

we report the biological impact of a novel truncating EBF2 variant. **METHODS/STUDY POPULATION:** Using 3T3-L1 and human primary subcutaneous preadipocytes, we performed loss-of-function and gene rescue experiments. All cells were cultured in DMEM with 10% bovine calf serum (Invitrogen) at 5% CO<sub>2</sub>. After lentivirus transfection, cells were grown to confluence and then exposed to adipogenesis induction media containing dexamethasone (0.25 ÅM), insulin (1 Åg/ml) and isobutyl methylxanthine (0.5 mM). Total RNA was extracted using RNeasy Mini Kit (Qiagen) and cDNA was synthesized using IScript (Bio-Rad). Real-time qPCR was performed using TaqMan probes for Pparg and Fabp4, two key adipogenesis markers. **RESULTS/ANTICIPATED RESULTS:** Patient was found to carry a heterozygous nonsense mutation in exon 6 of EBF2, causing the premature termination of the protein at amino acid position 165. Adipogenesis was significantly suppressed in 3T3L1 cells when endogenous Ebf2 was suppressed with siRNA and lentiviral shRNA. Adipocytes with suppressed Ebf2 expression showed marked reduction of intracellular lipid content and Pparg and Fabp4 expression (>80% reduction). With lentiviral gene transfer, EBF2 fully rescued adipogenic potential, whereas the truncated variant EBF2 did not. Of note, 3T3-L1 cells transfected with the EBF2 variant displayed impaired adipogenesis, suggesting a dominant-negative effect of the EBF2 variant on adipogenesis. We confirmed the dominant effect of the EBF2 variant in human adipocyte differentiation. **DISCUSSION/SIGNIFICANCE:** Our data suggest that EBF2 is indispensable for adipogenesis. The loss of function and dominant-negative effect of the truncating variant of EBF2 likely plays a pathogenic role in PL. Whole exome sequencing of PL patients and ex-vivo functional analysis help identify novel gene variants and better understand the molecular pathogenesis of PL.

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### Early Neurorehabilitation of Disorders of Consciousness after Acute Hemorrhagic Stroke

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**OBJECTIVES/GOALS:** Accurate classification of disorders of consciousness (DoC) is key in developing rehabilitation plans following brain injury. The Coma Recovery Scale-Revised (CRS-R) is a sensitive measure of consciousness. We explore feasibility, safety and impact of CRS-R guided rehab in hemorrhagic stroke patients with DoC and evaluate predictors of recovery. **METHODS/STUDY POPULATION:** Consecutive patients with non-traumatic hemorrhagic stroke, defined as subarachnoid hemorrhage (SAH) or intracerebral hemorrhage (ICH), receiving serial CRS-R assessments during their ICU stay at University of Maryland Medical Center from 2017-2021 were retrospectively identified. Outcomes of interest included the association with CRS-R and discharge disposition, therapy-based function and mobility and occurrence of safety events during CRS assessment. We also examined the association between CRS-R and physiological and anatomical injury pattern on electroencephalography (EEG) and magnetic resonance imaging (MRI), respectively. **RESULTS/ANTICIPATED RESULTS:** 76

patients with ≥2 CRS-R assessments were identified (22 SAH, 54 ICH, median age = 59, 50% female). Median CRS-R completed was 3 with no SAEs identified during sessions. We identified 4 patterns: persistent VS/UWS (49%), persistent MCS or better (13%), emergence from VS/UWS to MCS or better (27%) and regression from MCS or better to VS/UWS (11%). Persistent low CRS-R correlated with older age in SAH ( $p=0.01$ ), female gender in ICH ( $p=0.04$ ), and history of diabetes ( $p=0.01$ ). 2% of patients with final CRS-R **DISCUSSION/SIGNIFICANCE:** Early neurorehabilitation guided by CRS-R appears to be feasible and safe acutely following hemorrhagic stroke complicated by prolonged DoC and may enhance access to inpatient rehabilitation with a lasting benefit on recovery. Further characterization of DoC patterns and their correlation to clinical markers, including EEG and MRI is needed.

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### Insights into the complex immune environment during pregnancy and association with the developing human connectome

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**OBJECTIVES/GOALS:** Maternal health and exposures during pregnancy play a major role in shaping the neurodevelopment of our offspring—one influence is maternal immune activation (MIA). Here we explore the association of MIA during pregnancy and the developing human connectome through analysis of 46 markers of activation. **METHODS/STUDY POPULATION:** 74 healthy women with singleton pregnancies underwent blood draws between 34-37 weeks gestation. 46 markers of maternal immune activation, both adaptive (e.g., IgG) and innate (e.g., cytokines and acute phase reactants), were collected. In addition, for preliminary analyses of MIA in relation to the newborn brain, we utilized 30 participants with MRIs between the ages of 0-6 months. **RESULTS/ANTICIPATED RESULTS:** Principal component analysis (PCA) identified the first 5 PCs explains ~68% of the variance and the first 10 explains ~83% (top PC is 42.1%). Using the top PC each edge in the connectome was correlated with the immune profiles. Several regions trended towards significance—one survived correction and included 359 edges, showing. The highest number of edges was observed in the inferior parietal lobe of the left hemisphere—a region associated with functions from basic attention to social cognition, suggesting that deviations in fetal exposure to MIA can longitudinally impact offspring behavior in areas essential for human interaction. **DISCUSSION/SIGNIFICANCE:** This is the first study in understanding how interruptions (i.e., MIA) influence later development. Identification of alterations, and long-term outcomes could lead to the development of mechanism-based health-care, facilitate timely referral for appropriate interventions and provide family support.