and Beery-Buktenica Visual-Motor Developmental Test. Parents/guardians were also surveyed with the Behavior Rating Inventory of Executive Function, Child Behavior Checklist, and for 22 socioeconomic/demographic factors.

RESULTS: Twenty patients (12 Yale, 8 CHOP) participated with a mean age of 12.1 years. All patients underwent cranial vault remodeling at a mean age of 8.0 months and included 55% female and 50% right-sided ULC craniosynostosis. ULC patients on average had mean academic achievement performance percentiles above the national mean (word reading 76.3%, reading comprehension 60.8%, reading composite 68.1%, spelling 61.4%) with the notable exception of numerical operations (47.2%); patients scored significantly lower for numerical operations compared to word reading (p=0.022). Mean verbal IQ (VIQ) was highest at 117.3 while mean performance IQ (PIQ) was lower at 106.4. Patients performed below average on all Beery-Buktenica visual-motor tests (visual-motor integration 42.5%, visual perception 49.6%, motor coordination 26.0%); motor coordination was poorer than both visual-motor-integration and visual-perception (p=0.027, p=0.005). Regarding behavioral surveys, patients performed the poorest on behavioral regulation (38.7%) and emotional control (39.4%) in the Behavior Rating Inventory of Executive Function survey. Patients performed the worst on externalizing problems (45.1%) in the Child Behavior Checklist. Surgery before 7 months trended towards improved motor coordination (p=0.067), and female patients had higher VMI (p=0.024). Breast-fed patients had higher overall PIQ (p=0.034), VMI (p=0.014), and visual perception scores (p=0.031). Significant correlations existed between, paternal education/visual perception (r=0.450; p=0.046), household income/VIQ (r=0.628; p=0.004), and birth weight/numerical operations (r=0.578; p=0.015). Right ULC patients had improved spelling compared to left ULC patients (p=0.033). While no significant differences between laterality were found for any other neuropsychological (or behavioral) score, subjects with right sided fusion scored higher on all language/verbal tests. Follow-up multiple regression between coronal sidedness and spelling scores was performed to control for all three IQ measures (VIQ, PIQ, FSIQ), all variables that significantly impacted performance (age at surgery, sex, breast-feeding status, paternal education, household income), age at testing, and race. Adjusted analysis revealed right-sided ULC still significantly predicted higher spelling scores (R² 0.650, p=0.033).

CONCLUSION: Our prospective multi-institutional evaluation of cranially-mature patients with ULC revealed improved language academic achievement in comparison to mathematics, higher VIQ than PIQ, and overall poor visuo-motor skills. Left-sided ULC may lead to deficit spelling performance possibly in response to left brain restriction. Breast feeding was associated with overall improved VMI, visual perception, and PIQ, and should be encouraged for patients diagnosed with ULC. Patients and families with ULC should be counseled on expected outcomes and early neurocognitive/motor exams should be included in patient care.

68

Tolerance Induction of Vascularized Composite Allografts Across a Class I Barrier in Swine

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PURPOSE: Vascularized composite allografts (VCAs) are an established option for restoration of complex soft tissue defects. The limiting factors for VCA include the risks of immunosuppression and the specter of chronic rejection. Therefore, the development of tolerance protocols remains a priority. We sought to optimize our mixed hematopoietic chimerism VCA tolerance protocol with the addition of co-stimulatory blockade, Tregulatory cell augmentation, inflammatory cytokine inhibition and augmentation of hematopoietic cell engraftment.

METHODS: Prior to VCA, MGH swine received non-myeloablative conditioning with 300 cGy total body and 700 cGy thymic irradiation on day -2. Osteomyocutaneous hind limb VCAs were transplanted into MHC class I-mismatched recipients (n=8). Tacrolimus was administrated for 45 days (target level 10–15 ng/mL). CTLA4-Ig (20 mg/kg) was administered on POD 0 and on days 2, 4, 6. Anti-IL6R (10mg/kg) was given on POD 0, 7, 14, 21, 28. VCA skin and muscle biopsies were performed on POD 30, 50 and 100. Systemic immune function and chimerism status
were assayed. Split thickness skin grafts were placed at POD 150 from self, donor, and third-party donor to assess acceptance/rejection of the original donor skin.

RESULTS: Three animals out of eight completed the protocol: swine 23645 became tolerant of the VCA, including the skin, and was terminated at 251 days, corresponding to the endpoint of the study. For other animals, we elected to extend the endpoint to 400 days. 24087 and 24356 has been tolerant up to POD 400. One episode of acute epidermal rejection occurred at POD 252 (after 207 days off immunosuppression) but resolved spontaneously without needing additional immunosuppression. All animals showed mixed hematopoietic chimerism in the blood. Split thickness skin grafts placed at POD 150 showed acceptance of self and donor skin grafts, whereas the third-party graft was rejected in the normal 8-12-day time frame. We observed an increase of anti-inflammatory cytokines in the blood stream.

CONCLUSION: We demonstrate tolerance in a Class I mismatch swine model of all components of the allograft, including epidermis. This VCA tolerance induction protocol is notable for the use of donor bone marrow as a hematopoietic cell source as well as clinically-approved induction agents. This protocol is being tested in full MHC mismatch swine in anticipation of clinical translation.

Acceleration Of Dermal Wound Healing By Regulation Of A Circadian Clock Gene, Neuronal Pas Domain 2 (Npas2)

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PURPOSE: Circadian rhythms that maintain cellular homeostasis during a 24-hour cycle have been shown to regulate a wide range of peripheral tissues. We have hypothesized that dermal wound healing is also under the regulation of circadian gene. In this study, we investigated the role of one of the circadian genes, Neuronal PAS domain 2 (Npas2) in the homeostasis of dermal structure using in vivoand in vitrowound healing models.

MATERIALS AND METHODS: Primary fibroblasts were isolated from homozygous knock out (KO) (Npas2-/-), heterozygous KO (Npas2+/-) and normal C57Bl6J (WT) mice. The expression of core clock genes was determined by quantitative RT-PCR (qPCR). Fibroblast behaviors were characterized in terms of cell proliferation (WST-1 test), cell migration (Scratch test), and cell contraction (floating collagen gel culture). In addition, gene expression of alpha-smooth muscle actin (alpha-SMA) and extracellular matrix collagens (type I, III, XII, and XIV) was determined by qPCR and in vitro collagen accumulation was evaluated by Picrosirius red staining. The time-course healing of full-thickness punched-out wounds was monitored in WT and Npas2 KO mice. Moreover, we screened 1,120 FDA-approved compounds for Npas2 expression and fibroblast migration. One candidate compound exhibited the increased fibroblast migration in vitro and the accelerated full-thickness dorsal skin punched-out wound healing in vivo.

RESULTS: There was no effect on the core clock gene expression by Npas2 KO mutation. The KO fibroblasts showed higher cell proliferation, migration and contraction capabilities. While alpha-SMA expression was not affected, FACIT collagen XII and XIV gene expression was significantly increased in Npas2 KO fibroblasts. Picrosirius red staining was strongly positive in Npas2 KO fibroblasts. Npas2-/- mice demonstrated faster dermal wound closure than the other groups (p<0.01). Furthermore, candidate compound-treated dermal wounds suggested accelerated wound healing.

DISCUSSION AND CONCLUSION: Our study demonstrated that Npas2 suppression in dermal fibroblasts modified cell behaviors demonstrated by accelerated cell proliferation, cell migration and cell contraction force in vitro. Moreover, Npas2 suppression resulted in accelerated dermal wound healing. This study suggests that Npas2 may be a novel therapeutic target for dermal homeostasis and wound healing.