

implantation, potentially leading to a new diagnostic or treatment target in early pregnancy failure. **OBJECTIVES/GOALS:** Studies suggest interferon signaling regulation is tightly balanced between physiologic and pathophysiologic growth in early pregnancy. We propose to determine the impact of interferon-mediated inflammation on embryo implantation and early pregnancy failure in normal conditions and chronic inflammatory diseases in a novel mixed-mouse model. **METHODS/STUDY POPULATION:** To probe the role of type-I interferons (IFNs) in implantation, we will utilize a mouse model and non-surgically transfer both *Ifnar1*^{-/-} and *Ifnar1*^{+/-} embryos into an immune-competent pseudopregnant wild-type female recipient. This will allow analysis of a litter with distinct genotypes within the same, immune-competent, uterine environment. Type-I IFN stimulation will be systemically induced with Poly-(I:C) at various time points around implantation. A similar approach will be used in mouse models of chronic inflammatory disease states associated with early pregnancy loss (e.g. systemic lupus erythematosus). With this model, we will be able to control for deficiencies in maternal immune response to specifically determine the embryonic response to inflammation during implantation and development. **RESULTS/ANTICIPATED RESULTS:** We anticipate the *Ifnar1*^{+/-} embryos - those able to respond to Type-I IFN - and their surrounding implantation sites will exhibit more maternal-fetal barrier dysfunction in the form of impaired trophoblast fusion, improper formation of the microvascular architecture, and increased permeability of the maternal-fetal barrier, compared to embryos unable to respond to IFN. We will also conduct similar analyses in mouse models of chronic inflammatory diseases. We hypothesize these mice to have baseline endometrial inflammation that stimulated the IFN-pathway in IFN-capable embryos, producing breakdown of the maternal-fetal barrier. In these mice, we predict *Ifnar1*^{-/-} embryos will show improved molecular outcomes when compared to *Ifnar1*^{+/-} embryos, and thus improved associated pregnancy outcomes. **DISCUSSION/SIGNIFICANCE OF FINDINGS:** This work can insight into the immunological mechanisms that govern embryo implantation and early placentation. This could provide more pointed means for management and intervention of early pregnancy failure and/or disease states that are commonly associated with poor reproductive outcomes.

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Massively Parallel Reporter Assay Reveals Functional Impact of 3'-UTR SNPs Associated with Neurological and Psychiatric Disorders*

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ABSTRACT IMPACT: Screening the effect of thousands of non-coding genetic variants will help identify variants important in the etiology of diseases **OBJECTIVES/GOALS:** Massively parallel reporter assays (MPRAs) can experimentally evaluate the impact of genetic variants on gene expression. In this study, our objective was to systematically evaluate the functional activity of 3'-UTR SNPs associated with neurological disorders and use those results to help understand their contributions to disease etiology. **METHODS/STUDY POPULATION:** To choose variants to evaluate with the MPRA, we first gathered SNPs from the GWAS Catalog that were

associated with any neurological disorder trait with p-value < 10⁻⁵. For each SNP, we identified the region that was in linkage disequilibrium ($r^2 > 0.8$) and retrieved all the common 3'-UTR SNPs (allele-frequency > 0.05) within that region. We used an MPRA to measure the impact of these 3'-UTR variants in SH-SY5Y neuroblastoma cells and a microglial cell line. These results were then used to train a deep-learning model to predict the impact of variants and identify features that contribute to the predictions. **RESULTS/ANTICIPATED RESULTS:** Of the 13,515 3'-UTR SNPs tested, 400 and 657 significantly impacted gene expression in SH-SY5Y and microglia, respectively. Of the 84 SNPs significantly impacted in both cells, the direction of impact was the same in 81. The direction of eQTL in GTEx tissues agreed with the assay SNP effect in SH-SY5Y cells but not microglial cells. The deep-learning model predicted sequence activity level correlated with the experimental activity level (Spearman's $\text{corr} = 0.45$). The deep-learning model identified several predictive motifs similar to motifs of RNA-binding proteins. **DISCUSSION/SIGNIFICANCE OF FINDINGS:** This study demonstrates that MPRAs can be used to evaluate the effect of non-coding variants, and the results can be used to train a machine learning model and interpret its predictions. Together, these can help identify causal variants and further understand the etiology of diseases.

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The Association Between Specialized Pro-resolving Lipid Mediators and Markers of Neuroinflammation and Disease Severity in Acute Traumatic Brain Injury: A Prospective, Observational Cohort Study

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ABSTRACT IMPACT: Characterizing specialized pro-resolving lipid mediators (SPMs) of inflammation in a cohort of adult patients with traumatic brain injury (TBI) will potentially identify novel biomarkers of disease and would generate hypotheses regarding the role of SPMs in the pathophysiology and recovery after brain injury **OBJECTIVES/GOALS:** Primary aim: evaluate the association between plasma and cerebrospinal fluid (CSF) SPM levels with inflammatory markers known to mediate blood brain barrier (BBB) disruption. Secondary aim: evaluate the association between SPM levels and disease severity, 14-day Glasgow Coma Scale score (GCS) and survival. **METHODS/STUDY POPULATION:** This is a single-center, prospective, observational cohort study (target N=50). Adult participants with moderate-to-severe non-penetrating TBI will have blood and CSF sampled serially for laboratory measures of SPMs (resolvins, protectins, lipoxins, maresins) and inflammatory peptides (TNF- α , IL-1 β , IL-6, MMP-9, TIMP-1) by liquid chromatography⁺ mass spectrometry and RT-PCR & ELISA, respectively. Baseline patient characteristics, admission GCS, and 14-day GCS and mortality will be prospectively collected. To evaluate the association between SPMs and other continuous variables, Pearson's and Spearman's correlation will be used. Cox regression will be used to evaluate the association between SPM lipidomes and 14-day survival. **RESULTS/ANTICIPATED RESULTS:** As the primary outcome measure, the association between SPM levels with assayed inflammatory markers will be determined at 24 and 72 hours post-TBI. We hypothesize that SPMs will be negatively correlated with TNF- α , IL-1 β , IL-6, MMP-9 and positively correlated with