PURPOSE: The availability of an objective outcome measure for facial reconstructive surgery remains elusive. Evaluations submitted by external raters or by patient self-report may be influenced by expert knowledge, emotional antecedent, or implicit attitude. These types of subjective ratings, or objective measures such as anthropometric analysis, may unrelaibly convey how one is perceived by others. We are interested in observers’ instantaneous, reflexive responses to the human face, and how those instinctive responses relate to subjective judgment of a given face. We explored the visual markers that lead to differential perception of patients before and after facial reanimation surgery. By examining the early stages of visual processing that occur, we intended to measure changes in the focus of impression formation, thereby helping surgeons and their patients prioritize areas for reconstruction.

METHODS: Pre and post operation (>3 months) photographs from 10 patients with unilateral facial nerve palsy who underwent cross facial nerve graft and free gracillis muscle transfer were obtained. Twenty lookzone regions were mapped onto each facial image, reflecting aesthetic units of the face. 40 observers examined each image while an infrared eye-tracking camera continuously recorded their eye movements. The observers were then asked to rate the image for character attributes (attractiveness, trustworthiness, sociability, healthy, and capability, 1–7 scale). Factorial ANOVA and student t-test analysis was performed to determine significance of differences between groups.

RESULTS: (i) The surgical intervention was found to decrease the observers’ attention to the upper lip on the unaffected (non palsy) side, thus equalizing attention to both sides of the lips. (ii) The surgical intervention was found to significantly increase the character ratings for all five attributes compared to pre op controls: (sociable 3.2 to 3.6, trustworthy 3.42 to 3.61, attractive 2.96 to 3.2, health 3.37 to 3.54, p<0.05. (iii) For those pre-operative images of facial palsy, observers’ attention was overwhelming drawn to the area of disproportion. (iv) Our eye tracking methodology clearly reflects a trend towards normalization of gaze attention following surgical intervention. This finding was associated with the improvement in character assessment of the images in the post-op cohort of images.

CONCLUSION: We provide data illustrating a novel and objective technique to evaluate the effect of reconstructive intervention for facial palsy. This information may be used to inform patients about how these areas of facial difference/aging are perceived, and the potential effect that surgical intervention may have on others’ perception of them. This work may assist patients and their surgeons to more meaningfully focus their surgical decision-making priorities.
formed Embryoid Bodies from the iPSC’s to investigate the expression of markers of craniofacial and eye development in order to understand the potentially shared role of ALX1 in both developmental processes.

RESULTS: We identified a missense L165F variant in the homeodomain of ALX1 leading to a loss-of-function of this transcription factor. This mutation was found to be heterozygous in the parents (ALX1+/−) and homozygous in the children born with FND (ALX1−/−). Using CRISPR, we generated mutant alx1−/− zebrafish which exhibited reduced gene expression and anomalies of the craniofacial cartilage of the median palate, corresponding to the frontonasal oblique facial cleft phenotype of the subjects. The alx1−/− zebrafish also formed smaller Meckel’s cartilage, analogous to human micrognathia. We also found that ALX1 mutant zebrafish displayed strong increases in the expression of ALX3 and ALX4a at early developmental stages relative to their wild-type counterparts, likely related to a redundancy of these genes. The reprogramming process into iPSC’s from the patient’s samples as well as those of the father and the control showed no differences pointing toward a role in later development of ALX1. Through the analysis of the EB’s developed from the iPSC’s, we found that ALX1 loss of function resulted in altered expression of genes relevant to neural crest differentiation, as well as PAX6 and its target gene SIX6, both key genes of eye development.

CONCLUSION: We found that ALX1 plays a vital role in the development of both facial and ocular structures, through regulation of cranial neural crest progenitors that contribute to facial and eye structures of the frontonasal process. Identifying the genetic basis of developmental disorders such as FND allows for both a deeper understanding of molecular processes that regulate midface development. This work also highlights translation of surgical care to mechanistic discovery, by applying stem cell and CRISPR gene editing approaches, underscoring the unique advantage of craniofacial surgeon-scientists.

8

Corneal Neurotization Rescues the Corneal Epithelium from Thinning in a Rat Model of Neurotrophic Keratopathy

Kira Antonyshyn1,2, Joseph Catapano, MD, PhD1, Jennifer Zhang, MD, PhD2, Tessa Gordon, PhD2, Gregory H. Borschel, MD, FACS, FAAP1,2

PURPOSE: The cornea is one of the most densely innervated tissues in the body, and in the absence of corneal sensory innervation, patients develop Neurotrophic Keratopathy (NK). NK is a disease characterized by corneal epithelial breakdown that can lead to progressive corneal scarring and ultimately, vision loss. Corneal neurotization uses nerve autografts to restore sensory innervation in the NK cornea.1,2 In our rat model of NK, we previously demonstrated that corneal neurotization with common peroneal and sural nerve grafts reduces epithelial breakdown, prevents scarring, and improves the rate of epithelial healing in the cornea.1 Central corneal epithelial thinning is present in corneal denervation and NK. In the present study, we evaluate the effect of corneal neurotization on corneal epithelial and stromal thickness in a rat model of NK.

METHODS: Previously validated models of NK and corneal neurotization were used in this study. The experimental groups were rats with i) NK (negative control) and ii) NK treated with corneal neurotization (treatment), and the control group consisted of rats with normally innervated corneas. In the experimental groups, NK was achieved with stereotactic ablation of the ophthalmic nerve. Four weeks after ablation, affected corneas (n=5 per group) were harvested. Three 10μm cross-sections from each cornea, sampled from representative areas of the cornea, were stained with Hematoxylin and Eosin for analysis. Each section was imaged with bright-field microscopy at 200X magnification. The epithelial and stromal thicknesses of each section were measured using ImagePro. Mean thicknesses were compared using the Kruksal-Wall test and unpaired t-tests.

RESULTS: The central epithelial thickness of the NK corneas (15.83±1.62) was significantly decreased compared to that of the central epithelium in the treated (25.07±3.89, p < 0.05) and normally innervated corneas (24.75±2.46, p < 0.05), the treated and normally innervated corneas not being significantly different. In contrast, the stromal thicknesses were insignificantly different in all three groups.

CONCLUSIONS: Corneal neurotization rescues the NK cornea from central epithelial thinning, suggesting the reinervating axons of the inserted graft restore corneal epithelial integrity. We are pursuing further research with the same rat models of NK and corneal neurotization to elucidate the underlying mechanisms.