in reduced peptide secretion at day 7 (44.4 ng/ml v. 55.75 ng/ml, $p<0.01$) and day 14 (22.09 ng/ml v. 39.66 ng/ml, $p<0.05$).

Conclusion: Our findings provide strong evidence that treatment with P188 can improve adipocyte survival after electroporation-mediated gene delivery. Specifically post-electroporation treatment with P188 results in elevated secretion levels which are sustained through 2 weeks in culture. Reduced peptide secretion observed with pre-electroporation P188 may be due to poor gene delivery into the cell caused by the improved cell membrane stabilization. The inclusion of post-electroporation P188 is likely to improve the efficacy of our desired cell therapy, and may have broader impact in laboratory approaches to cell transfection.

QS73. Comprehensive Analysis of Potential Downstream Target Genes of the Interaction of Twist1 Mutation and Environmental Factors in Craniosynostosis

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Purpose: Craniosynostosis is a congenital defect characterized by the premature fusion of calvarial sutures. While the genetic basis of craniosynostosis can be identified in about a quarter of patients, the pathophysiology underlying development of this disease is mostly unknown. Mutation in *TWIST1* leads to Saethre-Chotzen syndrome with coronal synostosis. Our recent study using a *Twist1+/-* mouse model has shown evidence that environmental factors, including *in utero* exposure to the selective serotonin reuptake inhibitor citalopram, can exacerbate disease incidence and severity. In order to understand the molecular regulatory mechanism of the synergistic effect of $Twist1^{+/-}$ and exposure to citalopram on the development of craniosynostosis, we have performed RNA sequencing analysis at P7 to uncover the downstream target genes affected in the coronal suture prior to its fusion.

Methods: Sixteen *Twist1+/-* mutant mice and wild-type (WT) mice with or without *in utero* citalopram exposure (20 mg/kg per day) were generated: WT (n=5), *Twist1+/-* (n=4), WT + citalopram (n=3), and *Twist1+/-* + citalopram (n=4). At P7, the mice were sacrificed, and the coronal sutures were dissected under a microscope and subjected to RNA extraction. The samples were analyzed for quality, and then Hitech sequencing was conducted at the core facility at University of California, Los Angeles. Post-sequencing data analysis was performed using Partek Flow and Ingenuity Pathway Analysis.

Results: *Twist1+/-* mice exposed to citalopram demonstrated significant upregulation of genes involved in the pathophysiology of osteoarthritis [*S100a8*: 4.616; *S100a9*: 3.815] in comparison to *Twist1+/-* mice without citalopram exposure. WT mice exposed to citalopram demonstrated a significant downregulation of genes in the Igf1 signaling pathway [*Igfbp4:* -1.476; *Igfbp5:* -1.447] compared to WT mice without citalopram exposure. *Twist1+/-* mice without citalopram treatment had significant downregulation of genes involved in Ephrin signaling [*Epha3:* -1.562; *Cxcl12:* -1.279; *Grin2b:* -2.357] in comparison to WT mice without citalopram treatment. Finally, *Twist1+/-* mice with citalopram exposure had significant downregulation of growth factor receptors in the STAT3 pathway [*Fgfr3:* -1.262; *Igf1r:* -1.246; *Igf2r:* -1.306] in comparison to WT mice with citalopram exposure.

Conclusion: RNA sequencing data provide valuable insights into the potential signaling pathways and regulatory mechanisms involved immediately preceding suture fusion. Future in vivo studies focusing on implicated pathways will guide further mechanistic investigation into the pathophysiology that leads to craniosynostosis. By analyzing the interplay of the environment and genetics involved, we can better understand how to prevent and treat this devastating disease. Supported by R01 DE030901, NIDCR, NIH

QS75. Bone Bending: Developing an Animal Model For Mechanical Properties of Human Infant Calvarium

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Purpose: Manipulation of the shape of infant calvarium is an integral part of surgery to correct craniosynostosis, and may involve cutting, drilling, weakening, or bending the bone selectively in order to achieve the desired outcome. Little is known of the mechanical properties of infant calvarium and its response to mechanical manipulation, beyond the surgeon's "feel" in the operating room. Knowledge of these mechanical properties can provide insight into its response to physical loads, limits for plastic/elastic deformation, and may inform future development of novel surgical techniques. As such, we sought to develop an animal model which most closely resembles the biomechanical characteristics of human infant calvarium, as the foundation for future research into cranial bone shape manipulation. In this study, we analyzed the bone's response to bending stimulus.

Methods: Candidate species models included macaque monkey, neonatal swine, beagle, and human adult calvarium. 8 specimens were tested for each of the planned tests. A universal test machine (UTM) with custom fixtures was used to perform a 3-point bend test on each specimen at a preload of 10N and a rate of 10mm/min until specimen fracture. Load-displacement curves and break forces such as peak load, stress, and strain were recorded during the test. The bending modulus was computed as the linear slope of the stress-strain plot, while the maximum point in this curve is defined as the bending strength. Our results were then compared with historical data for the human infant calvarium.

Results: An unpaired t-test was used to compare bending modulus and strength from different species. The mean bending modulus was found out to be 3.03 GPa (SD 1.4) for adult human calvarium, 1.73 GPa (SD 0.85, p=0.06) for canine, 1.71 GPa (SD 0.73, p=0.05) for macaque, and 0.82 GPa (SD 0.14, p=0.001) for pig. The mean bending strength for the adult human calvarium was 61 MPa (SD 12.8), 74.12 MPa (SD 37.85, p=0.4) for canine, 74.43 MPa (SD 27.6, p=0.26) for macaque, and 29.05 MPa (SD 7.49, p=0.001) for pig. The elastic modulus of composite human cranial bone in bending is 1.37 GPa at around the time of birth.

Conclusion: Our results in this study show that the neonatal piglet model shows bending properties closest to that of a 1-week-old human infant (E=820.9 MPa at a loading rate of 2540mm/min). While canine and macaque models show similar properties, the adult human has a bending modulus that is 3.7 times that of the pig. This study is the first in a series examining the mechanical properties of various animal skull bone models. These results will be collated with finite element analysis and subjective testing to inform the optimal choice of model for future development of surgical techniques for cranial bone shape manipulation.

QS76. A Novel Approach for Quantifying Skin Growth in Patients Undergoing Expander-based Breast Reconstruction

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Purpose: Tissue expansion protocols for breast reconstruction are highly variable among surgeons and institutions. Animal studies in a porcine model of tissue expansion have relied on skin tattooing to standardize and predict expansion-induced skin growth, as estimated by the amount of total *in-vivo* deformation calculated by multi-view stereo and isogeometric analysis (IGA). However, an investigation of expansion-induced skin growth has not been performed in humans, and studies are limited by absence of a standardized methodology. Here, we will utilize our knowledge of IGA in the porcine model to develop a standardized approach for tracking skin growth in human patients undergoing expander-based breast reconstruction without the use of invasive tattoo-based methodology.

Methods: Female Yucatan minipigs underwent tattooing of 10-by-10 cm grids and placement of subcutaneous tissue expanders below the grids. Female human patients were marked at several fixed bony anatomic landmarks that remain unchanged throughout reconstruction, including the clavicle, sternal notch, xyphoid, and midline connecting the notch and xyphoid. Four additional marks were made between the nipple and base of the breast in each quadrant to further orient the 3D camera (VECTRA®H2 from Canfield