55th Annual Midwest Student Biomedical Research Forum

Saturday, March 2, 2024

ROOM 3048

1:45 p.m. **O-19** EXPLORING THE RELATIONSHIP BETWEEN KNEE BIOMECHANICS, PHYSICAL ACTIVITY, AND SERUM BIOMARKERS BEFORE AND AFTER ACL RECONSTRUCTION
Presenter: Lorena Fuentes-Rivera, UNMC

2:00 p.m. **O-22** KNEE EXTENSION RANGE OF MOTION DEFICITS POST-ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION ASSOCIATE WITH EARLY SIGNS OF KNEE OSTEOARTHRITIS
Presenter: Jayden Harrington, Clarke University

2:15 p.m. **O-26** EVALUATING THE MOLECULAR DETERMINANTS OF PDAC RACIAL HEALTH DISPARITIES
Presenter: Anthony Johansen-Sallee, UNMC

2:30 p.m. **O-45** IMPACT OF GRAFT TYPE AND MENISCAL STATUS ON PATELLOFEMORAL CARTILAGE EARLY AFTER ACL RECONSTRUCTION
Presenter: Samuel Mormino, UNMC

2:45 p.m. **O-60** GENOTYPE-PHENOTYPE ANALYSIS OF VALOSIN-CONTAINING PROTEIN DISEASE VARIANTS
Presenter: Sarah Robinson, Creighton University

3:00 p.m. **O-63** VIDEO ANALYSIS OF MULTI-LIGAMENT KNEE INJURY IN NFL ATHLETES
Presenter: Ethan Ruh, UNMC

3:15 p.m. **O-66** SUBSPECIALTY FACULTY IN OBSTETRICS AND GYNECOLOGY: A WORKFORCE ANALYSIS
Presenter: Morgan Steffen, UNMC

3:30 p.m. **O-70** BEDSIDE-TO-BENCH: GRANULOCYTE HETEROGENEITY DURING HUMAN CRANIOTOMY INFECTION
Presenter: Zachary Van Roy, UNMC

3:45 p.m. Break

Title: EXPLORING THE RELATIONSHIP BETWEEN KNEE BIOMECHANICS, PHYSICAL ACTIVITY, AND SERUM BIOMARKERS BEFORE AND AFTER ACL RECONSTRUCTION

Authors: Lorena Fuentes-Rivera, Mikayla McKee, Mazie Atteberry, David Werner, Alyx Jorgensen, Matthew Tao, Elizabeth Wellsandt

Background: Anterior cruciate ligament (ACL) injuries are a leading cause of early knee osteoarthritis (OA), with approximately 50% of individuals developing the condition within a decade of injury. Early detection of OA following an ACL injury remains a significant challenge, as traditional diagnostics often recognize the disease too late for interventions. Recent advancements spotlight the potential of biochemical markers in the early detection and pathogenesis of OA after ACL injury. Specifically, N-terminal crosslinking telopeptide of type I collagen (NTX-I) is indicative of increased bone turnover, while matrix metalloproteinase 3 (MMP3) and cartilage oligomeric matrix protein (COMP) are markers for cartilage degradation. Higher values of MMP3 and COMP are associated with disease progression. Further, both gait biomechanics and physical activity (PA) levels have emerged as markers for risk of OA development. While studies have investigated biochemical markers individually with gait biomechanics, their relationship with PA levels remains unknown. The purpose of this analysis was to evaluate the relationship between gait biomechanics, PA levels, and serum biomarkers before and within six months after ACL reconstruction (ACLR).

Design: In a prospective cohort study, we enrolled 35 individuals aged 14-35 within one month of ACL injury with no previous history of knee injury or surgery. A 7-mL blood draw was collected before and 6 months after ACLR. The serum was isolated and analyzed using ELISA kits for the following biomarkers: NTX-I, MMP-3, and COMP. Gait biomechanics and PA were measured before ACLR and 2, 4, and 6 months after ACLR. Biomechanics were collected using an 8-camera motion capture system (Qualisys AB, Gothenburg, Sweden) sampled at 120 Hz and two embedded force plates (Bertec Corporation, Columbus, OH) sampled at 1080 Hz. Participants walked a 5.4-meter pathway at a self-selected speed established during pre-operative testing and maintained ±5% during post-operative testing sessions. Knee joint moments were determined using an inverse dynamics technique within Visual3D software (C-Motion, Bethesda, MD) and normalized to body mass and height. The variable of interest was the external knee flexion moment (KFM) impulse measured using the trapezoidal rule during stance phase. Participants wore a tri-axial accelerometer (wGT3X-BT, Actigraph LLC, Pensacola, FL) during waking hours of the 7 days following each testing session. A valid week of wear required 10 hours of wear for at least four days. Periods of inactivity were defined as 90-minute intervals with zero activity counts. PA variables of interest included daily step counts and daily minutes of moderate-to-vigorous physical activity (MVPA). Cumulative knee joint loading was calculated as the product of KFM impulse and daily step counts to provide an estimate of total knee joint loading during a single day. Pearson correlations were used to evaluate the relationship between biomarkers, biomechanics, and PA. For statistically significant correlations, linear regression models were used to determine the strength of the association after adjusting for sex, body mass index (BMI), and age. A p-value of 0.05 was set *a priori*.

Results: Participants were 19.0±4.6 years old, 62.9% female, and had an average BMI of 24.9±4.5 kg/m^2. Changes in serum biomarker values between before and 6 months after ACLR (Δ) are presented in Table 1. Mean gait biomechanics and daily PA levels are provided in Table 2. Pearson correlations between serum biomarkers with KFM impulse, MVPA, daily steps, and daily cumulative knee joint loading showed that a higher KFM impulse at both 2 and 4 months after ACLR correlated with lower 6-month COMP levels but not ΔCOMP levels from before to 6 months after ACLR. Higher levels of MVPA at all four times points and daily steps at 4 months were correlated with greater decreases in NTX-I. Conversely, greater MVPA and daily steps at 4 months was associated with a greater increase in COMP, while greater MVPA and daily steps at 6 months was associated with higher levels of MMP3 at 6 months. Further, more daily steps at 4 months were correlated with a greater increase in MMP3. Finally, greater cumulative knee joint loading at 2 and 4 months was correlated to lower COMP levels at 6 months after ACLR. After adjusting for age, BMI, and sex, nearly all biomechanical and PA variables of interest remained significantly associated with 6-month levels of COMP, NTX-I and MMP3.

Conclusion: Our findings underscore the significant relationships between gait biomechanics, PA levels, and early biochemical markers of joint health after ACL reconstruction. Higher knee joint loading during walking (KFM impulse) was associated with lower (better) COMP levels at 6 months after surgery. Further, our findings suggest that higher levels of MVPA are associated with improved NTX-I levels at 6 months. However, higher step counts were associated with higher (worse) levels of COMP and MMP3. Future research is needed to further understand the complex relationships between joint biomechanics and PA levels in relation to early OA development to optimize joint health after ACLR.

Table 1. Serum Biomarker Values	**COMP (nM)**	**NTX-I (nM)**	**MMP3 (nM)**
	Mean ± Standard Deviation [95% Confidence Interval]		
6 months	138.0 ± 44.1 [123.4 - 152.6]	15.9 ± 6.7 [13.6 - 18.2]	11.1 ± 6.3 [8.8 - 13.2]
Δ Pre-ACLR to 6 months	4.0 ± 48.7 [-12.4 - 20.4]	-8.0 ± 11.2 [-11.8 - -4.2]	0.7 ± 4.6 [-0.9 - 2.3]

Abbreviations: nM, nanomole; Δ, change; ACLR, anterior cruciate ligament reconstruction.

Table 2. Gait Biomechanics and PA Before and After ACLR	**Pre-ACLR**	**2 months**	**4 months**	**6 months**
	Mean ± Standard Deviation			
KFM Impulse (N·m /kg·m·s)	0.05±0.02	0.03±0.02	0.03±0.03	0.03±0.02
Daily MVPA (minutes)	23.8±19.5	20.1±14.3	37.9±22.4	38.3±24.4
Daily Steps	5690.4±2568.7	5354.1±1722.8	7449.6±2543.7	7832.4±2729.1
Cumulative Knee Joint Loading	301.6±183.7	142.7±130.1	240.0±210.9	216.7±154.9

Abbreviations: N, Newton; m, meter; kg, kilogram; s, second; min, minute, ACLR, anterior cruciate ligament reconstruction.

KNEE EXTENSION RANGE OF MOTION DEFICITS POST-ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION ASSOCIATE WITH EARLY SIGNS OF KNEE OSTEOARTHRITIS

*Harrington J, Weaver B, Manzer M, Tao M, Wellsandt E.
*Clarke University, Dubuque, IA

Background: Early knee osteoarthritis (OA) is a common consequence of anterior cruciate ligament (ACL) injury. The mechanisms of early OA development following ACL injury are poorly understood, emphasizing the necessity of early interventions to prevent and delay articular cartilage breakdown. Restoration of knee range of motion (ROM) is an important clinical milestone early after ACL reconstruction. The purpose of this study was to determine if knee extension ROM deficits at two months after ACL reconstruction (ACLR) associate with cartilage T2 relaxation times at six months after ACLR.

Significance of Problem: Fifty percent of patients develop knee OA within 12-14 years of ACLR. The common clinical symptoms of knee OA are disability and pain.

Hypothesis: We hypothesized that extension deficits two months after ACLR would be associated with longer (worse) T2 relaxation times in the tibiofemoral articular cartilage at six months after ACLR.

Experimental Design: Thirty participants (15-35 years old) were enrolled within 1 month of ACL injury before ACLR. Exclusion criteria included a previous injury or surgery to either knee, concomitant grade III tear to other knee ligaments, meniscus tear with anticipated meniscectomy by the treating orthopaedic surgeon, acute chondral lesions or pre-existing degenerative changes, or open growth plates requiring altered ACLR technique (i.e., physeal-sparing). Participants completed quantitative MRI before ACLR and six months after ACLR. Mean T2 relaxation times, a measure of water content and marker of OA, was calculated in regions of interest in the tibial and femoral cartilage (Figure 1). The MRI variable of interest was the percent change in mean T2 relaxation from before to six months after ACLR. Active knee extension ROM was measured at two months after ACLR using a standard goniometer with the participant in supine and a bolster placed under the ankle. An extension difference greater than 3º of the injured limb compared to the uninjured limb was considered abnormal and thus classified as an extension deficit. Independent t tests were used to determine if percent change in cartilage T2 relaxation time in the injured knee were different between those that did and did not restore knee extension ROM at two months after ACLR.

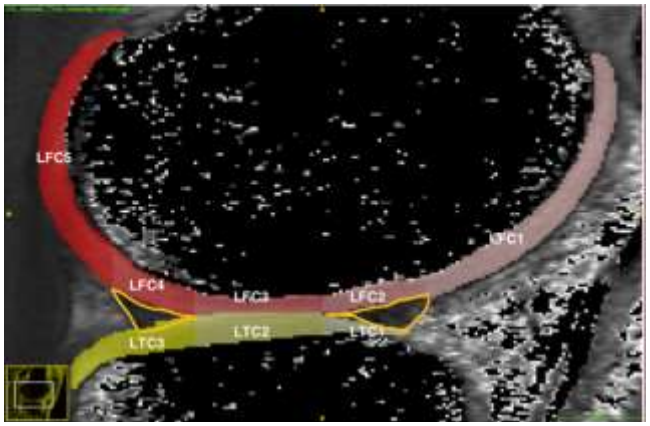

Figure 1: Segmentation of cartilage. Region 1 represents anterior cartilage, combined regions of 2, 3, and 4 represent central weightbearing cartilage, and region 5 represents posterior cartilage in each tibiofemoral compartment.

Results: Participants were 18.8±3.9 years old and 63.3% female. Fifteen of 30 participants (50%) had a knee extension ROM deficit in the injured limb at 2 months after ACLR. At six months after ACLR, participants with a knee extension deficit had significantly greater (worse) increases in T2 relaxation times in the anterior cartilage of the medial trochlea ($p=0.003$; Extension Deficit: 16.2±9.3%; No Extension Deficit: 6.0±8.2%) and the central weightbearing cartilage of the medial femoral condyle ($p=0.004$; Extension Deficit: 12.6±4.7%; No Extension Deficit: 6.5±5.8%). T2 relaxation times were not different between groups in other cartilage regions (all $p>0.05$).

Conclusions: The results of the study partially support our hypothesis that knee extension ROM deficits after ACLR are associated with longer (worse) cartilage T2 relaxation times. Extension deficits at two months after ACLR were associated with worse MRI markers of cartilage health in the injured limb at six months after ACLR in the anterior and weightbearing compartments of the medial femoral condyle. Our findings support previous work indicating the risk of OA after ACL injury is high in the medial tibiofemoral and patellofemoral compartments. Regaining knee extension ROM after ACLR is a critical clinical target that may decrease the risk for later OA development.

EVALUATING THE MOLECULAR DETERMINANTS OF PDAC RACIAL HEALTH DISPARITIES

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Pancreatic cancer is the 3rd leading cause of cancer-related deaths with a 5-year survival rate of just 12%. An estimated 64,000 new cases of pancreatic cancer would be diagnosed, and around 51,000 patients would die from pancreatic cancer in 2023. In addition to the poor survival rate, pancreatic cancer is typically diagnosed at the distant and regional stages of disease, around 52% and 30% respectively. Pancreatic ductal adenocarcinoma (PDAC), accounting for over 90% of pancreatic malignancies, has the highest incidence rate, mortality rate, and shortest survival times in non-Hispanic black (defined here as African and/or African American ancestry) patients compared to other races. Due to the racial health disparities of PDAC, this study seeks to quantify the proteomic signatures associated with tumor racial origin to identify the underlying molecular pathways that could contribute to these disparities. Using mass spectrometry on primary PDAC tumor samples collected from 30 Caucasian and 12 African American patients, 183 proteins were differentially expressed between these groups. The most over-expressed protein in African American tumors was Ring Finger Protein 2 (RNF2) with a log2 fold-change greater than six. RNF2 had previously been found to regulate GATA Binding Protein 6 (GATA6) in embryonic stem cells. Additionally, GATA6 is also an important determinant in PDAC subtyping with higher GATA6 expression leading to greater chemosensitivity. To expand upon these findings, chromatin immunoprecipitation and sequencing (ChIP-seq) was performed on MIA-PaCa2 to identify binding sites for RNF2 in PDAC cells. Peaks were called with MACS2 (q <0.01) and a final peak list with an irreproducible discovery rate (IDR) of less than 0.05 across two replicates was generated. From the 1,142 replicated peaks identified, RNF2 peaks were localized over the transcription start site of 644 unique transcripts, including *GATA6*. Modulating RNF2 levels by exogenous expression or short hairpin RNA (shRNA) knockdown confirms that *GATA6* is a target of RNF2 repression in PDAC. To further identify its downstream targets in PDAC, RNF2 knock down in MIA-PaCa2 was analyzed by RNA-seq. Differential gene expression analysis revealed RNF2 knockdown decreased expression of genes related to innate immunity, as well as inflammatory signaling, which we have previously identified as a marker of the aggressive Inflammatory PDAC subtype using proteomics. Of the 4,763 differentially expressed genes (P-adj < 0.05), 226 had RNF2 binding sites, demonstrating the scope of RNF2 regulation in MIA-PaCa2. Our work identifies differences in the underlying proteome of PDAC tumors as a function of racial origin, with overexpression of RNF2 in African American tumors potentially contributing to inflammation and subtype delineation. Because PDAC subtypes are predictive of therapeutic response, additional research is necessary to determine whether targeting RNF2 can mitigate oncogenic phenotypes that contribute to racial health disparities.

IMPACT OF GRAFT TYPE AND MENISCAL STATUS ON PATELLOFEMORAL CARTILAGE EARLY AFTER ACL RECONSTRUCTION

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Background, Significance, Hypothesis: Anterior cruciate ligament reconstruction (ACLR) restores passive knee stability but does not reduce the rate of osteoarthritis (OA). Meniscal status, requiring repair or meniscectomy, is a known component of degenerative progression. Regarding the patellofemoral joint (PFJ) specifically, traditional imaging modalities have shown evidence that patellar tendon autograft (BPTB) is associated with an increased risk of OA. T2 relaxation time using quantitative MRI (qMRI) is an established method to identify early cartilage changes. However, T2 relaxation in the PFJ as a function of graft type and meniscal status has not been previously evaluated. We aimed to evaluate the effect of autograft type (BPTB, hamstring [HT] and quadriceps tendon [QT]) as well as concomitant meniscal surgery (repair or meniscectomy) at the time of ACLR on T2 relaxation time in PFJ cartilage. We hypothesized that BPTB grafts, concomitant meniscal repair, and meniscectomy would result in higher (worse) T2 relaxation times at 6 months after ACLR. We also evaluated the effect of abnormal joint loading during gait on the PFJ cartilage. We hypothesized that T2 relaxation times would not differ in any group after controlling for knee joint loading during walking.

Methods and Study Design: Thirty-four participants aged 15-35 years were enrolled prospectively within 1 month of ACL injury. Exclusion criteria included prior knee injury or surgery, concomitant grade III tear to other knee ligaments that was symptomatic or required surgical intervention, and presence of chondral damage. Sagittal qMRIs were obtained within 1 month of injury (but prior to ACLR) and 6 months after ACLR. Articular cartilage of the PFJ was manually segmented using ITK-SNAP software (Penn Image Computing and Science Laboratory, University of Pennsylvania) and was confirmed for accuracy by a board-certified musculoskeletal radiologist. Mean T2 relaxation times were extracted from the trochlear and patellar cartilage. Non-physiological T2 relaxation times less than 10 ms and greater than 90 ms were excluded. Gait biomechanics were captured using two in-ground force plates (Bertec Corporation, Columbus, OH) sampled at 1,080 Hz and retroreflective markers with an 8-camera motion capture system (Qualisys, Göteborg, Sweden) sampled at 120 Hz. Data was processed using Visual 3D software (C-motion, Bethesda, MD). Using an inverse dynamic approach, joint angle and force plate data were used to calculate the knee flexion moment (KFM) impulse normalized to mass and height during the stance phase. Separate one-way ANOVAs were used to evaluate the relationship between graft type and concomitant meniscus surgery with the percent change in T2 relaxation time in PFJ cartilage from baseline to 6 months after ACLR. To determine the influence of gait biomechanics on T2 relaxation time across groups, general linear models were used with the interlimb ratio (involved/uninvolved) of KFM impulse as a covariate.

Results: The participants had a mean age of 19.0±4.7 years, and 21 (61.8%) were female. Graft type included 22 BPTB, 4 HT and 8 QT. Seventeen participants (50.0%) had a concomitant meniscus repair and 3 (8.8%) had a meniscectomy. Overall, T2 relaxation time increased 8.7±7.3% in the patellar cartilage (pre-ACLR: 39.1±2.6 ms, 6 months: 42.4±3.6 ms, $p<0.001$) and 7.1±7.3% in the trochlear cartilage (pre-ACLR: 46.3±2.6 ms, 6 months: 49.5±2.7 ms, $p<0.001$).

The change in T2 relaxation time 6 months after ACLR did not differ based on graft type in either the patellar ($p=0.729$) or trochlear cartilage ($p=0.525$) (Figure 1). Group differences remained insignificant after controlling for knee loading (KFM impulse) during gait (patellar: $p=0.594$; trochlear: $p=0.222$).

Meniscal status was also not associated with a change in T2 relaxation time in either the patellar ($p=0.392$) or trochlear cartilage ($p=0.190$) (Figure 2) although there was a trend toward higher T2 relaxation times in those with a concomitant meniscus repair or meniscectomy. Group differences remained statistically insignificant after controlling for the KFM impulse during gait (patellar: $p=0.400$; trochlear: $p=0.197$).

Conclusion: Our findings provide insufficient evidence that graft type is associated with worse quantitative chondral changes within the PFJ at 6 months following ACLR. While meniscal status is a known risk factor for increased contact pressure in the involved compartment and subsequent degenerative progression, our data does not demonstrate statistically significant changes to the PFJ cartilage when meniscal tears were present, likely due to the limited sample size and large variation within groups. Future work with larger cohorts is needed to confirm our findings and further investigate the role that graft type and meniscal status may play within the PFJ after ACLR.

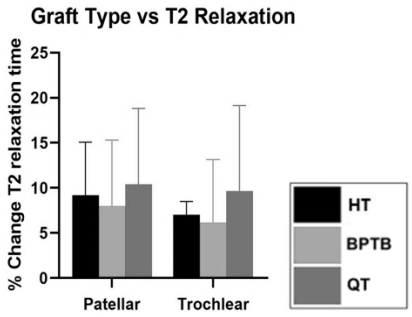

Figure 1. Cartilage T2 relaxation time across graft types.

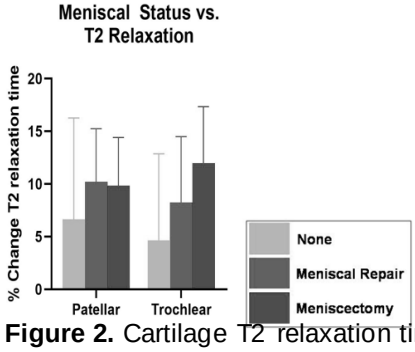

Figure 2. Cartilage T2 relaxation time across concomitant meniscus procedures.

Title: GENOTYPE-PHENOTYPE ANALYSIS OF VALOSIN-CONTAINING PROTEIN DISEASE VARIANTS

Authors: Sarah E. Robinson[1], Chris Weihl, Andrew Findlay

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Background: Valosin-containing protein (VCP) disease is condition caused by mutations in the p97/VCP ATPase. Mutations in VCP disrupt autophagy and trafficking and increase its ATPase activity due to structural changes. Clinically, VCP disease presents as Paget's disease of bone (PDB), frontal temporal dementia (FTD), and inclusion body myositis (IBM). Over 20 unique mutations have been identified, but the correlation between genotype and phenotype remains poorly understood.

Significance of Problem: VCP disease is a rare condition with no treatment and varying courses of onset and progression. As several therapeutics currently being developed, there is a need for tests or biomarkers that can predict disease onset and assess its severity. Currently there are no such tests, so availability of such tools is crucial for achieving clinical trial readiness.

Hypothesis or Problem or Question: Does in vitro ATPase activity of the most common p97/VCP mutations correlate with age of disease onset, clinical features, or disease severity?

Experimental Design: Previously collected international retrospective data and a review of literature were assessed and patients with the R155C, R155H, R93C, and R159H (Fig.1 A, B) mutations in the VCP gene were pulled out. A database was generated and analyzed phenotypic differences among the variants, including age of onset and loss of ambulation, and weakness patterns. For each VCP mutation, phenotypic data was correlated with VCP ATPase activity as measured by an *in vitro* assay using purified human VCP protein. VCP mutations were generated by site-directed mutagenesis of a VCP plasmid (TCB197) to create the above variants. One-way ANOVAs with multiple comparisons were run to assess significance between mutations.

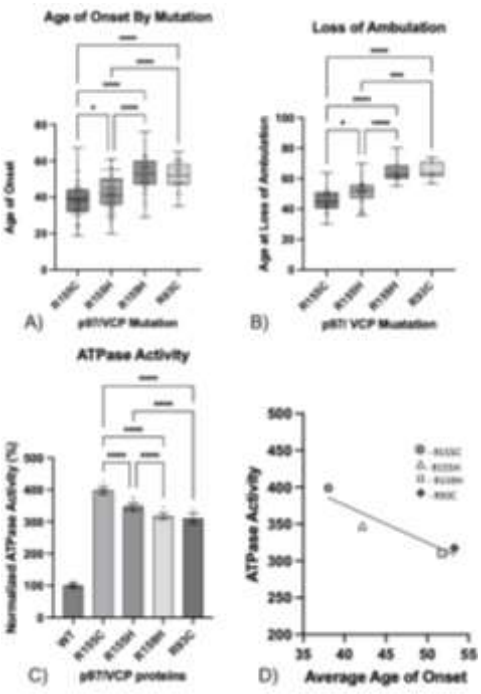

Figure 1. A) Age of onset by genotype. B) Age at loss of ambulation by genotype. C) Normalized ATPase activity by p97/VCP protein mutation. D) Linear regression of ATPase activity with age of onset by VCP genotype (r=-0.9376). (<0.05, **<0.01, ***<0.001, **** <0.0001)*

Results: Retrospective clinical data indicated that R155C has a significantly earlier onset and age at loss of ambulation than R155H, R159H, and R93C mutations. Similarly, the R155C protein had significantly higher ATPase activity than the other mutations (Fig.1C) R155H had a significantly earlier age of onset and loss of ambulation with higher ATPase activity than R159H and R93C (Fig.1 A,B,C). No difference was detected between R159H and R93C in either ATPase activity or clinical age of onset. Those genotypes appear to have later disease onset with lower overall ATPase activity. ATPase activity inversely correlates with average age of onset by mutation (r^2=0.879) (Fig 1.D). It appears that R155C has the earliest age of disease onset of the three with correspondingly high ATPase activity. Weakness patterns and respiratory involvement data are still being analyzed.

Conclusion:

VCP ATPase data inversely correlates with VCP disease progression as characterized by age of onset and age at loss of ambulation. The R155C mutation has an early onset and early age at loss of ambulation which has previously been recognized as a risk factor associated with death. These findings underscore the potential of in vitro ATPase assays as a tool for predicting the clinical spectrum of VCP disease variants and may serve as a biomarker for assessing disease prognosis and severity.

VIDEO ANALYSIS OF MULTI-LIGAMENT KNEE INJURY IN NFL PLAYERS

O-63

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Background: The multi-ligament knee injury (MLKI), is defined as a tear of more than one ligament in the knee. Previous research utilizing video analysis on ACL injuries in NFL players found over 70% occurred in non-contact or indirect contact mechanisms and identified patterns of knee, foot, and hip position at the time of injury. Video analysis of MLKIs has been limited and MLKIs are far less understood than isolated ACL injury.

Significance of Problem: A review of fifty NFL athletes that sustained a MLKI identified a return-to-play rate of 64%, though it was dependent upon which ligaments were involved. Athletes who suffered MLKIs involving the ACL and MCL had a return-to-sport rate of 70.8% though only a 43.5% chance of returning to their original level of play. In contrast, athletes that sustained MLKIs involving the ACL and LCL or PCL had return-to-sport rates of 55.6%, with only an 18.5% chance of returning to their original level of play.

Hypothesis, Problem, or Question: We hypothesized that the mechanism of multi-ligament knee injury will vary based on its categorization.

Experimental Design: A retrospective video analysis of official NFL game footage spanning 1997-2022 was performed. Players with MLKIs were identified from publicly available NFL injury surveillance data and confirmed via web search of team injury reports. Athletes were placed into categories based upon ligaments injury pattern: Group 1: ACL + MCL, Group 2: ACL + LCL and ACL + PCL, Group 3: ACL + MCL + PCL and ACL + LCL + PCL. Video analysis was performed to categorize injuries based on non-contact, indirect contact, or direct contact mechanisms while also determining the position of the hip, knee, and foot at the time of injury. Differences across MLKI groups were identified by Fisher Exact Test.

Results/Data: 35 MLKIs were identified and utilized in video analysis. MLKIs most often occurred from direct contact to the affected limb (80%), with the second most common injury mechanism being indirect contact (14.3%). The most common MLKI was injury to the ACL and MCL (65%), the second most common was injury to the ACL and LCL (15%). Direct contact MLKI most often resulted in a valgus and external rotational force about a flexed knee (58%). There were statistically significant differences noted in location of contact, position of the hip and knee by injury category (Table 1).

Conclusions: Multi-ligament knee injuries in the NFL most frequently occur from direct contact forces, often while a ball carrier is being tackled. Combined ACL and MCL injury was the most common multi-ligament knee injury pattern. Combined ACL and MCL injury most commonly occurred with knee flexion, valgus, and external rotation of the tibia. Multi-ligament injuries involving three ligaments occurred only from direct contact.

Table 1. Differences in Location of Contact, Knee Position and Hip Position in Video Analysis. Data presented as Count (Percentage of Category).

Parameter	Categorization	Category I ACL + MCL	Category II ACL + LCL ACL + PCL	Category III ACL + LCL + PCL ACL + MCL + PCL	P value
Location of Contact	Anterior/Anteromedial	0 (0.0%)	2 (50.0%)	0 (0.0%)	0.009
	Lateral/Posterolateral	16 (84.2%)	2 (50.0%)	2 (40.0%)	
	Anterolateral	3 (15.8%)	0 (0.0%)	3 (60.0%)	
Knee Flexion/Extension	Flexion	19 (82.6%)	1 (16.7%)	4 (80.0%)	0.009
	Extension	4 (17.4%)	5 (83.3%)	1 (20.0%)	
Knee Valgus/Varus	Valgus	23 (100%)	2 (33.3%)	4 (80.0%)	<0.001
	Varus	0 (0.0%)	4 (66.7%)	1 (20.0%)	
Knee Rotation	Internal	3 (13.6%)	4 (66.7%)	3 (60.0%)	0.002
	External	19 (86.4%)	2 (33.3%)	2 (40.0%)	
Hip Rotation	Internal	21 (91.3%)	2 (33.3%)	5 (100%)	0.009
	External	2 (8.7%)	4 (66.7%)	0 (0.0%)	

SUBSPECIALTY FACULTY IN OBSTETRICS AND GYNECOLOGY: A WORKFORCE ANALYSIS

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Background: A recent Obstetrics and Gynecology subspecialty workforce analysis in 2017 by Rayburn et al. demonstrated an increasing trend in subspecialization, with one out of four residents pursuing subspecialty training and one in three expected to pursue subspecialty training by 2020. Among residents, a subspecialist mentor is an essential factor in deciding to pursue fellowship. Additionally, the Accredidation Council for Graduate Medical Education requires a designated faculty member for each subspecialty.

Significance of Problem: The Obstetrics and Gynecology subspecialist workforce census, diversity and distribution have implcations for residency training programs as they work to meet Accredidation Council for Graduate Medical Education program requirements and recruit residents, for the subspecialty workforce and also for pateint care as the diversity and distribution of the workforce increase and change.

Hypothesis, Problem, or Question: What percentage of the subspecialty workforce is involved in the clinical training of Obstetrics and Gynecology residents? What are the demographics and distribution of the subspecialists workforce involoved in the clinical training of Obstetrics and Gynecology residents?

Experimental Design: This cross-sectional, observational study used public data collected from July 1, 2022, through August 31, 2022. A list of Obstetrics and Gynecology residency programs, their sponsoring sites, and affiliated sites, was compiled from the American Medical Association's Fellowship and Residency Electronic Interactive Database. Faculty subspecialists' names were collected by manually searching each program's website. Demographics were collected from The National Plan and Provider Enumeration System. To be included in the study, subspecialists had completed an Obstetrics and Gynecology residency, were fellowship trained and/or subspecialty-boarded.

Results/Data: A total of 4,659 subspecialist faculty were identified from 278 residency programs, representing 81.5% of the total subspecialist workforce in Obstetrics and Gynecology (n=5,716). Of the subspecialists identified, 2,838 were faculty at sponsoring sites, representing 49.7% of the entire subspecialist workforce—the remainder work with residents at affiliate sites. Our results showed 59.9% of subspecialists were female and 40.1% were male. Subspecialists were 97.0% allopathic. The largest proportion of subspecialists were between ages 40-49 (36.6%). Subspecialists were present in 45 states, with the exception of Alaska, Idaho, Montana, North Dakota, South Dakota, and Wyoming.

Conclusions: The majority of the Obstetrics and Gynecology subspecialty workforce is involved in training Obstetrics and Gynecology residents, with half of the workforce on faculty at the residency program sponsor site. The subspecialty faculty workforce is primarily female, has an allopathic degree, is mid-career, and is geographically diverse.

BEDSIDE-TO-BENCH: GRANULOCYTE HETEROGENEITY DURING HUMAN CRANIOTOMY INFECTION

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Background: Craniotomies are among the most common neurosurgical procedures and involve the removal of a skull segment (bone flap), allowing intracranial access. Infection is one of the most common and devastating complications following craniotomy, which has been reported to occur in 1-7% of cases and is most often associated with *Staphylococcus aureus* (*S. aureus*). *S. aureus*, and other pathogens, form a biofilm on the bone flap surface which confers immunologic and antibiotic tolerance to the infection. Despite the severity of this clinical picture, a detailed analysis of patient demographics and immune profiles associated with human craniotomy infection has not been conducted. To study disease pathology, our laboratory has developed and extensively utilized a novel mouse model of craniotomy infection, which is characterized by robust neutrophil (PMN) and granulocytic myeloid-derived suppressor cell (G-MDSCs) infiltrates. G-MDSCs are pathologically activated granulocytes with immune suppressive activity mediated through IL-10 production, which inhibit T cell activation and PMN antibacterial activity. G-MDSCs have been implicated in various pathologies, including cancer, infection, and chronic inflammation, and have been shown to exacerbate bacterial burden during craniotomy infection.

Significance of Problem: Craniotomy infections have high fatality and poor clinical prognosis if not treated promptly, which requires additional surgery and months of medical management. While rodent models have been demonstrated to be useful tools in the study of this pathology, an analysis of patients with craniotomy infection that can assess human-specific phenomena was lacking. In the face of increasing antibiotic resistance and limited therapeutics, a better understanding of human immune responses during craniotomy infection may inform the generation of novel immunomodulatory therapies which, in conjunction with antibiotics, may resolve infection and avoid the need for additional surgeries in the future.

Questions: What characteristics typify patients that experience craniotomy infection from a demographic, pathological, and biochemical perspective? Do G-MDSCs also dominate the craniotomy infection site similar to the mouse model? If so, how do they differ in abundance or function from PMNs?

Experimental Design: We first conducted a retrospective study of 2,503 craniotomy patients at the University of Nebraska Medical Center (UNMC) between 2012 and 2023 using a deidentified research network with an electronic health record (EHR) common data model. We then recruited a cohort of 16 patients with craniotomy infection at UNMC to characterize leukocyte infiltrates, inflammatory mediator expression, and transcriptomics by flow cytometry and single-cell RNA sequencing (scRNA-seq), using matched blood samples as a control. Lastly, utilizing a 'bedside-to-bench' approach, we translated findings from this clinical investigation to our mouse model to mechanistically dissect pathways of interest.

Results: The most common indications for craniotomy at UNMC included resection of malignancy, hematoma evacuation, and decompression, with procedures for bone flap removal, treatment of brain infection, and exploratory surgeries at disproportionate risk of infection. Infection rate was similar across age and ethnicity, with females at a modestly higher risk. Staphylococcal species were implicated in over 75% of cases, with *S. aureus* responsible for 47% of infections. Craniotomy infection patients displayed a large influx of G-MDSCs and PMNs at the site of infection, whereas G-MDSCs were absent from the blood. Additionally, 15 key cytokines and chemokines were elevated in infected tissues compared to the blood of each patient. scRNA-seq identified large subpopulations of G-MDSC infiltrates, in agreement with our flow cytometry results, that were typified by increased unfolded protein response, reactive oxygen species production, and HIF-1a activation pathways. In a mouse craniotomy infection model, local delivery of a HIF-1a inhibitor using a novel microparticle delivery approach resulted in significant increases in immune infiltrates at the site of infection concomitant with reduced cytokine production compared to vehicle-treated animals.

Conclusions: This work establishes that *S. aureus* is the predominant causative pathogen in craniotomy infection, but the clinical, immunological, and biochemical landscapes are profoundly diverse across patients. It also highlights the similarities in immune responses between craniotomy infection in humans and the animal model, including the presence of G-MDSC infiltrates, the localized nature of craniotomy infection, and transcriptional heterogeneity in infiltrating granulocytes. Among inter- and intra-subject variation, HIF-1a activation emerged as a conserved infection response across patients and cell types, which was validated in the mouse craniotomy infection model, resulting in phenotypic alterations in disease pathology. Given this, ongoing studies may identify HIF-1a activation as a promising target for the development of immunomodulatory treatment of craniotomy infection in the future.