55th Annual Midwest Student Biomedical Research Forum

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FUNCTIONAL ANALYSIS OF HUMAN INTERLAMINAR ASTROCYTES FOLLOWING DEVELOPMENT IN THE MOUSE CORTEX

A. ANDING, M. BURKOVETSKAYA, K. HOFFMAN, P. RAGUNATHAN, A. DUNAEVSKY

University of Nebraska Medical Center

Omaha, Nebraska

Interlaminar astrocytes (ILAs) are primate-specific astrocytes that are located in the superficial layer of the cortex and project long processes that run perpendicular to the surface and transverse multiple layers of the cerebral cortex. Astroglia are known to be highly versatile and multi-faceted regulators of multiple brain functions and the extracellular environment, in additional to having an expansive networking capability. However, due to the lack of accessible experimental models, the functional properties of ILAs, the most abundant subtype of astrocyte in cortical layer 1, and their role in regulating neuronal circuits remain unclear. We have previously shown that human ILAs can develop in humanized glial chimeric mice. Following engraftment of astrocytes differentiated from human-induced pluripotent stem cells (hiPSC) into the mouse cortex, hiPSC-astrocytes develop morphological features characteristic of ILAs observed in the human brain and express canonical astrocyte markers.

Here we have extended this model to study the functional properties of ILAs. Since one of the hallmarks of astrocyte function is the intracellular calcium activity, ILAs expressing the calcium sensor GCaMP6s were imaged in cortical brain slices six months following engraftment. The spatiotemporal dynamics of calcium signals along the long interlaminar processes in response to purinergic and neuromodulatory stimulation were imaged with time-lapsed two-photon microscopy. In vivo calcium activity of ILAs in awake head-restrained mice are also examined by two-photon imaging through a cranial window. The result of our engraftment and imaging experiments demonstrate successful generation of a hiPSC-based chimera model that results in the development of functional ILAs. This model can also be used to study the functional properties of ILAs from individuals with developmental disorders such as Fragile X Syndrome (FXS), the most common monogenic form of inherited intellectual disability and leading monogenic cause of autism spectrum disorders. Comparison between activity of ILAs derived from FXS and control hiPSCs is ongoing. Our hypothesis is that FXS ILAs have altered functional properties that contribute to FXS pathogenesis.

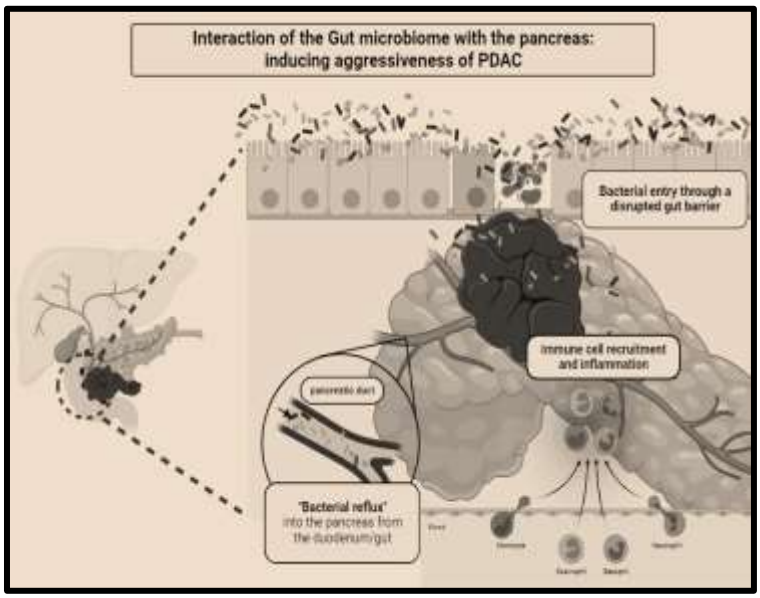
THE GUT CONNECTION: INTERPLAY OF THE MICROBIOME AND STEM CELL POPULATION IN PDAC

Kirtana Arikath[1], Seoung-Ryoung Choi[2], Prabagaran Narayanasamy[2], Zahraa Wajih I Alsafwani[1], Surinder.K.Batra[1] and Moorthy.P.Ponnusamy[1]

[1] Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE-68198

[2] Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE-68198

BACKGROUND: Pancreatic Ductal Adenocarcinoma (PDAC) is a malignant epithelial neoplastic tumor of the pancreas with a less than ten percent survival rate. While genetic anomalies have long been associated with cancer, recent studies have revealed a significant role played by the microbiome in the initiation and progression of various types of cancer. This implies that a better understanding of the microbiome's influence on cancer can lead to more effective treatments and therapies. Microbes can cause cancer-promoting inflammation and produce DNA-damaging toxins and carcinogenic metabolites, generating tumors that are more resistant to chemotherapy and immune responses. There is an altered microbiome in PDAC patients embedded within the highly desmoplastic tumor microenvironment (TME), which also contains cancer-associated fibroblasts (CAFs), immune cells, and, in particular, cancer stem cells (CSCs). The association between tumor aggression and microbial induced inflammation in the basal like subtype of PDAC has previously been shown. The degree of stress and inflammation in the surrounding microenvironment, brought on by the different additional components in the TME, is crucial for maintaining CSCs. It is thus reasonable to speculate that the microbiome is another essential factor influencing the evolution of CSCs.



SIGNIFICANCE: CSCs are crucial for metastasis, recurrence, tumorigenicity, and drug resistance. The human body houses a variety of microorganisms, including bacteria, fungi, and viruses, which comprise the microbiome. Each organ in the human body has its unique microbiome, with the gut microbiome receiving the most attention in recent times. Due to either "bacterial reflux" from the gut via the pancreatic duct or seepage of the gut microbiome into the pancreas via a breached epithelial barrier, the TME of PDAC is home to a dysbiotic microbiome. This dysbiotic environment may create a niche beneficial to the CSC population. Thus, modulating the microbiome in the PDAC TME can be of practical therapeutic efficacy in treating patients.

HYPOTHESIS: The microbiome plays a crucial role in enriching the cancer stem cell population, thereby promoting the aggressiveness of PDAC.

EXPERIMENTAL DESIGN: We Analyzed the existence of bacteria using Lipopolysaccharide (LPS) staining on human and mouse PDAC and Kras;PdxCre (KC) tumor samples along with dual staining of CSC markers. Fluorescent In-situ hybridization (FISH) was used to detect bacterial species in human and mouse PDAC and standard tissue samples. LPS treatment and treatment with bacterial supernatant and bacterial cell-free lysate with PDAC and regular pancreatic cell lines showed enrichment in particular CSC markers. This treatment established an environment where cancer cells are exposed to bacterial metabolites and toxins. A Co-culture model of three different bacterial species with mammalian PDAC and regular pancreatic cell lines was established to see enrichment in cancer stem cell maintenance by analyzing CSC gene expression through immunofluorescence.

RESULTS/DATA: Analysis of publicly available datasets showed a disrupted microbiome in the basal-like subtype compared to the classical subtype of PDAC, along with differential expression of certain bacterial species**.** We observed gram-negative bacteria in human and mouse PDAC samples using Lipopolysaccharide staining. We also observed the dual expression of stem cell markers CD44 and LPS. The presence of bacterial species in PDAC and normal pancreatic samples from humans and mice was confirmed using Fluorescent In-situ hybridization of bacterial 16srRNA. *In vitro*, treatment of cells with LPS and bacterial supernatant showed specific enrichment of certain stem cell markers and proliferation markers. Co-culture of three different bacterial species (Pseudomonas et al. baumanii, Sphingopyxis macrogoltabida) with mammalian PDAC cell lines enriched cancer stem cell markers, CD44 and Sox9.

CONCLUSION: Overall, our preliminary results validate our hypothesis, demonstrate the presence of bacteria in the PDAC TME, and shed light on the intricate connection between the gut microbiome and PDAC development, specifically through cancer stem cell population enrichment.

EHD1-MEDIATED REGULATION OF MICROTUBULE DYNAMICS IN PRIMARY CILIOGENESIS

Bazella Ashraf, Shuwei Xie, Naava Naslavsky, Steve Caplan

Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha NE 68198

Background: The primary cilium is an immotile, microtubule-based sensory organelle that can arise from the mother centriole (m-centriole) during the G0/G1 phase in almost every cell type. A key event in this crucial process is the removal of the capping protein, CP110, from the m-centriole. CP110 undergoes proteasomal degradation upon ubiquitination by the E3-Ub ligase HERC2, which is delivered via microtubule-based transport of centriolar satellites, a process reliant on the function of the endocytic regulatory protein, EHD1. Depletion of EHD1 prevents primary ciliogenesis, whilst compromising centriolar satellite movement. However, the precise mechanism by which EHD1 might regulate the centriolar satellite movement to the m-centriole is still unclear.

Significance of Problem: The sensory roles of the primary cilium are critical to normal homeostasis and development, owing to which defects in the process of ciliogenesis lead to a variety of diseases (ciliopathies), including cancer. Interestingly, a mutation in EHD1 also leads to a form of ciliopathy. Thus, elucidating the mechanism of EHD1-mediated regulation of centriolar satellite movement can greatly inform our understanding of these diseases and help unveil therapeutic targets. It could also reveal a new function for EHD1 as a microtubule-associated protein.

Hypothesis: Based on the manifest effects EHD1 depletion has on the localization and movement of centriolar satellites, we hypothesize that EHD1 regulates microtubule dynamics to control centriolar satellite movement and promote primary ciliogenesis.

Experimental Design: To explore the effect of EHD1 on microtubule dynamics, we exposed mock- and EHD1-siRNA treated HeLa cells to nocodazole for 30-40 minutes to depolymerize microtubules, followed by a washout to allow for microtubule recovery. The cells were then fixed and stained for alpha tubulin, and microtubule recovery was assessed by measuring the radius of the microtubule extension from the centrosome in individual cells. This was repeated with normal parental and EHD1$^{-/-}$ mouse embryonic fibroblast (MEF) cells. Next, to investigate the potential interaction between EHD1 and microtubules, coimmunoprecipitation between EHD1 and tubulins (α, β1, β3, and γ) was done in HeLa and retinal pigmented epithelial (RPE-1) cells. To verify the possibility of direct interaction and identify domains involved, GST-EHD1 fusion proteins (including GST attached to the EH domain only) were prepared, for use in an in-vitro microtubule binding assay.

Results: Our results show that the knockout of EHD1 in MEF and HeLa cells impedes microtubule regrowth after nocodazole-induced depolymerization. Additionally, we identified novel interactions between EHD1 and the predominant tubulins which constitute microtubules: α, β1, β3, and γ, in HeLa and RPE-1 cells.

Conclusions: Our observations of tubulin-EHD1 interactions and the effect of EHD1 on microtubule dynamics suggest a role in microtubule regulation, which in turn controls centriolar satellite movement required for ciliogenesis. Further studies are aimed at dissecting EHD1-microtubule interactions and understanding precisely how EHD1 factors in microtubule regulation to influence primary ciliogenesis, which may be crucial for understanding how EHD1 mutations lead to ciliopathy.

THE RELATIONSHIPS BETWEEN TIME TO ACL RECONSTRUCTION, SYNOVIAL FLUID BIOMARKERS, AND EARLY POST-OPERATIVE OUTCOMES

Authors: Atteberry M; Weldon N; Mormino S; Werner D; Fuentes-Rivera L; Tao M; Wellsandt E.; UNMC, Omaha, NE

Background: The amount of initial inflammation and knee effusion after anterior cruciate ligament (ACL) injury can be substantial, which has led to controversy between expediting ACL Reconstruction (ACLR) to hasten recovery or delaying surgery until a quiet knee is present (i.e., minimal pain, effusion, and motion deficits).

Significance of Problem: Patients who sustain ACL injury are at increased risk of meniscal and chondral tears, ACL reinjury, and knee osteoarthritis (OA); therefore, it is imperative to minimize these risks by reducing inflammation.

Question: This study aimed to examine the relationship between concentrations of inflammatory and joint health biomarkers in synovial fluid at the time of ACLR with the amount of time between ACL injury and ACLR and with 2-month post-operative pain, symptoms, activities of daily living (ADLs), range of motion (ROM), and gait biomechanics.

Experimental Design: This study is a secondary analysis of an observational longitudinal cohort study. The 36 participants were between the ages of 13 and 35, undergoing ACLR, and without previous knee injury or surgery. Synovial fluid was collected by the treating surgeon at the time of ACLR. Concentrations of biomarkers interleukin-6 (IL-6), matrix metalloproteinase-3 (MMP-3), and cartilage oligomeric matrix protein (COMP) were analyzed using ELISA assays.

Pain, patient-reported outcomes, range of motion (ROM), and gait biomechanics were evaluated 2 months after ACLR. Gait biomechanics were measured using an 8-camera Qualisys system (120 Hz; Göteborg, Sweden) with 2 embedded Bertec force plates (1080 Hz; Columbus, Ohio) and a forty-five retroreflective marker set. Following a static standing calibration, participants completed 5 gait trials that were used for analysis. Self-selected gait speed was maintained within ±5% during each trial at 2-month testing. Visual3D (C-Motion Inc.; Boyds, MD) was used to create an individualized model for each participant and calculate joint angles and external joint moments. Variables of interest included the peak knee flexion angle (PKFA) and peak knee flexion moment (PKFM) during the loading response and the peak knee adduction moment during the first 50% of stance (PKAM). Limb symmetry was determined by calculating the interlimb differences of each variable by subtracting the involved from the uninvolved limb. Participants completed the Knee injury and Osteoarthritis Outcome Score (KOOS) using the Research Electronic Data Capture (REDCap) system to evaluate knee pain, symptoms, and ADLs. Active knee flexion and extension ROM were measured using a standard goniometer. Pearson correlational analyses were used to quantify relationships between concentrations of synovial fluid biomarkers with a) time between ACL injury and ACLR and b) 2-month post-operative outcomes. *A priori* α was set to 0.05.

Results: Participant characteristics are presented in **Table 1**. In 11 participants, the treating surgeon was unable to collect a synovial fluid sample due to lack of knee effusion. Independent t-tests demonstrated that the average time to ACLR for the 25 participants with enough effusion to obtain a synovial fluid sample was shorter (38.2±13.4 days) compared to the 11 participants without knee effusion present (63.5±31.1 days) (p=0.001). At 2 months after ACLR, the groups with and without a synovial fluid sample at ACLR had no differences in pain, symptoms, ROM, or gait biomechanics (all p>0.05) (**Table 2**). In the 25 participants with a synovial fluid sample, a longer time between injury and ACLR correlated with lower concentrations of IL-6 (r=-0.40, p=0.045) and COMP (r=-0.40, p=0.045) but not MMP-3 (r=0.25, p=0.22). The concentration of IL-6 was negatively correlated with 2-month ADLs (r=-0.42, p=0.039). Greater MMP-3 levels trended toward worse 2-month symptoms (p=-0.38, p=0.064) and a lower PKFM during gait in the injured limb (r=-0.39, p=0.078). No other relationships between synovial fluid biomarkers and 2-month outcomes were present (all p>0.05).

Conclusion: Increased time between ACL injury and reconstruction correlated with lower markers of inflammation and cartilage breakdown in the synovial fluid at the time of surgery. Higher IL-6 in the synovial fluid at ACLR correlated to worse function with ADLs but not other outcomes at 2 months. MMP-3 may correlate with worse 2-month symptoms and gait patterns, whereas high concentrations of COMP appear to have no negative consequences on 2-month outcomes. Further work examining synovial fluid biomarkers at the time of ACLR in relation to early chondral changes, long-term functional outcomes, and return-to-sport outcomes is needed to better inform decision-making for timing of ACLR.

Table 1: Demographic and participant characteristic data.

	Mean (standard deviation) or Frequency
Age (years)	19.0 (4.6)
Sex (Female:Male)	24:12
Meniscal Repair Status (yes:no)	23:13
Graft Type (PT:QT:HS:Allo)	22:8:5:1
Synovial Fluid Sample (yes:no)	25:11

Abbreviations: PT, patellar tendon autograft; QT, quadriceps tendon autograft; HS, hamstring tendon autograft; Allo, allograft.

Table 2: Time to surgery and post-operative functional outcomes between participants with and without synovial fluid samples. Data presented are means with standard deviations in parentheses.

	Without SF	With SF	p-value
Time to Surgery (days)	63.5 (31.1)	38.2 (13.4)	0.001
KOOS Pain Score	76.9 (15.4)	79.0 (10.8)	0.656
KOOS Symptoms Score	62.5 (22.2)	64.9 (15.0)	0.717
KOOS ADL Score	85.6 (12.1)	87.4 (8.6)	0.629
Knee Extension Angle Difference (°)	-3.2 (3.1)	-2.6 (4.2)	0.680
Knee Flexion Angle Difference (°)	-11.4 (11.8)	-11.9 (13.5)	0.913
PKFA Difference (°)	-1.6 (5.2)	-4.6 (5.1)	0.140
PKFM Difference (N×m/kg×m)	-0.18 (0.19)	-0.22 (0.13)	0.422
PKAM Difference (N×m/kg×m)	-0.095 (0.16)	-0.074 (0.097)	0.659

Abbreviations: SF, synovial fluid; KOOS, Knee injury and Osteoarthritis Outcome Score; ADL, activities of daily living; PKFA, peak knee flexion angle; PKFM, peak knee flexion moment; PKAM, peak knee adduction moment during the first 50% of stance.

ANALYSIS OF CNS-DERIVED BLOOD EXOSOMES AS A POTENTIAL SOURCE FOR BIOMARKERS IN PARKINSON'S DISEASE

Ji Hye Bahn[1], Hyunha Kim[1], Melinda Burnett[2], and Jee-Yeon Hwang[1].

[1]Department of Pharmacology and Neuroscience, Creighton University School of Medicine, Omaha, NE, USA
[2]Department of Neurology, Creighton University School of Medicine, Omaha, NE, USA

Parkinson's Disease (PD) is the second most common neurodegenerative disease worldwide. PD is caused by severe loss of nigrostriatal dopaminergic neurons and is characterized by increases in cytoplasmic inclusions of α-synuclein. Identification of reliable biomarkers is critical for a timely diagnosis of PD. Although a recent study has reported αSyn-SAA technique with the high diagnostic accuracy of PD using cerebrospinal fluid, suitable laboratory tests are still quite limited and there are no blood-based tests available. In recent years, research on biofluid markers in PD has expanded, with particular emphasis on CNS-derived exosomes. Exosomes are nanovesicles that carry various proteins, lipids, mRNAs and microRNAs. Exosomes produced in the CNS can cross the blood-brain-barrier, making them a highly attractive source of biomarkers that can be isolated from peripheral blood. Several studies have shown a dysregulation of CNS-derived exosomes in PD patients' blood. These preliminary findings demonstrate the potential for clinical use of CNS-derived exosomal biomarkers in PD; however, research related to further validation and a large independent cohort study is needed for relevancy and accuracy. We conducted a pilot study to examine whether PD-related proteins, including α-synuclein, phosphorylated α-synuclein and DJ-1 in CNS-derived blood exosomes are significantly altered in PD patients *vs.* healthy controls. Toward this end, we isolated CNS-derived exosomes from blood samples of PD patients and controls and first, optimized the isolation protocol using healthy control blood samples. We confirmed successful isolation of CNS-derived exosomes with Western blot analysis on purified protein samples using antibodies against L1CAM. The results indicate that neuronal exosome marker L1CAM is detected only in CNS-derived exosomes. Next, to examine whether PD-related proteins are altered in CNS-derived blood exosomes, we performed Western blot analysis using the previously validated antibodies for α-synuclein, phosphorylated α-synuclein, PINK1, Parkin, and DJ-1. In our pilot study, we observed a significant increase in Parkin and a significant decrease in DJ-1 in the CNS-exosomes of PD patients compared to the control samples. These are preliminary findings from a small group of samples within our total sample pool. As we expect to continue blood collection in these next few months, we will report our progress from further investigation. Ultimately, our study may provide additional insight into the effort to find novel biomarkers for diagnosis and monitoring of PD.

RELATIONSHIP OF WOMEN'S REPRODUCTIVE STAGE AND PATIENT REPORTED LEG CRAMPS IN AN URBAN WOMEN'S HEALTH CLINIC

Authors: Emily Baxter, Jenenne Geske, Jennifer Griffin-Miller, and Birgit Khandalavala

Affiliations: University of Nebraska Medical Center, Omaha, NE

Background

This study describes the prevalence and impact of leg cramps in a female population. Muscle cramps are involuntary, spasmodic muscle contractions that most commonly occur in the lower extremity at rest. Leg cramps are most often reported in the calf or foot, and these painful muscle contractions can last from seconds to minutes. Most leg cramps occur at night, but daytime cramps are also commonly reported. Previous studies estimate the prevalence of leg cramps to be around 40%, and this value increases with age. Leg cramps are also associated with lower quality sleep and a decrease in overall health parameters. Additionally, there is an association between cardiovascular disease and an increased incidence of leg cramps. The etiology, prevalence, and treatment of leg cramps has been underexamined especially in women.

Experimental Design

A survey was given to patients at an urban midwestern women's health clinic during the months of June and July, 2023. The survey was available both as a paper copy and as a QR code for online self-entry. Women under the age of 19 and those who could not speak English were excluded from this study. The survey was developed based on a literature review of previous similar leg cramp research. Leg cramps were defined as "spasmodic, painful, involuntary muscle contractions when resting, lasting from a few seconds to minutes, usually affecting the calf and foot; a.k.a. "Charley horse". Patients were grouped based on reproductive stage which included pregnant women, menopausal women, and women who were neither pregnant nor menopausal.

Results / Data

Of the 260 total respondents, 45.2% reported having leg cramps. Women who were pregnant or who were in menopause reported a higher incidence of leg cramps. The prevalence of leg cramps also increased significantly in the final trimester of pregnancy. Most leg cramps were reported with rest, and a majority occurred at nighttime.

Conclusions

Our study aimed to discover the prevalence of leg cramps in women across different reproductive stages. There is a connection between leg cramps and both pregnancy and menopause which should prompt further research. It is unclear if there are any specific risk factors for developing leg cramps during pregnancy. Additionally, there is no clear effective treatment for leg cramps. Leg cramps are associated with poor sleep and a decline in health-related quality of life, so it is important for clinicians and researchers to address this widespread issue.

HIPPOCAMPAL VOLUME AND ANXIOUS TENDENCIES IN PERIADOLESCENT CHILDREN: PRELIMINARY FINDINGS FROM THE PRANK STUDY

Authors: Marie Bernard, Connor Phipps, Meghan Ramirez, Jennifer Sexton, Abi Heller-Wight, Abigail Zatkalik, Varun Sharma, Leah Heyen, Anna Wilhelm, Arthur Maerlender, Vaishali Phatak, Daniel Murman, David E. Warren
Affiliations: University of Nebraska Medical Center, Omaha, NE, University of Nebraska, Lincoln, NE

Background

Anxiety is characterized by intrusive, worrisome thoughts that are typically associated with changes in physiology, such as increased heart rate. These feelings and behaviors can manifest early in life and may persist unless treated. Periadolescence, a period of significant brain development, is a critical period where feelings of anxiety, changes in brain structure, and developing cognition may have relationships that have not been fully characterized. Certain brain regions, including the amygdala, have been the focus of investigations related to anxiety. Other brain regions that are structurally and functionally connected may also have a relationship with anxiety. The hippocampus has been implicated in context-dependent learning and conditioning, and the ventral hippocampus may play a role in emotion and stress. Prior research suggests decreased hippocampal volume as a potential risk factor for anxiety in adolescents.

Significance of Problem

The hippocampus develops heterogeneously throughout development. Despite the suggested relationship between total hippocampal volume and anxiety, the association with hippocampal subfields has not been determined. Additionally, if anxiety can affect the development of the hippocampus, it is possible that it can affect the development of normal memory function. The present study utilizes a large sample size (N=109) and hippocampal subfield volumes to fill these gaps and advance the state of the research.

Hypothesis

Anxious tendencies will significantly predict differences in hippocampal volume.

Experimental Design

The present study utilized participant data from the Polygenic risk of Alzheimer's disease in Nebraska Kids (PRANK) project. PRANK is an NIH/NIA funded study (R01 AG064247) focused on measuring the association between genetic risk factors for Alzheimer's disease, brain development, and cognitive abilities in periadolescent children. The current sample includes 109 periadolescents (57F) aged 8-13 that completed Child Behavior Checklist (CBCL) and an MRI study including data sufficient for the estimation of hippocampal volume. Anxiety was quantified using participant responses on CBCL prompts relating to heightened levels of fear, worry, and dependence/attachment to parents. Prompts are answered as "Not True" (0), "Somewhat or Sometimes True" (1), or "Very True or Often True" (2). Numeric values of answers were added together for a total anxiety behaviors score. Notably, participants are excluded from the PRANK study if they have a clinical diagnosis of anxiety. Therefore, participants answering affirmatively to these prompts demonstrate no more than subclinical anxious tendencies. MRI data were collected at UNMC's Core for Advanced Magnetic Resonance Imaging (CAMRI) using its Siemens 3T Prisma MRI instrument and a 32-channel head coil. Structural MRI data were collected, including a T2-weighted, turbo-spin-echo sequence which allowed for high-resolution imaging of the hippocampus. Automated FreeSurfer segmentation was used to identify the total volume of the hippocampus. Automated Segmentation of Hippocampal Subfields (ASHS) toolbox software was used for segmentation of hippocampal subfields. Segmentation was manually checked using a protocol developed in the Detroit Aging Brain Study, which has since been validated by other studies. Subfields were segmented into the entorhinal cortex, subiculum, cornu ammonis 1 (CA1), cornu ammonis 2 and 3 (CA2/3), and dentate gyrus (DG). Statistical analyses between anxious tendencies and hippocampal volumes were conducted using a regression model, with hippocampal volume as the dependent variable and anxiety scores as the independent variable, while controlling for age and gender, and correcting for intracranial volume.

Results

Total anxiety behaviors were a significant predictor of corrected left hippocampal volume, $t(100) = 2.47$, $p = .02$, such that an increase in total anxiety behaviors predicted a decrease in corrected left hippocampal volume. Total anxiety behaviors were also a significant predictor of corrected right hippocampal volume, $t(100) = 2.38$, $p = .02$, meaning that an increase in total anxiety behaviors predicted a decrease in corrected right hippocampal volume. Total anxiety behaviors were also a significant predictor of decreased volumes in various hippocampal subfields, including corrected left CA1 $t(100) = 2.81$, $p < .01$; corrected left subiculum, $t(100) = 2.32$, $p = .02$; and corrected left DG, $t(100) = 2.10$, $p = .05$.

Conclusions

The results suggest that subclinical anxious behavior is associated with a decrease in hippocampal volume. The hippocampal subfield data, specifically, suggest that subclinical anxious behavior is associated with decreased volume of the left CA1, left subiculum, and left DG. Further research of the relationship between anxiety and hippocampal subfields is critical to understanding the possible impact of anxiety on brain development and hippocampal-dependent memory.

THOMBOXANE-PROSTANOID RECEPTOR INHIBITORS ATTENUATE INFLAMMATION

Romilia Castillo [1], Vinothkumar Rajamanickam [1], Cyrus Desouza, and Viswanathan Saraswathi [1]

[1]Division of Diabetes, Endocrinology and Metabolism, Department of Internal Medicine, University of Nebraska Medical Center and VA Nebraska-Western Iowa Health Care System, Omaha, NE 68105, USA

Inflammation is critical for tissue recovery and fighting infections. Although this mechanism is part of the body's immune defense, chronic inflammation has been implicated in the pathophysiology of many obesity-related co-morbidities including non-alcoholic fatty liver disease, cardiovascular disease and diabetes mellitus. The transmembrane G-protein-coupled receptor, thromboxane A2 receptor (TXA2R), and the enzyme producing, TXA2 synthase (TXAS), may play a role in mediating an inflammatory response in a variety of cells. TXA2R and TXAS are widely expressed in different organs such as the heart, lungs and kidneys. TXA2R is well known to regulate vasoconstriction and hemostasis. In obesity-related chronic inflammation, free fatty acids (FFAs) are important mediators. However, the interaction between FFAs and TXA2R in mediating an inflammatory response remains unknown. Therefore, we sought to determine the role of TXA2R signaling in LPS or stearic acid-induced inflammatory response in peripheral blood mononuclear cells (PBMCs) and THP-1 macrophages. PBMCs and THP-1 cells were treated with TPR antagonist (SQ29548) or TXAS inhibitor, Ozagrel. Subsequently, the PBMCs were stimulated with stearic acid (SA), a saturated FFA, or lipopolysaccharide (LPS) to induce an inflammatory response. The inflammatory response was assessed by measuring the media levels cytokines and chemokines. Our results revealed that agents inhibiting TXA2R signaling attenuated LPS- and SA-induced secretion of tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β), interleukin-6 (IL-6) and Monocyte chemoattractant protein-1 (MCP-1/CCL2). These results suggest that TXA2R may present a therapeutic target to control inflammation and its complications.

Keywords: Cytokines, Inflammation, PBMC

3D PRINTED INTERVENTIONS AND THEIR CORRELATION WITH OVERALL SMOKING WITHIN PREGNANCY: A PILOT STUDY

Remington Del Coté[a], Daniele Dilsaver[b], Amy Badura-Brack[a], Pooja Doerman[b]John Joseph Coté[b]
[a]Creighton University, [b]Creighton University School of Medicine, Omaha, NE and Phoenix, AZ

Background: Researchers have tried to examine why some pregnant women can decrease or quit smoking in pregnancy and others don't. Recent research on the psychological processes involved in how smoking behaviors change within pregnancy has focused on maternal-fetal attachment (MFA). MFA, as defined by Condon, is the emotional tie or bond between a pregnant woman and her fetus (Condon & Corkindale, 1997). Initially, Magee et al. found that lower MFA scores were associated with greater smoking in pregnancy (Magee et al., 2014), and extended their findings by showing significant differences in attachment scores between smokers, non-smokers, and women who quit smoking (Massey et al., 2015). This association has more recently been substantiated by research showing significantly lower MFA scores in pregnant women who smoke compared to those who don't, and showing a negative relationship existed between nicotine addiction levels and MFA scores in pregnant women who smoked (Kartal & KOCATAŞ, 2023; Özçoban et al., 2020). Because of these associations, researchers have postulated that interventions that strengthen MFA may help facilitate less smoking or even increase the probability of smoking cessation while an individual is pregnant (Jussila et al., 2020; Kartal & KOCATAŞ, 2023; Slade et al., 2006).

Significance of Problem: In the United States, the percentage of mothers who smoked cigarettes at any time during pregnancy declined from 7.2% to 4.6% from 2016-2021 and in Nebraska, 6.5% of pregnant mothers smoked in 2021 (Martin et al., 2023). While we should celebrate this exceptional decrease in smoking in pregnancy, this does not negate the fact that smoking is one of the most preventable risk factors for poor pregnancy and birth outcomes. Smoking in pregnancy increases the risk of pre-term delivery, low birth weight babies, neonatal intensive care unit (NICU) admissions, miscarriages, congenital anomalies, and intrauterine fetal demises (IUFD) (Di et al., 2022; Sequí-Canet et al., 2022; Tarasi et al., 2022; Yang et al., 2022). While pregnant patients have proficient cessation treatments available to them, healthcare providers often are lacking in their provision of smoking cessation care during pregnancy (Bar-Zeev et al., 2019; Higgins et al., 2022)

Hypothesis, Problem, or Question: The aims of the present study were to (i) establish the relationship between maternal-fetal attachment scores and amount of smoking within pregnancy, and (ii) determine the effect 3D ultrasonography and 3D-printed models have on the overall amount of smoking in pregnancy.

Experimental Design: We performed a randomized clinical trial of pregnant smokers (n=33) using an intent to treat protocol. We recruited pregnant smokers and provided timeline follow back (TLFB) interviews from 27 weeks of pregnancy until 6 weeks post-partum. Patients answered Maternal antenatal attachment scale (MAAS) questionnaires upon entrance into the study and then one week after they were presented with a randomly assigned 3D image or 3D model of their fetus.

Results/Data: Thirty-three patients were randomized to 3D picture (n=16) versus 3D model (n=17). There was no difference in the demographic make-up between the groups regarding race, marital status, gestational age, education, or insurance. There was no significant difference between the groups regarding maternal-fetal attachment (MFA) scores after the intervention (86.4 +/- 7.3 vs 83.2 +/- 5.5 p= .177). The average cigarettes smoked per day was not statistically different between the groups before or after the interventions (p=.838 before; p=.868 after). The average cigarettes smoked per day WAS statistically different within the individual groups and as a collective before and after the interventions (p=.004 picture; p=.004 model; p=.00003 combined).

Timeline follow back (TLFB) and psychological characteristics stratified by intervention

	Pre		Post	
	3D Picture (*n* =15)	3D Model (*n* =17)	3D Picture (*n* =15)	3D Model (*n* =17)
TLFB				
Cig/day				
Total	7.5 ± 4.2	7.9 ± 7.5	5.9 ± 4.2	6.2 ± 6.4

Note Data presented as mean ± SD, median [IQR], or percent

Weight of Baby (g)	3147±240	3109±257	0.775
EGA at Delivery	39.2±0.8	38.4±1.4	0.065
Weight %	38±16	34±29	0.732
Hypertension	38	31	0.893

Conclusions: While only a pilot and neither intervention was statistically different pre and post administration, smokers in pregnancy on average smoked less cigarettes per day after the 3D interventions and had better outcomes.