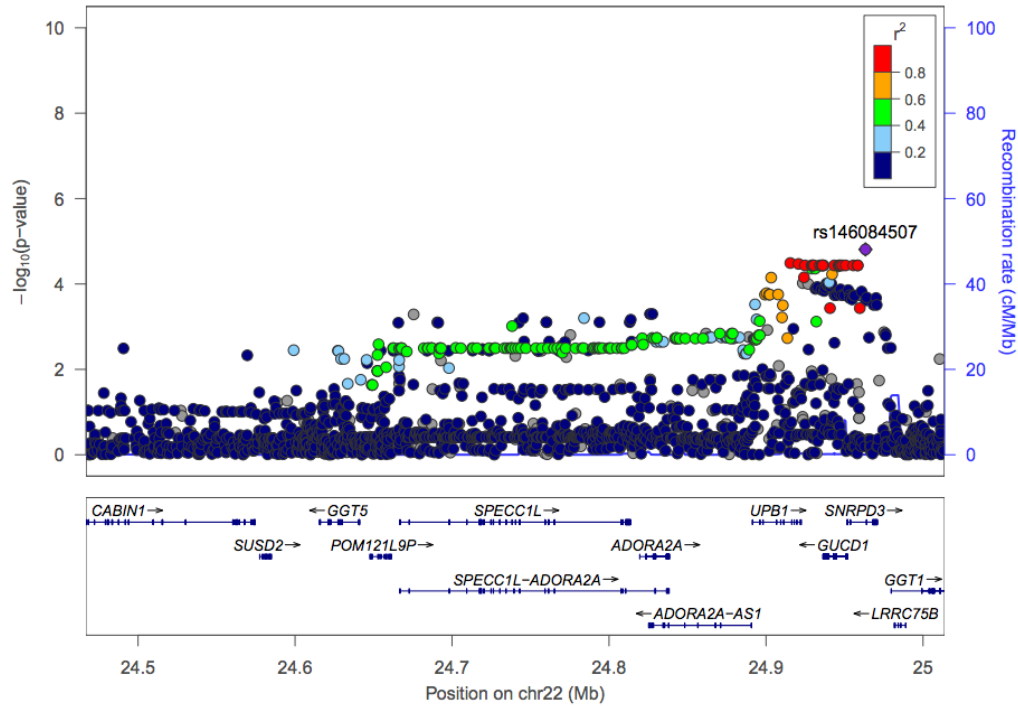


Figure 7: LocusZoom Plot for Significant SNP Associated with Outer-Canthal Distance

Figure 7 shows the suggestive SNP ($p=1.55 \times 10^{-5}$) 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 complex⁷³.

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 syndrome⁷⁴. Craniofacial malformations such as hypertelorism are implicated in this polygenic syndrome⁷⁴.

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 proliferation⁴⁴. Knowing that this SNP

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 ($p=9 \times 10^{-9}$) for nose bridge breadth, which is a similar measurement to intercanthal distance⁷⁵. 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 Xq13.2³. 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 ($p < 4.05 \times 10^{-6}$) 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 *SPECCIL*. 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 hypertelorism⁷⁶. 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

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

Page 1 of 1



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

3500 Fifth Avenue
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(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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