RT-QuIC and FRET assay, we expect EVs isolated from DLB patient samples to support seeded aggregation, whereas EVs from AD and HC will not. DISCUSSION/SIGNIFICANCE: To date, no diagnostic or less invasive biomarkers can distinguish DLB from AD. The successful completion of the aims outlined in this proposal will identify characteristics of bpEVs that differentiate DLB from AD or HC and support the development of bpEVs as a non-invasive, early biomarker to diagnose patients presenting with dementia from DLB.

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Epicardial adipose tissue and cardiometabolic health in youth-onset type 2 diabetes undergoing vertical sleeve gastrectomy

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OBJECTIVES/GOALS: The goal of this study is to investigate the potential independent relationship between epicardial adipose tissue (EAT) and cardiometabolic health in youth-onset type 2 diabetes (T2D) and explore changes in EAT as a potential mediator of changes in cardiometabolic health in response to vertical sleeve gastrectomy (VSG). METHODS/STUDY POPULATION: We will assess glycemic control, insulin sensitivity and secretion in youth with T2D before and 3 months after VSG. Fasting labs, anthropometrics, and a 4-hour, frequently sampled liquid mixed meal tolerance test (45g carbohydrates, 14g fat, and 14g protein) were performed. Calculations included glucose, insulin, and GLP-1 area under the curve (AUC), Matsuda Index, HOMA-IR, and oral disposition index (DI). These cardiometabolic outcomes will then be assessed for associations between total EAT volume, measured from cardiac MRI. RESULTS/ANTICIPATED RESULTS: Previous studies have shown that individuals with obesity have higher EAT than lean controls, and adults with T2D have even higher EAT than obese controls. Therefore, we anticipate that our participants will have higher volume of EAT than what has been reported in the literature and that they will have worsening cardiometabolic outcomes without MBS. Our anticipated results will include: Weight and BMI, hemoglobin A1c, diabetes medications, Matsuda Index, HOMA-IR, DI, and glucose and insulin AUC during an MMTT. Cardiac MRI's are being analyzed and will give total EAT volume and will be analyzed for correlations with the cardiometabolic outcomes of body composition, aortic stiffness, blood pressure, cardiac structure and function, as well as lipid panel and insulin sensitivity. DISCUSSION/SIGNIFICANCE: This study is the first to specifically assess EAT in adolescents with T2D. The assessment of EAT will be done with gold-standard MRI and correlated with cardiometabolic health assessed by gold-standard methods. Together, the results will give insight into EAT as a potential independent cardiometabolic risk factor in adolescents undergoing VSG.

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Fractalkine isoforms using gene therapy differentially regulate microglia activation and vascular damage in the diabetic retina

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OBJECTIVES/GOALS: Retinal inflammation caused by the activation of resident macrophages (microglia) during diabetes exacerbates glial cell dysfunction, resulting in neuronal loss. The goal is to use rAAV gene therapy to deliver neuronal-derived fractalkine (FKN), minimizing inflammation and vascular damage in the diabetic retina. METHODS/STUDY POPULATION: The human microglial receptor (CX3CR1) binds to FKN, a protein that is expressed on neuronal membranes (mFKN), and undergoes constitutive cleavage to release a soluble domain (sFKN). Deficiencies in CX3CR1 or FKN showed increased microglial activation and elevated retinal pathology. To understand the mechanism by which mFKN and sFKN regulate microglia function, recombinant adeno-associated viruses (rAAVs) expressing mFKN or sFKN were delivered to intact retinas during diabetes. Markers of neuronal loss, vascular damage, and inflammation were analyzed. We hypothesize that the administration of rAAV-sFKN but not rAAV-mFKN will prevent vascular and neuronal damage, and improve visual function. RESULTS/ANTICIPATED RESULTS: rAAV-sFKN minimized microglial activation, blood vessel rupture, fibrinogen deposition, and prevented neuronal loss, compared to mice treated with rAAV-mFKN in a mouse model of diabetic retinopathy (DR). rAAV-sFKN treated mice showed improved visual acuity using a two-choice discrimination task through learning-based behavior. rAAV-sFKN treatment correlated with the success rate of the mice finding the reward based on their ability to distinguish visual cues. Future studies will test the effects of rAAV-sFKN and rAAV-mFKN on microglia inflammatory cytokine release, optic nerve damage and synaptic neurotransmission, peripheral immune responses, and transcriptomic changes in microglia during diabetes. DISCUSSION/SIGNIFICANCE: Current therapies for DR are ineffective in restoring vision. rAAVs-sFKN delivery appears to act as a neuroprotective approach in the diabetic retina. sFKN serves as an alternative pathway to implement translational and therapeutic approaches, minimizing pathology and improving visual function.

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HMGB1 Localization as a Driver of Carcinogenesis in RDEB-Associated Squamous Cell Carcinoma

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OBJECTIVES/GOALS: This study investigates whether localization of high mobility group box 1 (HMGB1) controls inflammatory signaling and DNA damage response in human keratinocytes, the cell of origin for squamous cell carcinoma (SCC). SCC is especially

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metastatic in chronic wounds, burns, and in patients with recessive dystrophic epidermolysis bullosa (RDEB). METHODS/STUDY POPULATION: We used CRISPR/Cas9 gene-editing to knock out HMGB1 in a keratinocyte line, p16INK4a-negative keratinocytes immortalized by ectopic hTERT expression (N/TERT-2G [46, XY]). Following gene editing, clonal keratinocyte populations were screened for knockout by PCR followed by TIDE analysis (Tracking of Indels by Decomposition) to identify indels that would result in a frameshift mutation. Total cell lysates for each clonal population were analyzed by immunoblot and immunofluorescence for HMGB1 protein. These cells will be used to assay for DNA damage sensitivity in the presence of genotoxic agents (etoposide, ultraviolet radiation, γ-irradiation). A lentiviral vector will then be used to express mutant forms of HMGB1 that localize to the nucleus (C23/45S) or cytoplasm (C106S) and DNA damage assays repeated. RESULTS/ANTICIPATED RESULTS: We have confirmed by sequencing, immunoblot, and immunofluorescence that HMGB1 is knocked out in a clonal population of N/TERT-2G human keratinocyte cells. We anticipate that cells with a complete absence of HMGB1 will have high sensitivity to DNA damaging agents, but little change in inflammatory signaling. We also expect that cells expressing mutant HMGB1 that localizes exclusively to the cytoplasm will demonstrate an increased sensitivity to DNA damage relative to wild-type controls, while mutant HMGB1 that localizes exclusively to the nucleus will be protected from DNA damage caused by exposure to genotoxic agents. DISCUSSION/SIGNIFICANCE: HMGB1 is a nuclear protein and damage associated molecular pattern that is elevated systemically in patients with RDEB, many of whom will go on to develop metastatic squamous cell carcinoma. The experiments described here investigate whether HMGB1 plays a mechanistic role in skin carcinogenesis via regulation of the DNA damage response.

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Identification of potential targets for immunotherapy in a cynomolgus macaque model of Ebola virus disease

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OBJECTIVES/GOALS: Ebola virus infection causes severe disease and liver injury in humans. Macrophages contribute to inflammatory signaling and are prevalent in the liver. We assessed the activation status, including therapeutic target expression, of hepatic macrophages. METHODS/STUDY POPULATION: We compared formalin-fixed, paraffin-embedded liver tissue from terminal Ebola virus-infected and uninfected control cynomolgus macaques, a gold-standard model for human disease. We characterized region-specific protein and whole transcriptome expression in these tissues using GeoMx Digital Spatial Profiling. Macrophage (CD68+) and leukocyte (CD45+) accumulation in liver tissue was quantified by immunofluorescence image analysis using digital pathology software. RESULTS/ANTICIPATED RESULTS: Macrophage-specific (CD68+) regions in the liver of Ebola virus-infected macaques demonstrated a shift towards an inflammatory gene expression profile, as compared to those from healthy control tissue. These regions showed differential expression of monocyte/macrophage differentiation, antigen presentation, and T cell activation gene sets, which were associated with decreased MHC-II allele expression. Moreover, macrophage-specific regions in the infected macaques showed enriched expression of genes or proteins associated with known immunomodulatory therapeutics, including S100A9, IDO1, and CTLA-4. DISCUSSION/SIGNIFICANCE: These data demonstrate that hepatic macrophages express an inflammatory phenotype, that their ability to present antigens to the adaptive immune system may be impaired, and that they express therapeutically targetable markers for immunomodulation of these cells during Ebola virus infection.

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Identification of Psychosis Risk Biomarkers in 22q11DS for future translational studies

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OBJECTIVES/GOALS: 22q.11 deletion syndrome (22q11DS) is a genomic syndrome that elevates risk for psychosis >20-fold. We used a battery of cognitive and psychophysiological psychosis-risk biomarkers in 22q11DS patients and healthy subjects in order to identify biomarkers of psychosis in 22q11DS that could be used as translational targets in intervention studies. METHODS/STUDY POPULATION: We recruited 15 22q11DS individuals (Mean age=30, M/F=9/6) and 19 healthy controls (HCs; Mean age=34, M/F=5/14). Each individual completed the MATRICS Consensus Cognitive Battery (MCCB), the Wechsler Abbreviated Scale of Intelligence, Second edition (WASI-II) Verbal IQ subtests, and the computerized Wisconsin Card Sorting Task (WCST). To examine auditory EEG responses, each participant completed the 'Double-Deviant' target detection paradigm, which presents a pseudorandom sequence of frequent standard tones and infrequent deviant tones. Mismatch negativity (MMN) metrics were generated from this assessment. Welch's t-tests were completed for neurocognitive variables. One-Way ANOVAs were completed to examine EEG results, with sex entered as a separate factor and age entered as a covariate. RESULTS/ANTICIPATED RESULTS: Significant group differences were found in 8 of the 9 neurocognitive measurements (FDR-adjusted p's< 0.02, average Cohen's d=1.62, average observed power= 0.91) indicating widespread cognitive deficits in 22q11DS subjects across multiple domains. The Double-Deviant MMN ERP response was significantly smaller in absolute magnitude in the 22q11DS group (FDR-adjusted p=0.048, Cohen's d= -0.864, observed power= 0.58). The MMN ERPs for the frequency and duration deviants were not significantly different (FDR-adjusted p's> 0.33). No group by sex interactions were observed in any of the measures. Neurocognitive variables were associated with psychosis positive, negative, general, and disorganized symptom scales. DISCUSSION/SIGNIFICANCE: Our results identify potential psychosis-risk biomarkers in 22q11DS. If replicated, these biomarkers could provide important translational targets for future clinical trials for individuals with 22q11DS and other individuals at-risk for psychosis syndromes.

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