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Copula-Based Regression Models for Correlated Bivariate Binary Outcomes: Application to Ophthalmologic Data Structures

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OBJECTIVES/SPECIFIC AIMS: To account for association between the pair of binary outcomes, we adopt the Clayton and Frank copulas to indirectly specify their joint distributions. METHODS/STUDY POPULATION: We propose a regression model for the joint modelling of correlated bivariate outcomes using copulas. RESULTS/ANTICIPATED RESULTS: develop full maximum likelihood inference.

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Defining the Extracellular Vesicle Content of Interstitial Fluid for Blood-Free Diagnostics; Extraction Methods and Initial Characterization

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OBJECTIVES/SPECIFIC AIMS: Recent advances in microneedle technology have enabled practical, in vivo dermal interstitial fluid (ISF) sampling. These minimally-invasive techniques allow for collection of ISF without damage to adjacent tissues and do not rely on blister formation. Initial reports of extracellular vesicle (EV) isolation from dermal ISF and paired blood samples suggest that EVs may be more abundant in ISF. Analysis of ISF-derived EVs may allow for more detailed study of intercellular communication at the tissue level, particularly in acute inflammatory conditions. The objective of this study is to describe the isolation and initial characterization of interstitial fluid-derived exosomes. METHODS/STUDY POPULATION: We apply electron microscopy, nanoparticle tracking analysis (NTA), immuochemical, and sequencing methods to describe and distinguish the EV content of interstitial fluid. We include apparently healthy adult human subjects with no active skin disease. We also study immunocompetent, CD-hairless rats to demonstrate the generalizability of the methods. RESULTS/ANTICIPATED RESULTS: We successfully isolated EVs from human and rat interstitial fluid using commercially available precipitation methods. The EVs were initially characterized using UV/Vis spectroscopy, electron microscopy, and NTA. While the study is ongoing, initial results suggest that the concentration and size distribution of EVs differs significantly between blood fractions and ISF. Further immunochemical and sequencing characterization is ongoing. DISCUSSION/SIGNIFICANCE OF IMPACT: We present here the initial characterization of EVs isolated from dermal interstitial fluid. This appears to be the first report of EV characterization using ISF collection methods that do not perturb adjacent tissues (such as with blister or microdialysis methods). The present study lays a foundation for further examination of ISF-derived EVs in acute inflammatory disease such as cellulitis or infectious neuritis. This may enable minimally invasive diagnostics and new research tools to understand intercellular communication in living organisms with increased spatial and temporal resolution.

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Delayed Administration Of Angiotensin Receptor (AT2R) Agonist C21 Downregulates Diabetes Induced Pro-Inflammatory Microglia Activation To Improve Cognitive & Functional Recovery Post Stroke: Therapeutic Indications For The Treatment Of Vascular Cognitive

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OBJECTIVES/SPECIFIC AIMS: The study aim was to 1) elucidate mechanisms contributing to the evolution of PSCI using a clinically relevant model of diabetes, a major risk factor for stroke and cognitive impairment, and 2) develop angiotensin type 2 receptor (AT2R) agonism as a therapeutic target. METHODS/STUDY POPULATION: Diabetes was induced in male Wistar rats by a HFD & low dose streptozotocin combination. At 12-14 weeks of age a total of 69 control & diabetic rats were subjected to 1 hr middle cerebral artery occlusion (MCAO) or Sham surgery. 3 days post-MCAO, rats that met the preset inclusion criteria were administered C21 or saline in drinking water at a dose of 0.12 mg/kg/day Adhesive removal task (ART) & 2-trial Ymaze were utilized to test sensorimotor & cognitive function at baseline as well as 1, 2, 4 and 8 weeks post-stroke. At week 8 post-stroke cell suspensions from freshly harvested brains were analyzed by flow cytometry utilizing antibodies against cell surface markers for M1 (CD11b+/CD45 low/ CD86+/TNFa+), M2 (CD11b+/CD45 low/ CD206+/IL-10+), and residential microglia (CD11b+/CD45+/ TMEM119+). RESULTS/ANTICIPATED RESULTS: Control rats progressively recovered from stroke-induced functional deficits by week 8, while diabetics still remained impaired ($P< 0.05$). 8 weeks post-MCAO only diabetic rats exhibited a decline in sensorimotor ($P< 0.05$) and cognitive function ($P< 0.05$) compared to Shams. Delayed administration of C21 on D2 post-stroke halted the decline and improved sensorimotor ($P< 0.05$) and cognitive function ($P< 0.01$). Flow cytometric analyses indicate that 8 post-stroke vehicle diabetics had an elevated M1/M2 ratio within the ipsilateral prefrontal cortex and hippocampus ($P< 0.01, 0.01$). They also had a larger percentage of non-residential microglia/macrophages, indicative of compromised blood brain barrier (BBB) integrity. Treatment with C21 significantly lowered the M1/M2 ratio ($P< 0.05$) and improved the BBB integrity. DISCUSSION/SIGNIFICANCE OF IMPACT: Taken together this study suggests that the use of comorbid disease models such as diabetes, may allow for more translational evaluations of PSCI. Higher translational relevance may also lead to a higher number of successful clinical trials and more FDA approved stroke therapies. It also suggests that C21 may serve as a potential therapeutic to modulate the development of PSCI.

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Development of a Contractile Fontan Circuit to Decrease Central Venous Pressures in Single Ventricle Patients

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OBJECTIVES/SPECIFIC AIMS: Children born with a single ventricle congenital heart defect requires three invasive open-heart surgeries in the first three years of life. The third operation, the

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Fontan procedure, includes connection of the vena cava (VC) to the pulmonary artery (PA) using a bio-inert conduit to reduce work required by the right ventricle (RV). While this operation greatly extends the lives of HLHS patients, the Fontan circuit eventually fails, and the only solution is a scarcely available donor heart. This failed circuit is explained by the "Fontan paradox" where central venous pressures build up over time, causing increased systemic resistance and congestion. The absence of the sub-pulmonary ventricle leads to abnormal hemodynamics associated with life-threatening complications. We believe that decreasing central venous pressures through the use of a tissue engineered contractile, patient specific conduit will decrease the amount and severity of complications caused by the "Fontan paradox." We will use amniotic fluid derived induced pluripotent stem cells (AF-iPSCs) differentiated into cardiomyocytes (CMs) to generate flow within a biodegradable conduit. Additionally, AF-iPSC will be differentiated into structural support cells (SSCs), including cardiac fibroblasts and epicardium. Several studies suggest advanced contraction and structure of CMs in specific ratios with SSCs, particularly mouse and human fetal fibroblasts. In combination, these cells have shown advanced tissue organization and function through mechanically and electrically aligned junctions. This allows them to have a magnitude higher contractile force than CMs alone, making them ideal for increasing pressure within a tissue engineered construct. This poster focuses on the differentiation and selection of SSCs. METHODS/STUDY POPULATION: AF-iPSCs differentiation began at roughly 80% confluency. Mesoderm formation occurred via WNT pathway modulation by supplementing RPMI+insulin media with 0.5 ng/mL BMP4 at day 0, followed by 3 ng/mL BMP4, 2 ng/mL Activin A, and 5 ng/mL BFGF for four days. Then, RPMI+insulin media was supplemented with 10 ng/mL of BMP4 until day fifteen for epicardial formation. Cells were lifted to induce epithelial-to-mesenchymal transition (EMT) and RPMI-insulin media was supplemented with 10 ng/mL BFGF for cardiac fibroblasts. They were then harvested and characterized using immunofluorescence. Planned experiments include RT-qPCR for further characterization of cardiac fibroblasts. Additionally, a fibroblast isolation plating technique will be utilized to obtain cardiac fibroblast from AF-iPSC CMs and AF-iPSC epicardium. Commercially obtained human cardiac fibroblasts will be utilized as a control for all studies. RESULTS/ANTICIPATED RESULTS: Immunofluorescence (IF) revealed positive expression of vimentin and α-SMA indicating a fibroblast and vascular smooth muscle phenotype after supplementation with 10 ng/mL of BMP4 after EMT induction. It is expected that IF of epicardial formation at day 15 will show positive expression of WT1, a well-known epicardial marker. We also suspect RT-qPCR will reveal high expression of cardiac fibroblast specific markers COL1A1, PDGFA, TCF21, and THSB1. We expect to yield a higher number of cardiac fibroblast from the small molecule AF-iPSC differentiation compared to a timed plating technique of AF-iPSC CMs and AF-iPSC epicardium (separately plated). Results will be quantified and compared using the aforementioned techniques. DISCUSSION/SIGNIFICANCE OF IMPACT: Discussion/significance of impact: Although fibroblasts make up a large portion of cells in the heart and greatly enhance CM function, they are poorly characterized in the literature and not easily obtained. This study will provide an efficiency comparison on the best method for acquiring cardiac fibroblast for cardiac tissue engineering applications as we move forward with translational cardiac pediatric research.

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Development of human engineered cardiac tissue (hECT)-based screening assay to explore cardiac contractile properties in response to pharmacological challenge with proarrhythmic drugs

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OBJECTIVES/SPECIFIC AIMS: The goals of this study were (1) to evaluate the effect of proarrhythmic drugs on calcium transient and (2) to use three-dimensional human engineered cardiac tissue (hECT) technology to evaluate cardiac contractile properties in response to pharmacological challenge with proarrhythmic drugs. METHODS/STUDY POPULATION: Calcium transient was measured in subject-specific iPSC-CMs by using the IonOptix system in Sotalol treated vs. untreated conditions. We fabricated human engineered cardiac tissues (hECT) in a custom designed bioreactor using low- and high-sentitive subject-specific iPSC-CMs. Contractile function of the hECT was evaluated at baseline and after Sotalol [300 μM] administration. The change in beat rate was recorded under spontaneous beating conditions; changes in other twitch parameters, including time to relaxation, were recorded under electrical stimulation. Time to relaxation served as an indicator of action potential duration (APD), which has a temporal correlation with the QT interval. RESULTS/ANTICIPATED RESULTS: The low-sensitive iPSC-CM showed a considerable drop in overall peak height of the calcium transient, in the presence of 100 μM Sotalol. The high-sensitive line, however, showed a more pronounced drop in peak height. Sotalol treatment also induced a more pronounced increase in the exponential decay time constant (tau) in the high-sensitive line compared to the low-sensitive line. The hECT fabricated with high sensitive hiPSC-CM showed a larger decrease in spontaneous beat rate in response to Sotalol (0.41 vs 0.23 fold decrease), with a higher increase in time to relaxation (1.8 vs 1.3 fold increase), compared to hECT from low sensitive hiPSC-CM. Moreover, while the low-sensitive hECT showed a positive correlation between time to relaxation and developed force, as expected after Sotalol stimulation; the high-sensitive hECT failed to show a positive inotropic response. DISCUSSION/SIGNIFICANCE OF IMPACT: Our findings suggest subject-specific iPSC-CMs and hECT, can be used to model functional abnormalities observed in diLQTS in response to Sotalol, and offer novel insights into human-based screening assays for toxic drug reactions. Success of this study may help identify key components underlying diLQT susceptibility to ultimately develop novel therapeutic agents.

3521

Distinct single cell gene expression in peripheral blood monocytes correlates with treatment response groups to TNF-alpha inhibition in rheumatoid arthritis

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OBJECTIVES/SPECIFIC AIMS: The cellular mechanisms that underlie the IFNβ/α ratio that predicts response are not known. Effects of IFN on single immune cells may be masked in whole blood

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