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Loading of the medial knee ligaments and anterior cruciate ligament during clinical tests of anteromedial rotatory instability

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OBJECTIVES/GOALS: Injury to the medial knee ligaments (sMCL, dMCL, POL) and anterior cruciate ligament (ACL) can cause anteromedial rotatory instability (AMRI). AMRI can cause knee instability and ACL graft failure, but it is unclear how the sMCL, dMCL, POL, and ACL resist AMRI. We aimed to characterize the in-situ forces of the sMCL, dMCL, POL, and ACL under loading conditions involved with AMRI. **METHODS/STUDY POPULATION:** We characterized the in situ forces of the sMCL, dMCL, POL, and ACL under 1) isolated external tibial rotation torque (ER), 2) isolated anterior tibial force (Ant), and 3) combined ER+Ant loading. Twenty-eight human cadaveric knees (18 male; mean age, 48±13; 21–65 years) were tested on a robotic manipulator with force sensing. Tibiofemoral kinematics were recorded under isolated ER (4Nm, 0–90°), isolated Ant (134N at 0–90°), and combined ER+Ant (4Nm +100N at 15, 30, 90°). The sMCL, dMCL, POL, and ACL were dissected in random order. The in situ force (N) in the sMCL, dMCL, POL, and ACL at the peak applied load for each loading condition was calculated using superposition and compared with Kruskal–Wallis tests with post hoc pairwise testing using a Bonferroni–Holm correction for multiple comparisons ($\alpha = 0.05$). **RESULTS/ANTICIPATED RESULTS:** Under isolated ER, the force in the sMCL (32–52N) from 0°–90° exceeded that of the ACL, dMCL, and POL at each flexion angle ($p < 0.05$). Force in the ACL was the second highest (26–6N from 0°–90°). Force in the dMCL and POL was low (≤ 12 N). Under isolated Ant, the ACL carried the highest force at all flexion angles (≥ 113 N) ($p < 0.05$), but at 90° the sMCL carried the highest force of all ligaments ($p < 0.05$). At 90°, force in the dMCL diminished. **DISCUSSION/SIGNIFICANCE OF IMPACT:** We show that the sMCL is the major stabilizer to external rotation torques and combined anterior and external loading conditions related to anteromedial rotatory instability across the arc of knee flexion, while the dMCL, POL, and ACL play a less prominent role, with the exception of the ACL and dMCL near full extension.

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Genomic return of research service core with the precision health function of ICTS

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OBJECTIVES/GOALS: Returning genetic research results to participants can improve community engagement and enhance health equity. Providing research investigators with a convenient and cost-effective pathway for returning genetic findings, along with ensuring the necessary criteria for validity and utility, may reduce the barriers to returning results. **METHODS/STUDY POPULATION:** The ICTS Precision Health Genomic Return of Research Service Core (ICTS PH gROR) developed a process of returning genetic findings to participants who have indicated their preference to be notified of any findings that may impact their health.

The service includes returning primary findings (actionable results discovered as part of the Investigator's approved IRB study) and/or secondary findings [clinically actionable genes by the American College of Medical Genetic and Genomics (ACMG)]. Participants with positive findings will receive a written report, generated by a board-certified clinician specializing in genetics or molecular genetic pathology. A visit with a genetic counselor provides additional resources and guidance on the identified health risks. **RESULTS/ANTICIPATED RESULTS:** Centralizing a service for the return of genetic results will ensure best-practices and minimize the burden. By offering results at no cost to the participant or their family, the service promotes accessibility and removes financial barriers that could otherwise prevent individuals from benefiting from genetic insights. Furthermore, by involving expert oversight committees and genetic counselors in the process, participants will receive accurate information and appropriate guidance, enhancing their understanding of the results and empowering them to address any potential health risks. Subsidizing the service with the CTSA grant keeps the costs predictable and manageable for investigators. **DISCUSSION/SIGNIFICANCE OF IMPACT:** This approach recognizes the importance of informed consent, ethical considerations, and the potential social implications associated with genetic findings. Through open communication, participants are actively involved in the decision-making process and have the opportunity to seek further resources and support.

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The role of anemia and its treatment with iron in the development of oxygen-induced retinopathy

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OBJECTIVES/GOALS: The objective of this research is to characterize the role of anemia and its treatment with iron in the development of oxygen-induced retinopathy and to identify possible mechanisms of action for future investigation. **METHODS/STUDY POPULATION:** Newborn rats were exposed to cyclic hyperoxia (50%) hypoxia (10%) for 14 days to induce oxygen-induced retinopathy (OIR). Half of the pups were phlebotomized to induce anemia. Half of anemic and control pups received high-dose iron, while the other half received standard, low-dose iron. Retinas were dissected at postnatal day 20, and vascular outcomes were measured to determine phenotype. Comparisons will be made by two-way ANOVA. Additional future studies are planned: 1) electroretinogram to measure retinal function, 2) measure of retinal hypoxia and angiogenic protein levels to evaluate the effect on hypoxia-sensitive angiogenesis, 3) serum and retinal iron measures to determine iron status, 4) flow cytometry and cytokine array to evaluate immune cell response, and 5) exploratory single-cell RNA sequencing. **RESULTS/ANTICIPATED RESULTS:** Preliminary vascular outcomes are pending. We anticipate that retinas of anemic pups treated with low-dose (standard of care) iron will exhibit iron deficiency and hypoxia and reduced severity of OIR. Retinas of anemic and non-anemic pups treated with high-dose iron will exhibit iron excess and increased severity of OIR. However, retinal function will be reduced in anemia and rescued with treatment with high-dose iron. We further anticipate that anemia and iron-deficiency will down-regulate overall immune cell fractions, which will correlate with reduced cytokine and chemokine levels, suggesting a reduced immune response. Finally, we expect vascular endothelial cells and pericytes in anemic pup retinas to show increased angiogenic gene

expression and neurons to show signs of impaired development. **DISCUSSION/SIGNIFICANCE OF IMPACT:** We expect that anemia has a differential impact on retinal vascular anatomy and neuronal function. While retinopathy severity may be improved, overall retinal function will be dampened. These findings and the mechanistic work of this study will generate targets for intervention to preserve improved retinopathy outcomes but rescue retinal function.

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Gut-brain mechanisms of COVID-19 in wild type mice

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OBJECTIVES/GOALS: SARS-CoV-2 infection has been shown to impact multiple organ systems, including the brain, and is associated with increased cognitive decline in vulnerable populations. The gut microbiome may play a significant role in modulating these effects, as shifts in microbiota composition have been linked to inflammation and systemic disease processes. **METHODS/STUDY POPULATION:** To explore these interactions, we conducted an acute COVID-19 study using 12-week-old C57 mice intranasally inoculated with 1×10^4 PFU of the Mouse Adapted 10 (MA-10) strain of SARS-CoV-2. On day 5 postinfection, we performed 16S ribosomal RNA sequencing on fecal samples, analyzed using QIIME2 and DeSeq2, to assess microbiome alterations and identify changes in metabolic pathways associated with inflammatory responses. **RESULTS/ANTICIPATED RESULTS:** Analysis revealed notable shifts in the gut microbiome during the acute phase of COVID-19 infection, with significant alterations in metabolic pathways related to inflammation and immune regulation. These changes suggest that early gut microbiome disruptions may contribute to the host response in acute COVID-19. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Our findings indicate that acute SARS-CoV-2 infection leads to rapid alterations in the gut microbiome, which could underlie inflammatory and immune pathways impacting disease progression. These insights highlight the potential for microbiome-targeted therapies to mitigate acute COVID-19 morbidity and warrant further investigation.

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Effects of butyrate supplementation in modulation of gut microbiome and its metabolites in new-onset rheumatoid arthritis

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OBJECTIVES/GOALS: The gut microbiome and its metabolites, such as short-chain fatty acids (SCFA), are dysregulated in rheumatoid arthritis (RA); however, the significance of this observation and

its implications in pathogenesis and therapeutics is unclear. Here, we explore the role of the SCFA, butyrate, in treatment efficacy in new-onset rheumatoid arthritis. **METHODS/STUDY POPULATION:** We designed a proof-of-principle study to determine the effects of butyrate supplementation in new-onset RA (NORA) patients that fulfilled 2010 ACR/EULAR RA criteria. We evaluated the effects of methotrexate (MTX) plus butyrate in NORA (n = 17; 1 gm butyrate, 3 times daily) compared to MTX alone (n = 19) over 4 months. MTX responders were defined by a change in disease activity score (DAS)-28 ESR of > 1.8 at 4 months. Fecal samples were collected at baseline and followed up for 16S rRNA sequencing and metabolite quantification by 1H NMR spectroscopy. Unpaired-t, paired-t, Wilcoxon and Fisher's exact test were performed as appropriate. **RESULTS/ANTICIPATED RESULTS:** MTX responders in the MTX-only group had a higher concentration of fecal butyrate than nonresponders at baseline (p = 0.045). Fecal butyrate concentration decreased over time in treatment responders in MTX group (p = 0.05), whereas butyrate concentration remained similar in MTX/butyrate group. Prior to treatment, both MTX and MTX/butyrate groups demonstrated similar levels of gut bacterial alpha diversity (Shannon index), yet only the MTX/butyrate group demonstrated a significant increase in alpha diversity by 4 months (p = 0.022). LeSe analysis demonstrated increased abundances of *Bacteroides*, *Clostridium*, and *Phascolarctobacterium* in responders in the MTX/butyrate group by 4 months. Ten (52.6%) patients in MTX and 11 (64.7%) in MTX/butyrate group were considered MTX responders by 4 months (p = 0.516). **DISCUSSION/SIGNIFICANCE OF IMPACT:** Butyrate supplementation increased gut microbial diversity in patients and led to increased abundance of *Bacteroides*, which has been implicated in efficacy of methotrexate, a first line medication in rheumatoid arthritis. Butyrate may have implications for the maximization of therapeutic effectiveness in rheumatoid arthritis.

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Identification of gut barrier protective fruits and vegetables for Latin American infants

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OBJECTIVES/GOALS: Early childhood obesity is a major concern for Latin American children in the U.S., with gut barrier dysfunction as a key risk factor. Diet plays a role in gut development, but few studies have focused on Latin American infants. Our objective is to identify culturally relevant introductory foods that promote in vitro gut barrier development and function. **METHODS/STUDY POPULATION:** Pooled human milk (2.5 mL) from 6-month postpartum Hispanic mothers was combined with fruit and vegetable baby food products (2.5 g) and subjected to a 3-phase in vitro digestion system that simulates oral, gastric, and intestinal digestion. Digested products were then anaerobically fermented for 24-hours