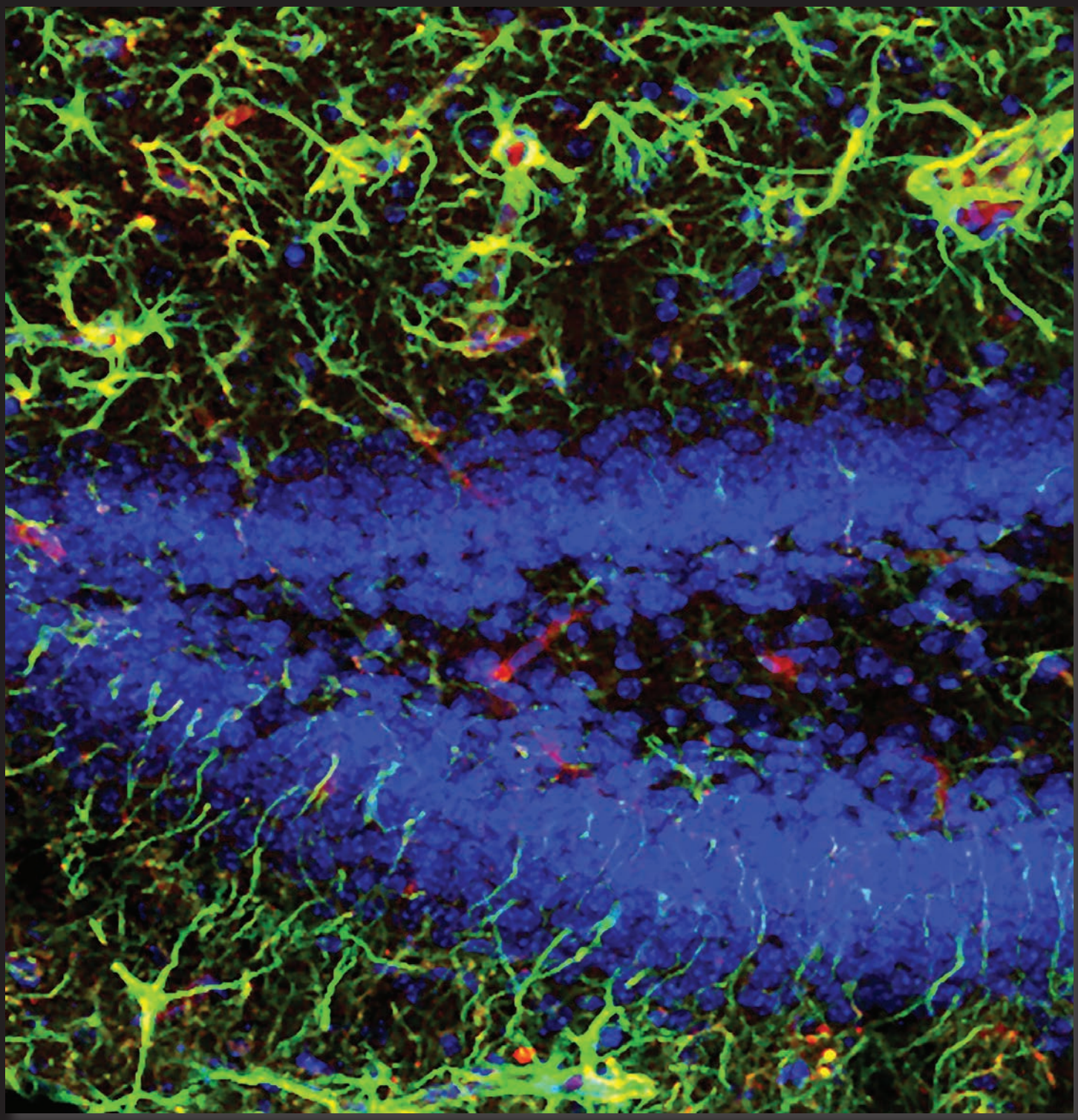
Fusion

A student-led research publication of the George Washington University School of Medicine and Health Sciences | Spring 2019, Volume XII



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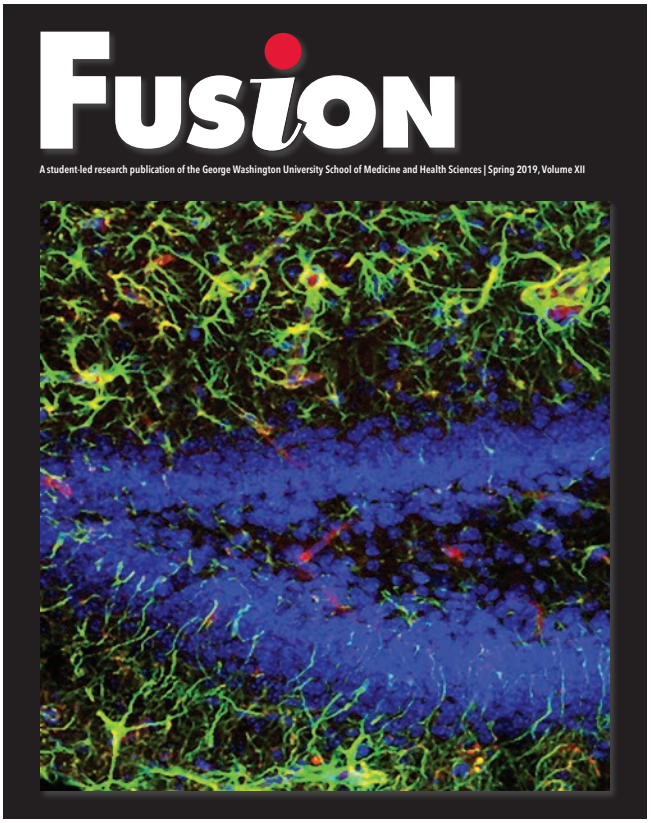
ON THE COVER

On the cover is a photograph of a section of healthy mouse hippocampus tissue (near the dentate gyrus) at 30 days of age. The tissue is stained for nuclei (blue), astrocytes (green), and glucose transporter GLUT8 (red). The image was produced during research conducted by Madhuri Rao, MSII.

Photos like Rao's, capturing the beauty and intricacy of science, are featured annually in the George Washington University (GW) School of Medicine and Health Sciences (SMHS) Art of Science Contest. The competition began in 2018 as a means of highlighting the research that SMHS medical students, graduate students, and postdoctoral fellows are conducting.

This year, more than 17 images were submitted, an increase from last year's competition, and five entries were chosen as winners.

A grand prize of $250 was awarded to Brett Eaton, a PhD student who submitted an image of the Marburg virus budding from infected dendritic cells. Prizes of $100 were awarded to four other entrants, all GW PhD students – Rachel Burga, Samantha Dow, Naemeh Pourshafie, and Jessica Schenck.



The winning images will be displayed in Ross Hall, alongside the winners from last year.

WILLIAM H. BEAUMONT RESEARCH PRIZE WINNERS

BASIC SCIENCE

CLINICAL RESEARCH

Fusion

is a publication of the George Washington University School of Medicine and Health Sciences William H. Beaumont Medical Research Honor Society.

This research journal is published by students in collaboration with the Office of the Dean and the Office of Communications and Marketing.

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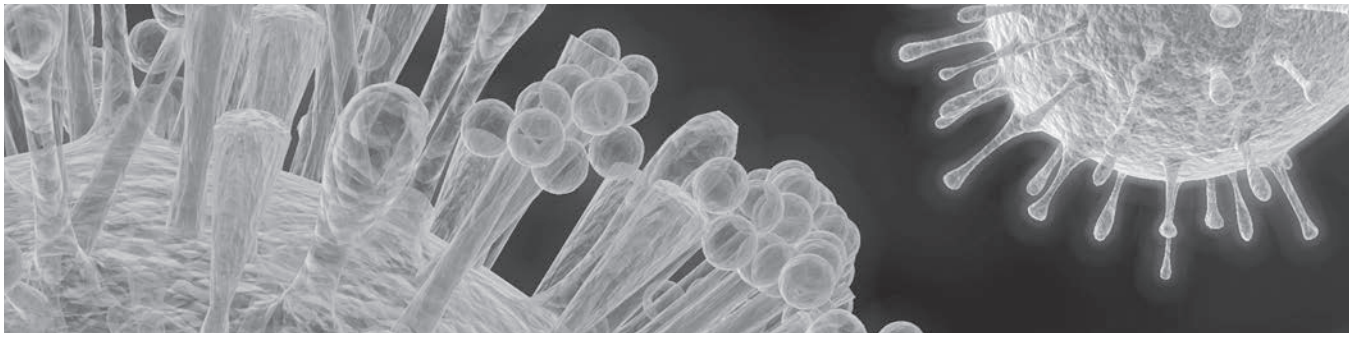
MEDICAL EDUCATION



From the Editors:

Neil Almeida, MSII

Abby Pepin, MSII

As part of the George Washington University (GW) School of Medicine and Health Sciences' (SMHS) mission to educate, research, and heal, GW medical students have the opportunity to conduct research in a multitude of fields. The William H. Beaumont Medical Research Honor Society aims to promote inquiry-based research to train physician-scientists for the future. This journal, run by the William H. Beaumont Society, is an entirely student-run publication including research conducted by GW medical students while in medical school. This year's collection of abstracts reflects a sample of the diverse student research being conducted at SHMS from basic science to clinical research to public health initiatives.

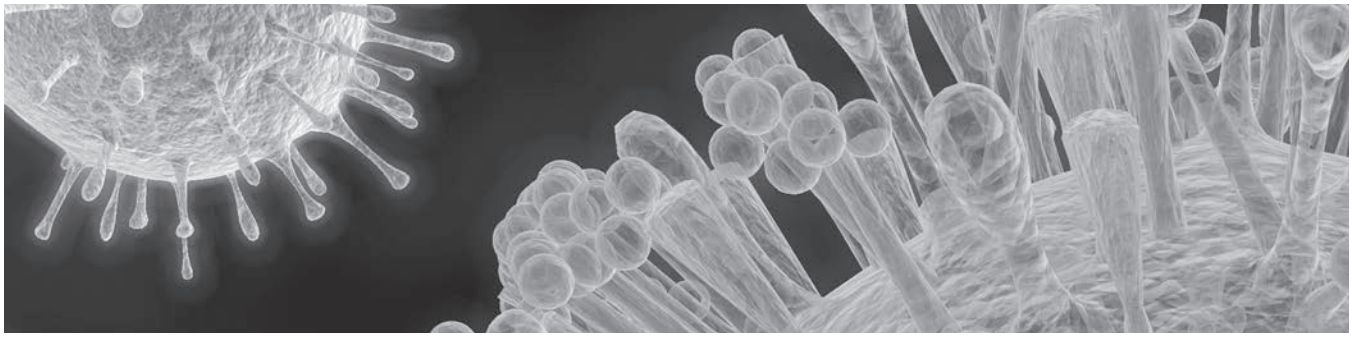
As co-presidents of the William H. Beaumont Medical Research Honor Society, we aim to provide a forum to learn about peer research, inspire future and matriculating medical students, and serve as an outlet to share research experiences of GW medical students. We hope that this journal inspires a dialogue to continue the sharing of new knowledge and passion for research amongst our students, faulty, and staff. We aim to support GW's goal to expand and enhance research experiences for its students as an opportunity to learn and become future leaders in medicine and health sciences fields.

We would like to offer our congratulations to this year's William H. Beaumont Medical Honor Society Student Research Award winners. These individuals and their research projects stood out as exceptional in quality among their peers.

This year's edition would not have been possible without the support of many individuals. We would like to thank former presidents, Janine Amirault and Sarah McCormack, for their continued involvement and support of this organization. We would like to thank our faculty adviser, Dean Alison Hall, for her wisdom and support. We would also like to thank the SMHS Office of Communications and Marking for the design and production of this year's edition. Finally, we would like to thank our classmates and peers who volunteered their time to edit the abstracts submitted, without whom this journal could not exist.

On behalf of the William H. Beaumont Medical Research Honor Society's executive board and members, we hope you enjoy the 2019 edition of Fusion!

Neil Almeida, MSII
Abby Pepin, MSII
Co-Presidents of the William H. Beaumont Medical Research Honor Society



From the Faculty Adviser:

It's an honor to assist with this edition of Fusion magazine that highlights research done by medical students at the George Washington University (GW) School of Medicine and Health Sciences.

You will find examples of the broad range of research pursued by students, from basic biomedical discovery to clinical and translational research with patients to initial translation to practice and communities, on every page of Fusion. In their submissions, medical students described numerous sophisticated approaches, including chart review, data collection, molecular biology techniques, field studies, study design, assisting in IRB applications, and statistical analyses to name just a few. It is always a challenge to select the abstract winners from a collection of such excellent projects.

I would also like to thank the research mentors who host students in their programs here at GW and in our partner institution Children's National Health Center, and in research programs at the nearby NIH campus and other institutions around the nation. Mentorship is key in engaging students and encouraging research careers in academic health centers.

Medical students have rich opportunities for involvement in research. About 20 percent of each class joins the clinical and translational research scholarly concentration, that includes longitudinal lectures, summer research with an abstract and poster, and additional elective clerkships in research. But you don't have to be in the scholarly concentration to do research! Many students compete for external research fellowships as well as internal WT Gill and Health Services Research summer fellowships. Students are encouraged to present — and to learn — at GW's Research Days in April each year.

Alison K. Hall, PhD

Fusion magazine is just one effort by the William H. Beaumont Research Honor Society to engage GW medical students in the excitement and impact of research. I welcome you to explore p. 4–5 and for additional ways to join this community of clinician-investigators.

Alison K. Hall, PhD
Associate Dean for Research Workforce Development, Professor of Neurology

Numerous Opportunities for Medical Students to Engage in Research + Scholarship

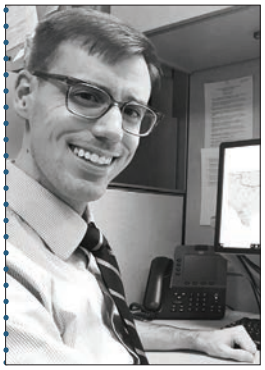
RESEARCH SCHOLARLY CONCENTRATION

Last year, 32 MD students elected to join the Clinical and Translational Research Scholarly Concentration. In this program, students conduct mentored research over the summer between first and second year, attend research lectures, and continue with research scholarships. For details, see https://tinyurl.com/ybpn74cl.

Taniya Walker, MSII, won a 2018 Gill Fellowship to conduct cardiology research on the effects of computed tomography scan findings on the outcomes of transcatheter aortic valve replacement.

YEAR-OUT

Stephen Langerman is taking the 2018–19 year off to conduct research as part of the National Institutes of Health (NIH)-Medical Research Scholars Program. He will study the influence of social factors, such as crime, poverty, and discrimination on health outcomes with Tiffany M. Powell-Wiley, MD, MPH, who heads the Social Determinants of Obesity and Cardiovascular Risk Laboratory at NIH.



GW RESEARCH FELLOWSHIPS

A number of competitive scholarship programs are available to assist in funding exceptional projects in health care and medicine. In 2018, 31 students won a Gill Summer Research Fellowship and 57 students won a Health Services Scholarship. For details,see https://tinyurl.com/ybu4z3w5 and https://tinyurl.com/y8ltewa9.

RESEARCH DAYS AT GW

GW Research Days is an April annual event that celebrates research across GW. Students and postdoctoral fellows present posters and oral presentations and compete for cash prizes.
See https://researchdays.gwu.edu/.

At Research Day 2018, Nicole Casasanta, Sarit Toltzis, Dara Baker, and Christina Pugliese accepted the William Beaumont Research Award from former Fusion editors, Sarah McCormack and Janine Amirault.

FUSION

Fusion is GW's annual medical student research journal. It is a forum to share research done by GW medical students with the larger research community. In 2019, 35 student research abstracts were published. The Beaumont Society seeks Fusion editors each year.

EXTERNAL FELLOWSHIPS

Medical students may elect to take a year off from the MD program to conduct research full-time at GW, NIH, or other institutions. This can be a great way to fully immerse yourself in a research project and form connections at other institutions. External fellowship opportunities are listed at https://tinyurl.com/y9vj54gj.

Third-year SMHS MD student Maggie Beatson, at left, is a Brown University Dermatoepidemiology Research Fellow, studying predictors of skin cancer.

WILLIAM H. BEAUMONT RESEARCH HONOR SOCIETY

The Beaumont Research Honor Society was established to educate and inform medical students about research. Any GW medical student interested in research is welcome to join. 2018-2019 Co-Presidents are Neil Almeida and Abby Pepin. See https://tinyurl.com/y7yuwr6w

METEOR PROGRAM

The Mentored Experience to Expand Opportunities in Research (METEOR) program is a competitive fellowship for underrepresented-in-medicine students. Students are supported for a pre-matriculation research summer and enroll in the research track. https://tinyurl.com/y86oxwmb

METEOR students Guido Pelaez, MS1, and Juan Nogues, MSII

CTSA

The Clinical and Translational Science Award (CTSA) is a joint NIH award between GW and Children's National Health System to conduct "bench to bedside" research.

ART OF SCIENCE IMAGE CONTEST

Students submit artistic images of their research for cash prizes. The award highlights the breadth and beauty of research done at GW. Aslam Akhtar, MSII, was last year's grand prize winner. See https://tinyurl.com/ybr8l26g

RESEARCH PRIZES

The 2018 Walter Freeman Research Award for best research paper was presented to (from left) Jason Chien and Peter Mullins by Lorenzo Norris, MD.

The 2018 Doris DeFord Speck and George Speck Endowed Prize was awarded to Laura Tiedemann (at right) for her research on neonatal intensive care management.

TRAVEL AWARDS

Medical students are encouraged to apply for outside funding for summer or year-out research, or for travel to meetings. Visit the Scholarly Concentration website to see a list of opportunities for summer research, travel, and yearlong fellowships.

See tinyurl.com/y9vj54gj

To learn more, please contact the Office of Student Professional Enrichment smhs.gwu.edu/ospe.

Evaluation of Longitudinal Antibody Responses in Zika-Infected Individuals from Colombia

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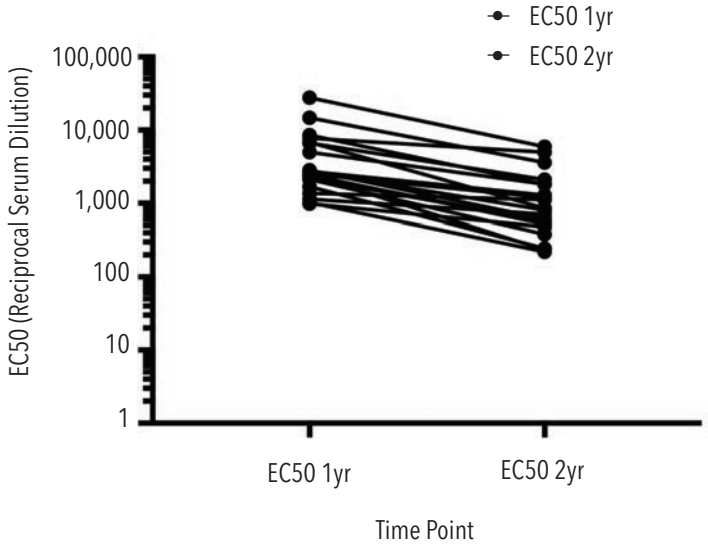

FIGURE 1: Comparison of antibody titers from patients in Colombia, South America, at one and two years post-Zika infection. Titers were evaluated through indirect ELISA assays. The reciprocal plasma dilution at which binding was at 50% is graphed (EC50). This plot depicts a gradual waning of the memory antibody response found within the plasma.

Since the Zika epidemic in 2015, there have been striking associations between Zika virus (ZIKV) infection and neurological complications, sequelae which are most devastatingly seen in fetal development.[1,2] As a result, there is an urgent need for a vaccine against Zika. However, in order to elicit protective, neutralizing antibodies there is a critical need-to-know of how pre-existing immunity to Dengue virus affects the antibody response to Zika infection. We have previously observed that people who developed Guillain-Barre syndrome (GBS) had higher titers of Dengue and Zika neutralizing antibodies than those who did not develop GBS during Zika infection.[3] This study hypothesizes that in regions endemic for flavivirus infection, individuals who developed GBS and had high neutralizing antibody titers will have antibodies that target different viral epitopes from those who do not. To conduct this study, clinically diagnosed plasma samples taken from Zika-infected persons living in Colombia, South America, were tested for the presence and strength of memory antibodies at two different time points after virus clearance. Sera were collected at one year (mean 1.3 years) and two years (mean 2.3 years) post-Zika infection and were tested against the ZIKV E (envelope) protein. As expected, all serum samples bound highly to ZIKV E monomer protein with detectable waning in titer over one year (Figure 1). In order to map the targeted viral epitopes, we ran competition ELISAs using known monoclonal antibodies, 4G2 and ZK67. These antibodies target the fusion loop (FL) and Domain III (DIII) of the E protein respectively. Interestingly, we found that the majority of sera contained antibodies targeting the FL but not DIII. Therefore, we investigated whether there were clinical differences in people who did or did not develop Guillain-Barre syndrome (GBS). We found that FL responses were significantly stronger in people who did not develop GBS, and that DIII responses

We found that FL responses were significantly stronger in people who did not develop GBS, and that DIII responses were undetectable in all patients.

were undetectable in all patients (Figure 2). Our future direction is to expand this dataset to map epitopes found on the ZIKV E dimer, which will better reflect the natural conformation of ZIKV E protein.

REFERENCES

1. Parra B, Lizarazo J, Jiménez-Arango JA, Zea-Vera AF, González-Manrique G, Vargas J, et al. Guillain-Barré Syndrome Associated with Zika Virus Infection in Colombia. N Engl J Med. 2016 Oct 20;375(16):1513–23.

2. da Silva IRF, Frontera JA, Bispo de Filippis AM, Nascimento OJMD, RIO-GBS-ZIKV Research Group. Neurologic Complications Associated with the Zika Virus in Brazilian Adults. JAMA Neurol. 2017 Aug 14.

3. Lynch RM, Mantus G, Encinales L, Pacheco N, Li G, Porras A, et al. Augmented Zika and Dengue Neutralizing Antibodies Are Associated with Guillain-Barré Syndrome. The Journal of infectious diseases. 2018 Aug 3.

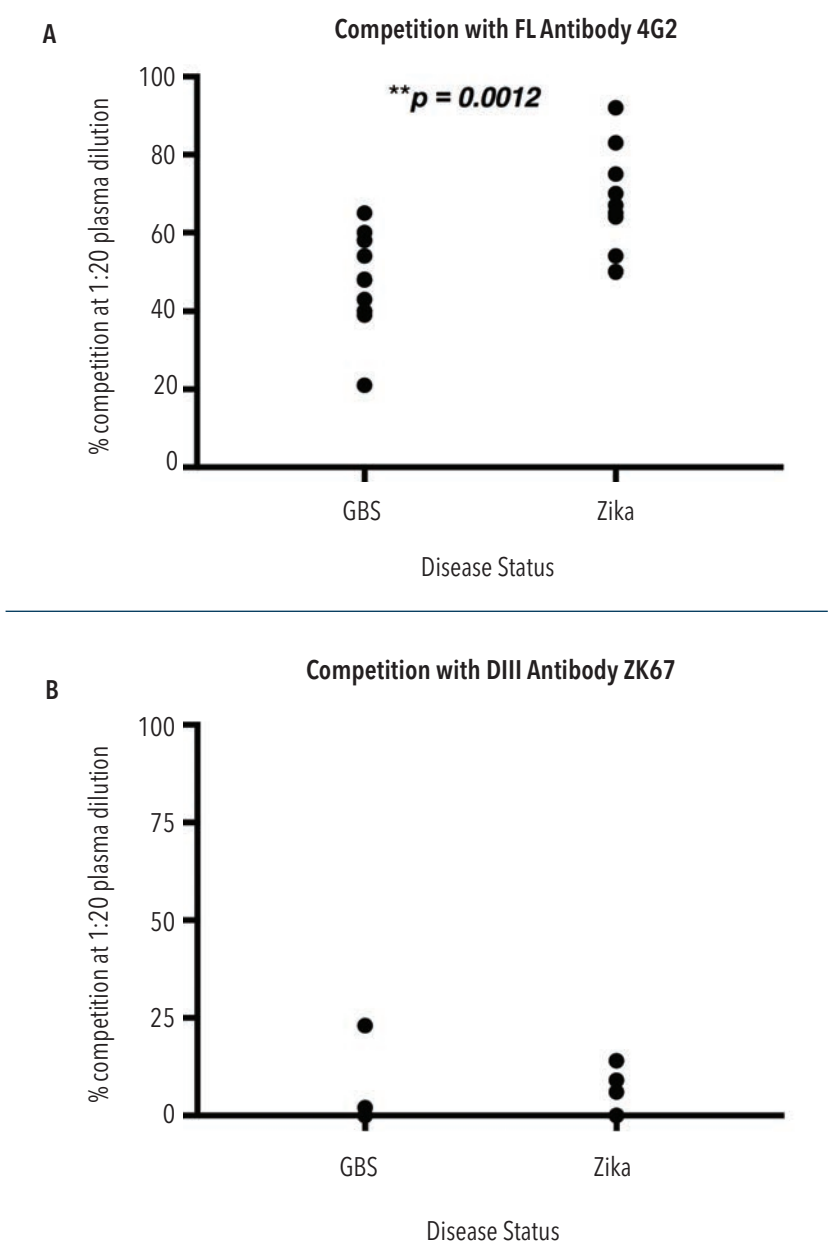

FIGURE 2: Graphs of Plasma Binding Competition to Zika E Monomer Protein with Two Known Antibidies. The percent binding competition to E protein at a plasma dilution of 1:20 is graphed to (A) anti-fusion loop antibody 4G2 and (B) anti-DIII antibody ZK67. Each dot represents an individual plasma and is graphed by disease status of having a diagnosis of GBS or Zika infection alone. There is significantly less competition (fusion loop antibodies) in the plasma from patients diagnosed with GBS by Mann-Whitney.

Prevention of Cardiovascular Thrombosis Through Inhibition of Coagulation Factor XII

Sharjeel Chaudhry, MSIII
ADVISER: Robert Flaumenhaft, MD PhD

Beth Israel Deaconess, Harvard Medical School

Discovery of an antithrombotic therapy that does not cause bleeding would be a transformative advance in the management of conditions such as myocardial infarction, stroke, and venous thromboembolic disease.[1] One promising approach is inhibition of factor XII (FXII/FXIIa), which has been demonstrated to be thromboprotective in preclinical models.[2] Importantly, severe congenital FXII deficiency is not associated with bleeding and FXII inhibition does not augment bleeding in animal models.[3-4] Despite its potential as a therapeutic target, the physiological mechanisms of FXII recruitment to sites of injury and its activation by platelets remains poorly understood.[5] To explore the binding and activation of FXII by platelets during thrombus formation, we stimulated platelet rich plasma with PAR-1 agonist peptide SFLLRN and measured the thrombin generating capacity of platelets by cleavage of a fluorogenic substrate in the presence of anti-FXIIa antibody, anti-TF antibody, and anti-FVIIa antibody. We also stimulated washed platelets with SFLLRN and tested the ability of the various platelet fractions, including from a patient with Hermansky Pudlak Syndrome (HPS), to generate thrombin and FXIIa. Similarly, we measured platelet-dependent FXIIa generation in the presence of CaEDTA, a specific zinc chelator, to test whether zinc was required for FXII binding and activation. We next interrogated whether phosphatidylserine (PS), a negatively-charged phospholipid expressed on the surface of stimulated platelets, was responsible for FXII activation using lactadherin, a phosphatidylserine blocker, and liposomes containing increasing concentrations of 80% phosphatidylcholine and 20% phosphatidylserine (PC/PS). We used platelet flow cytometry with FITC-labeled FXII, bead-immobilized immunoprecipitation, and mass spectrometry to investigate a potential platelet binding partner for FXII. We found that stimulated, but not resting platelets, are able to support thrombin generation via a FXII-dependent

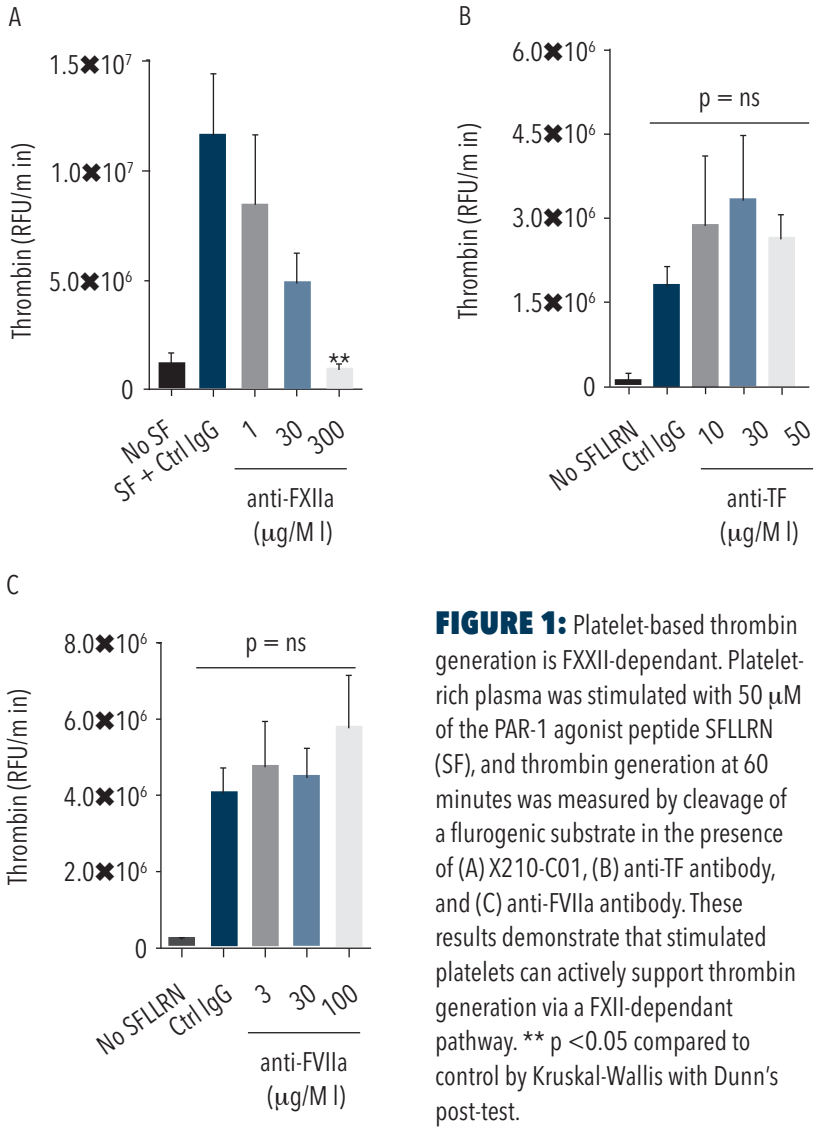

FIGURE 1: Platelet-based thrombin generation is FXXII-dependant. Platelet-rich plasma was stimulated with 50 μM of the PAR-1 agonist peptide SFLLRN (SF), and thrombin generation at 60 minutes was measured by cleavage of a flurogenic substrate in the presence of (A) X210-C01, (B) anti-TF antibody, and (C) anti-FVIIa antibody. These results demonstrate that stimulated platelets can actively support thrombin generation via a FXII-dependant pathway. ** p <0.05 compared to control by Kruskal-Wallis with Dunn's post-test.

pathway (Figure 1). We demonstrated that the FXIIa-generating capacity of platelets is contained in the insoluble fraction, not the releasate (Figure 2). Evaluation of the interaction between FXII and the platelet surface by flow cytometry showed that FXII-FITC binds to stimulated platelets in a specific manner requiring cationic zinc. Platelet-dependent FXIIa generation was inhibited by the presence of CaEDTA (Figure 3). We found that PS blockade with lactadherin prevents platelet-dependent FXIIa generation, while the addition of PS-containing liposomes to plasma did not trigger FXIIa production (Figure 3). These results suggest that PS is necessary but not sufficient for FXII activation

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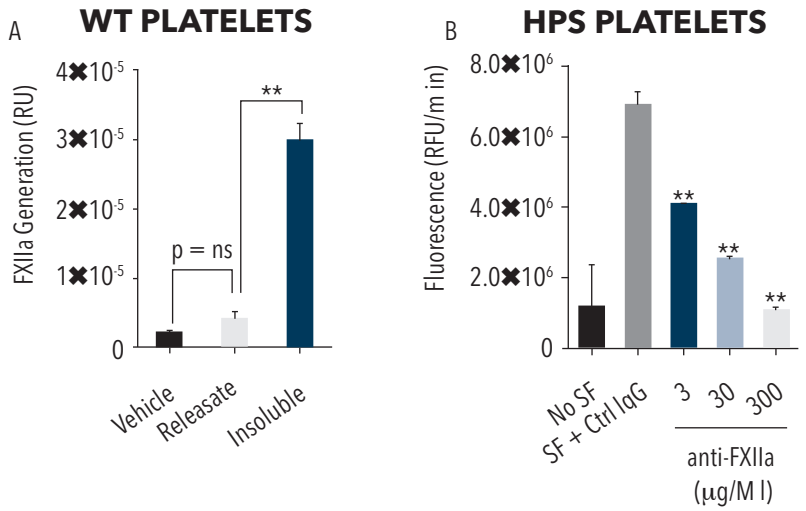

FIGURE 2: The FXIIa and thrombin-generating activity of platelets is not contained in releasate. (A) Washed platelets (2.7 x 109) were stimulated with 100 µM SFLLRN, and the ability of the indicated fractions to generate FXIIa was measured by a FXIIa-specific chromogenic substrate. **p<0.05 Mann-Whitney U test. (B) Platelet-rich plasma (PRP) from a patient with Hermansky Pudlak Syndrome (HPS) was stimulated with 50 µM SF and thrombin generation at 60 minutes with measured by cleavage of flurogenic substrate in the presence of increasing concentrations of X210-C01. **p<0.05 compared to control by Kruskal-Wallis with Dunn's post-test.

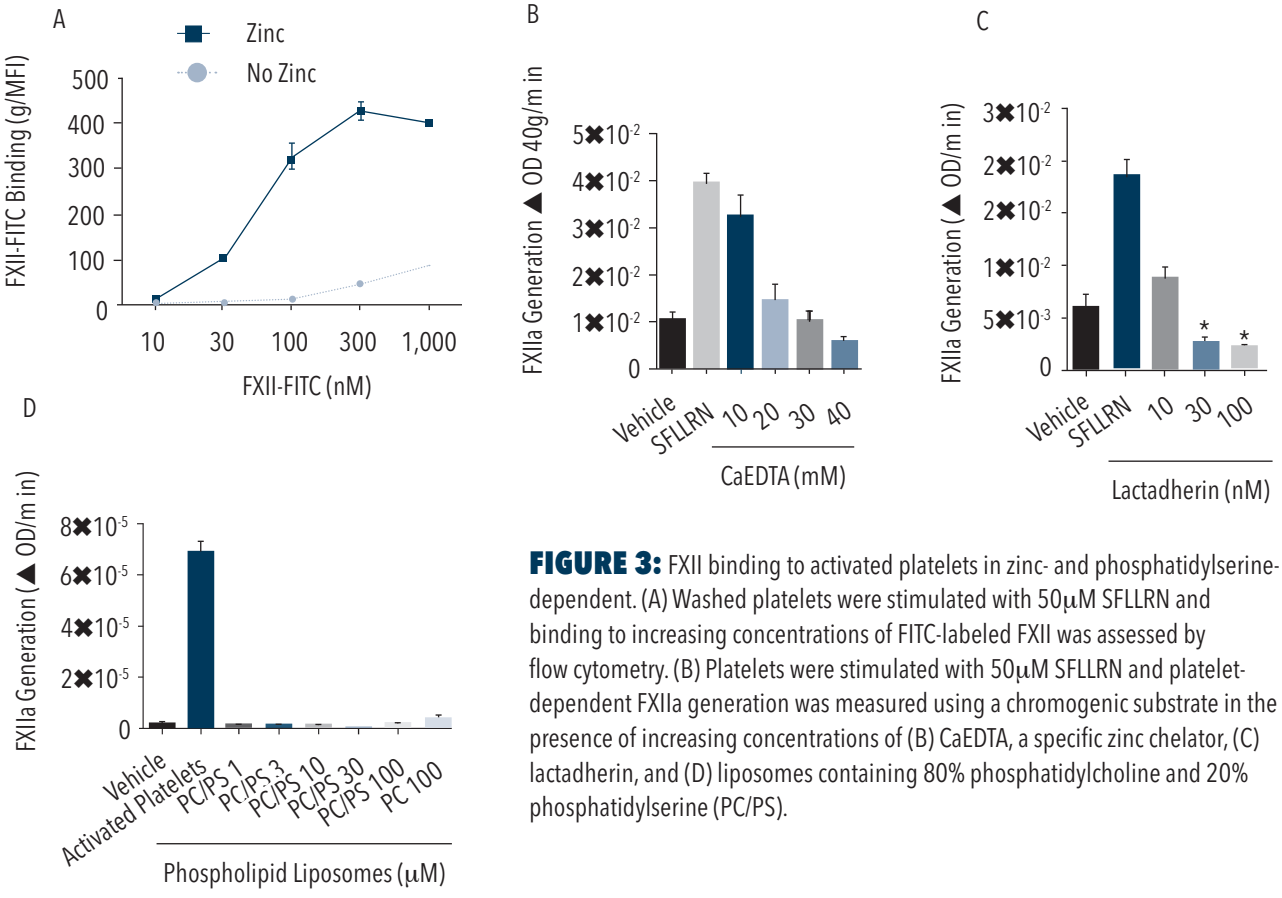

FIGURE 3: FXII binding to activated platelets in zinc- and phosphatidylserine-dependent. (A) Washed platelets were stimulated with 50µM SFLLRN and binding to increasing concentrations of FITC-labeled FXII was assessed by flow cytometry. (B) Platelets were stimulated with 50µM SFLLRN and platelet-dependent FXIIa generation was measured using a chromogenic substrate in the presence of increasing concentrations of (B) CaEDTA, a specific zinc chelator, (C) lactadherin, and (D) liposomes containing 80% phosphatidylcholine and 20% phosphatidylserine (PC/PS).

Continued from p. 9

and that another platelet surface constituent is required. Using co-immunoprecipitation of FXII with binding partners from platelet lysate followed by mass spectrometry, we identified candidate platelet surface proteins that bind with FXII in the presence of zinc. Factor XII binds the platelet surface in a specific, zinc-dependent manner, which is followed by its activation in a process that requires PS. Together, these results may explain the longstanding clinical observation that platelets are crucial for arterial thrombosis but relatively less important in venous clotting. Greater insight into this mechanism would help address an important scientific question and aid in the much-needed development of antithrombotic medications that carry minimal risk of hemorrhage.

REFERENCES

1. Leading Causes of Death. CDC National Center for Health Statistics; 2016. Available from: www.cdc.gov/nchs/fastats/leading-causes-of-death.htm
2. Kleinschnitz C, Stoll G, Bendszus M, Schuh K, Pauer HU, Burfeind P, Renne C, Gailani D, Nieswandt B, Renne T. Targeting Coagulation Factor XII Provides Protection from Pathological Thrombosis in Cerebral Ischemia Without Interfering with Hemostasis. J Exp Med 2006;203: 513-8.
3. Renne T, Pozgajova M, Gruner S, Schuh K, Pauer HU, Burfeind P, Gailani D, Nieswandt B. Defective Thrombus Formation in Mice Lacking Coagulation Factor XII. J Exp Med 2005;202: 271-81.
4. Muller F, Gailani D, Renne T. Factor XI and XII as Antithrombotic Targets. Curr Opin Hematol 2011;18: 349-55.
5. Key NS. Epidemiologic and Clinical Data Linking Factors XI and XII to Thrombosis. Hematology Am Soc Hematol Educ Program 2014;2014: 66-70.

CyTOF Analysis Provides Insight into Immune Response to H3.327M Neoantigen Peptide Vaccine in Glioma Patients

Neil D. Almeida, MSII, and Jared Taitt, BS[1]

ADVISERS: Payal Watchmaker, PhD, and Hideho Okada, MD, PhD[1]

[1] University of California, San Francisco

Gliomas are the most common brain tumor found in children and comprise half of all pediatric central nervous system (CNS) tumors.[1] Diffuse midline glioma, H3 K27M-mutant is a novel entity describing a classification of tumors of the CNS, predominantly arising in pediatric patients, located within midline structures, and carrying a poor prognosis. K27M mutations in H3F3A or HIST1H3B/C, encoding the histone variants H3.1 and H3.3, have been shown to be critical for gliomagenesis.[2] The Okada lab recently implemented a trial in which newly diagnosed children with diffuse midline glioma who are positive for HLA-A2 and the H3.3K27M mutation that underwent radiation therapy, received a specific H3.3K27M peptide vaccine, combined with the tetanus toxoid (TT) peptide, emulsified in Montanide. Poly-ICLC, which is a Toll-like receptor 3 agonist, was given

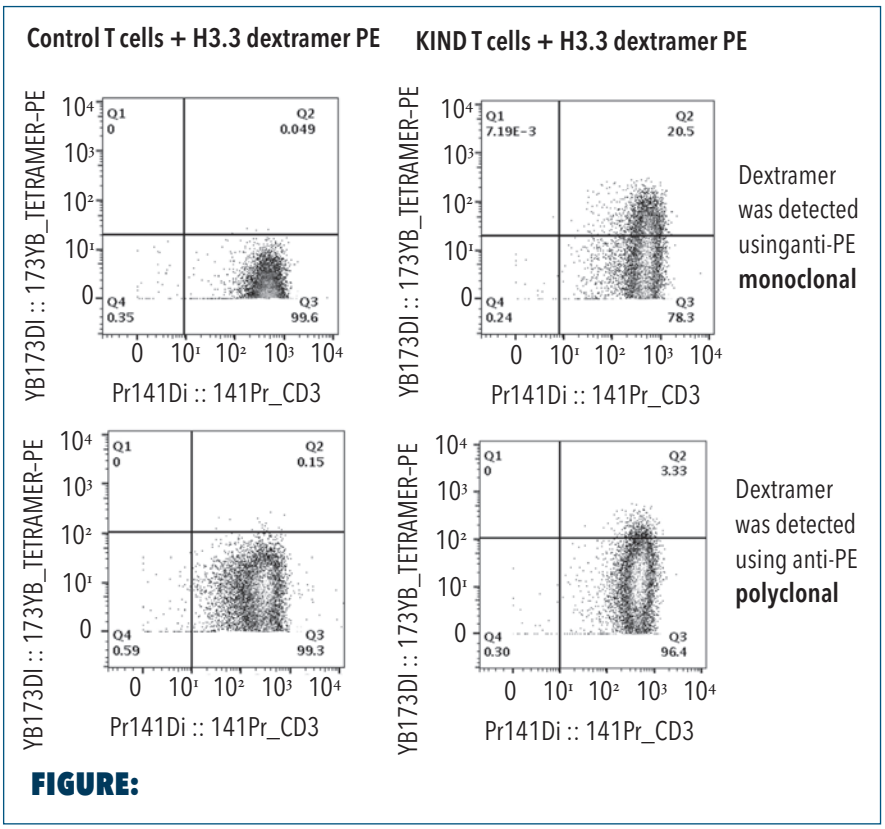

FIGURE:

concurrently to improve the therapeutic effects of the vaccine. Vaccine was administered every three weeks for the first 24 weeks. If there was stable or improved disease, vaccine was administered every 6 weeks for a total treatment period of 96 weeks. My objective was to develop an immune evaluation system to gain insight into immune response to H3.327 peptide vaccine. Peripheral blood mononuclear cells were obtained at each of these time points and analyzed utilizing mass cytometry (CyTOF). CyTOF is an emerging single-cell analysis platform that uses atomic mass spectrometric analysis to quantify up to 42 metal-tagged antibodies per cell. The large number of analytes per cell allows the simultaneous monitoring of multiple immune subsets from small tumor samples, making it ideal for this clinical trial. Dextramer technology involves utilizing heterogenous polymers conjugated to MHC molecules that bind to T-cells and can subsequently be detected using CyTOF.

Control T-cells and K27M TCR transduced T-cells were treated with dasatinib to reduce TCR internalization and subsequently stained with cisplatin. This was followed with staining with H3.3 dextramer with conjugated PE fluorophore and metal conjugated CD3 (141Pr). H3.3 dextramer-PE was detected on CyTOF using anti-PE monoclonal or polyclonal antibody conjugated 173Yb. These preliminary results indicate that TCR-specific T cells can be detected using dextramer-PE and metal conjugated anti-PE monoclonal antibody with minimal non-specific staining. Furthermore, this validated the efficacy of utilizing dasatinib as a method of increasing TCR cell surface expression. This will serve as a powerful tool to learn about how the anti-tumor immune response could be activated and the impact of tumor microenvironment on the functional status of vaccine-induced T-cells both systemically and locally.

REFERENCES:

1. Minturn, Jane E. Gliomas in Children. Current Treatment Options in Neurology June 2013; 15 (3); 316.

2. Karremann, Michael. Diffuse High-Grade Gliomas with H3 K27M Mutations Carry a Dismal Prognosis Independent of Tumor Location. Neuro Oncology. Jan. 2018; 20 (1); 123.

A Machine Learning Approach to Mapping Co-regulated Variant Loci and Gene Expression over Time

Arjun Panda, MSII
ADVISER: Anelia Horvath, PhD, MSc

The George Washington University School of Medicine and Health Sciences

We developed a machine learning analysis pipeline to discover functional gene variants by examining the effect of RNA containing single nucleotide variants (SNVs) on gene expression at cis- and trans- genomic locations over time. This reflects a hypothesis of genetic co-regulation where, as the relative presence of a particular variant allele seen in RNA transcription changes over time (due to changing cellular requirements), gene expression elsewhere in the genome is affected as a result (Figure 1). We believe this analysis pipeline can give novel mechanistic insights into a wide range of basic and translational cell biology questions, particularly on the evolution of drug resistance in cancer cells.

In order to measure changing cellular requirements in cancer cell lines, we conducted paired-end RNA sequencing on human melanoma cell line WM164 under four experimental conditions: with and without histone deacytelase (HDAC) inhibition, and with and without IFN-gamma treatment. This was done over eight time points each, for a total of 32 samples. We then aligned the RNA-sequencing reads to the human genome and called variants.[1]

Measuring the regulation of variant containing RNA is a problem

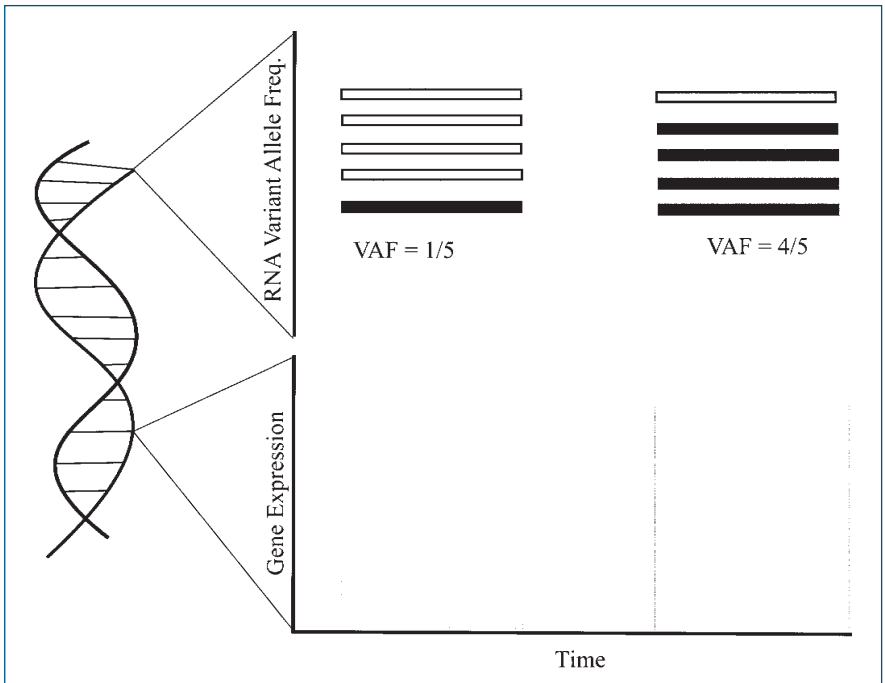

FIGURE 1:

the Horvath lab has tackled using a novel metric called the Variant Allele Frequency (VAF).[2] The variant allele frequency (VAF) measures the RNA expression of SNV-containing loci ($VAF_{RNA} = n_{var} / (n_{var} + n_{ref})$), where nvar and nref are read counts containing the variant and reference SNV (Figure 1). Whereas a DNA allele count can model the effect of a discrete dose of variant harboring alleles ($N \in \{0,1,2\}$ for diploid genomes) on a phenotype such as transcription, the VAF is a continuous measurement with a support of $N \in [0,1]$. Thus the VAF accounts for the true allelic prevalence at multi-allelic loci and reflects molecular regulation events such as imprinting, post-transcriptional regulation by RNA binding molecules, as well as RNA editing. From here, we needed to see if temporal VAF regulation mirrored absolute gene expression trends elsewhere in the genome, elucidating functional relationships between gene variants and gene expression.

Our pipeline starts with clustering in Graphia Professional, which uses a graph-based approach to build relationships between genes.[3,4] Each VAF or gene expression "time-course" groups with another if it meets a minimum Pearson correlation of 0.96. As a result, genes with similar expression trends and VAF trends are connected to each other in a structured graph (Figure 2). We then used a machine learning method called the Markov Cluster algorithm (MCL) to partition the graph into formal clusters by looking for packs of highly interconnected genes.[5] We then built custom R modules to scan through the clusters to find pairs of VAF and gene expression profiles that

cluster together in two samples or more. Once found, each pair was categorized as a cis- relationship if they were less than one million base-pairs apart, and trans- otherwise.

All 60,963 VAF and gene expression profiles were clustered into 3,730 clusters, where each cluster represents a certain pattern of RNA regulation through time. Using custom R scripts, we discovered 440 co-regulated VAF containing positions and gene expression profiles. Further work will include Protein-Protein Interaction (PPI) analyses to validate findings, especially in a larger data set.

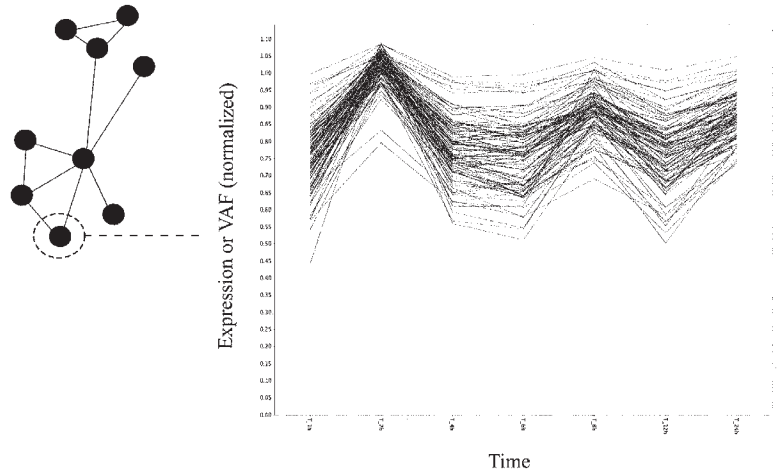

FIGURE 2:

REFERENCES

1. Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., and 1000 Genome Project Data Processing Subgroup (2009). The Sequence Alignment/Map format and SAMtools. Bioinforma. Oxf. Engl. 25, 2078–2079.

2. Movassagh, M., Alomran, N., Mudvari, P., Dede, M., Dede, C., Kowsari, K., Restrepo, P., Cauley, E., Bahl, S., Li, M., et al. (2016). RNA2DNAlign: Nucleotide Resolution Allele Asymmetries Through Quantitative Assessment of RNA and DNA Paired Sequencing Data. Nucleic Acids Res. 44, e161.

3. Freeman, T.C., Goldovsky, L., Brosch, M., van Dongen, S., Mazière, P., Grocock, R.J., Freilich, S., Thornton, J., and Enright, A.J. (2007). Construction, Visualisation, and Clustering of Transcription Networks from Microarray Expression Data. PLoS Comput. Biol. 3.

4. Theocharidis, A., van Dongen, S., Enright, A.J., and Freeman, T.C. (2009). Network Visualization and Analysis of Gene Expression Data Using BioLayout Express(3D). Nat. Protoc. 4, 1535–1550.

5. Van Dongen, S. (2008). Graph Clustering Via a Discrete Uncoupling Process. SIAM J. Matrix Anal. Appl. 30, 121–141.

Association of SREBF1 and TOM1L2 Polymorphisms with Bone and Muscle Phenotypes

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It is well established that muscle and bone have a rich and complex biomechanical relationship. In particular, according to the mechanostat hypothesis, bone growth and bone loss are stimulated by the local mechanical elastic deformation of bone. Thus, bone mineral density (BMD) increases in response to muscle use and strengthening due to increased load-bearing of the affected bone.[1] Originally, it was thought that the strain of the muscle on the bone was the primary impetus causing the increased BMD, however, more recently, researchers have concluded that a paracrine/endocrine relationship between muscle and bone is the more likely explanation. From the onset of life, muscle and bone cells are highly similar due to their common mesodermal origin.[2] At the height of their development, these tissues make up the majority of our body mass and exhibit complex endocrine relationships with the rest of the body.[3] While the proteins that are secreted from muscle and bone are well described, the genetic variants that influence these relationships are not fully understood. In this study, we focused on two single nucleotide polymorphisms (SNPs) (rs1889018 and rs7501812) found in genes SREBF1 and TOM1L2, respectively. Our goal was to explore their individual and possible pleiotropic effects on bone and muscle phenotypes in one cohort of African American children (Bone Health) and two cohorts of young adults (FAMuSS and AIMMY).

All cohorts were genotyped using the Applied Biosystems Taqman allelic discrimination assays and the QuantStudio 7 Flex Real-Time PCR System. After testing for Hardy-Weinberg equilibrium (HWE), data was stratified by sex and cohort and analyzed using analysis of covariance (ANCOVA) applying both an additive and dominant model. Where appropriate, post hoc pair-wise comparisons were performed and the resulting p-values adjusted for multiple comparisons using the Sidak method. For the SNP-SNP interaction analysis, linear regression models were used and included gender, appropriate covariates, and an interaction term quantifying the number of minor alleles present for each SNP pair.

The role of SREBF1 has been well described in recent literature as a regulator of myogenic regulatory factors (MRFs) and causes muscle atrophy when overexpressed both in vivo and in vitro.

The role of SREBF1 has been well described in recent literature as a regulator of myogenic regulatory factors (MRFs) and causes muscle atrophy when overexpressed both in vivo and in vitro.[4] Based on this finding, we expected the SREBF1 SNP rs1889018 to be significantly associated with the muscular phenotypes in each cohort. This expectation was confirmed using an additive model in Caucasian females from the AIMMY cohort for right hand grip strength; however, this finding was not extended to the other cohorts. TOM1L2 is often studied in conjunction with SREBF1 as they share a locus. Our data confirm the impact that TOM1L2 rs7501812 has on a variety of phenotypes including total body BMD, grip strength, VO2 max and baseline 1-repetition maximum strength in more than one cohort and in both Caucasians and African Americans, thus meeting our expectations based on the existing literature. The pleiotropic features of TOM1L2/SREBF1 have been explored in conjunction with other loci affecting BMD and lean mass.[5] In this study, we found that the two SNPs had a statistically significant pleiotropic relationship with the phenotypes VO2 max and bone volume of the non-dominant arm in the FAMuSS cohort, while the relationship in the dominant arm approached significance.

REFERENCES:

1. Sugiyama T, Yamaguchi A, Kawai S. Effects of Skeletal Loading on Bone Mass and Compensation Mechanism in Bone: A New Insight into The "Mechanostat" Theory. J Bone Miner Metab. 2002;20(4):196-200.

2. Brotto M, Bonewald L. Bone and Muscle: Interactions Beyond Mechanical. Bone. 2015 Nov;80:109-114.

3. Cianferotti L, Brandi ML. Muscle-bone Interactions: Basic and Clinical Aspects. Endocrine. 2014 Mar;45(2): 165-77.

4. Dessalle K, Euthine V, Chanon S, Delarichaudy J, Fujii I, Rome S, et al. SREBP-1 Transcription Factors Regulate Skeletal Muscle Cell Size By Controlling Protein Synthesis Through Myogenic Regulatory Factors. PLoS One. 2012;7(11).

5. Medina-Gomez C, Kemp JP, Dimou NL, Kreiner E, Chesi A, Zemel BS, et al. Bivariate Genome-Wide Association Meta-Analysis of Pediatric Musculoskeletal Traits Reveals Pleiotropic Effects at the SREBF1/TOM1L2 Locus. Nat Commun. 2017 Jul 25;8(1):121.

FAM210A and SOST Polymorphisms are Associated with Musculoskeletal Phenotypes in Healthy Young Adults

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Genetic variants in the muscle-specific gene FAM210A (rs4796995), and the bone-specific gene SOST (rs4792909), which codes for the osteokine sclerostin, have been shown to be associated with total BMD and fracture risk. FAM210A knockout mice have low grip strength, as well as high levels of matrix metalloproteinase-12 (MMP-12), a bone-resorption related peptide. Sclerostin suppresses the Wnt/b-catenin signaling pathway in osteoblasts and osteocytes by binding to LRP5/6 coreceptors, thereby inhibiting osteoblast development. The purpose of this study is to explore the influence of these genetic variants on musculoskeletal phenotypes in three previously developed cohorts.

Cohorts: AIMMY: 153 Caucasian young adults aged 18-35 (82 M, 71 F; avg. age 23.2 yrs) and 75 African American young adults aged 19-25 (21 M, 54 F; avg. age 19.2); FAMuSS: 368 young adults (135 M, 233 F, avg age 23); Bone Health: 73 healthy African-American children, (38 male, 35 female; ages 5-9) Phenotypes: AIMMY: right and left arm grip strength, maximal oxygen consumption (VO2 max), and body mass index (BMI). FAMuSS: baseline isometric strength and 1-RM strength in the non-dominant (ND) and dominant (D) arms, and total bone and cortical humeral bone volumes. Bone Health: height-adjusted total BMD minus head z-score. Genotyping: Applied Biosystems Taqman allelic discrimination assays and the QuantStudio 7 Flex Real-Time PCR System were used to perform genotyping. Statistical Analysis: Hardy-Weinberg equilibrium was assessed. Phenotypes were tested in gender specific cohorts using ANCOVA, where phenotype was the independent variable, genotype was the dependent variable, and weight and/or age were covariates. Post hoc pair-wise comparisons were performed and the resulting p-values adjusted using the Sidak method. For the SNP-SNP interaction analysis, linear regression models were used and included gender, appropriate covariates, and an interaction term quantifying the number of minor alleles present for each SNP pair. IRB: This project was approved by the IRB of the Children's National Health System and the University of Calgary.

SOST was found to be out of Hardy-Weinberg equilibrium in the FAMuSS ($p=0.034$), Bone-Health ($p=0.006$), and AIMMY cohorts ($p<0.001$). Among men of FAMuSS, statistically significant associations were found between variants of FAM210A and D arm baseline bone+marrow volume ($p=0.006$), D arm baseline cortical bone volume ($p=0.001$), and ND arm baseline cortical bone volume ($p=0.009$) using an additive model. Statistically significant associations were found between FAM210A and the right ($p=0.022$) and left ($p=0.007$) grip strengths of African-American women of AIMMY. Caucasian males of AIMMY were found to have a statistically significant association between VO2 max and FAM210A in both the additive ($p=0.037$) and dominant ($p=0.002$) models. No significant association was found between FAM210A the Bone-Health phenotypes. SNP-SNP interaction analysis between SOST and FAM210 showed statistically significant interactions in FAMuSS on ND arm baseline 1-RM strength (coefficient=0.567, $p=0.046$) and ND arm baseline cortical bone volume (coefficient=352, $p=0.040$). A

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statistically significant interaction was also found in the Caucasian subgroup of AIMMY in VO2 max (coefficient=1.529, p=0.037).

Early identification of individuals at risk for developing lower peak bone mass and possibly increased fracture risk as seniors has the potential to set the stage for the development of personalized medicine protocols/interventions designed to reduce long term fracture risk by helping these individuals maximize the use of appropriate fitness, nutrition, and other health maintenance strategies.

Programmed Death Ligand 1 Is a Negative Prognostic Marker in Recurrent Isocitrate Dehydrogenase-Wildtype Glioblastoma

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The glioblastoma microenvironment is particularly immunosuppressive, including many secreted and cell-based immune suppressive mechanisms.[1] Programmed death ligand-1 (PD-L1) is a labile, inducible transmembrane receptor ligand that facilitates immune system evasion through co-ligation of its receptor, PD-1, on activated T-cells.[2]

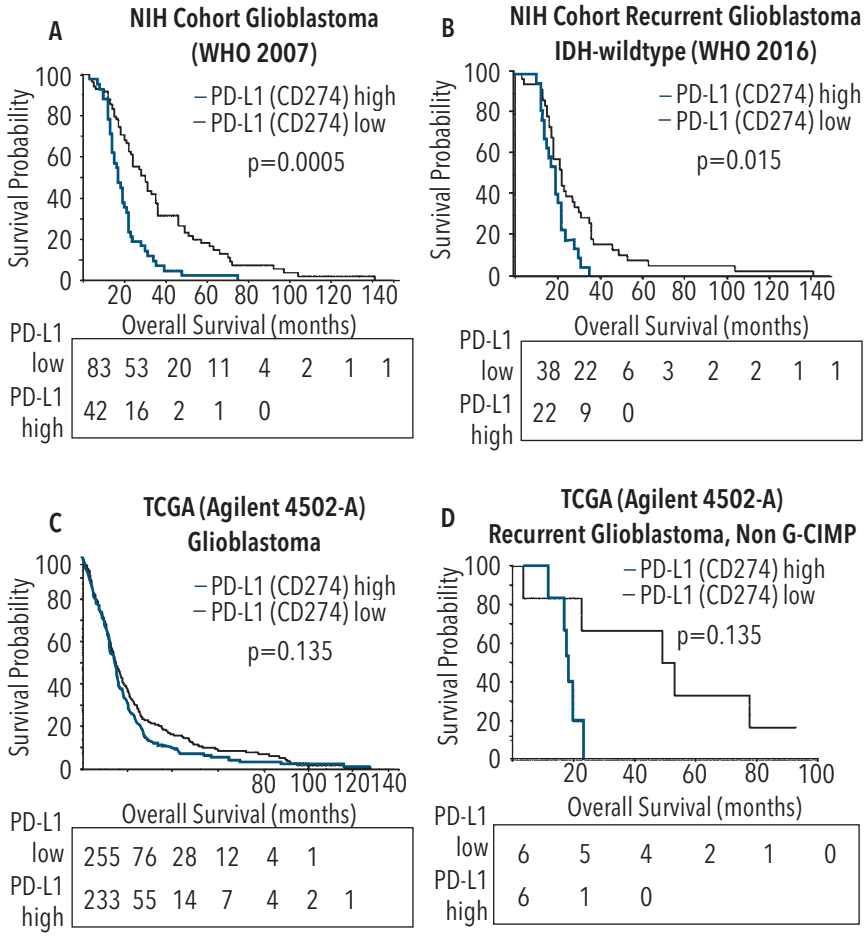

Figure: Kaplan–Meier survival estimates comparing high and low PD-L1 expression in glioblastoma. (A) Differences in survival curves from tumors meeting the 2007 WHO histologic criteria for glioblastoma (5% PD-L1 cut-off). (B) Differences in survival curves from recurrent IDH-wildtype glioblastoma reclassified according to the 2016 WHO nomenclature (5% PD-L1 cut-off). (C) TCGA survival curves, classified only as glioblastoma, stratified by median PD-L1 mRNA expression. (D) Survival curves from TCGA patients after filtering for recurrent, non–G-CIMP (IDH-wildtype) tumors (median PD-L1 mRNA cut-off).

Upregulation of PD-L1 on tumor cells has been proposed as a mechanism of immune escape in gliomas,[3] and its detection at the protein level has been previously demonstrated.[4,5] However, study characteristics (grading, sample size) and technical considerations (assays, cut-offs) have resulted in highly variable rates of expression — ranging from 6.1% to 88% in larger studies.[4] Furthermore, the role of PD-L1 as a prognostic marker in gliomas, independent of predicting treatment response, remains contentious. A specific cut-off for PD-L1 expression has not been established in glioblastoma, and it remains to be seen what role PD-L1 expression has in patient selection in future clinical trials which include PD-1/PD-L1 blockade — as either a predictive or prognostic marker. Here, we sought to address the prognostic role of PD-L1 in recurrent glioblastoma in a large tumor cohort according to the updated 2016 World Health Organization (WHO) classification of diffuse gliomas. Additionally, we sought to localize cellular expression of PD-L1 in the tumor microenvironment using multiplex immunofluorescence in post-treatment glioblastoma. Checkpoint inhibition has demonstrated clinical efficacy in a variety of solid tumors. Reports of PD-L1 expression in glioblastoma are highly variable (ranging from 6% to 88%) and its role as a prognostic marker has yielded conflicting results. To validate the prevalence and prognostic role of PD-L1 expression in a large cohort of diffuse gliomas according to the 2016 revised WHO classification. Using tissue microarrays, we compared 5 PD-L1 monoclonal antibodies (n = 56) and validated expression (n = 183) using quantitative immunohistochemistry (IHC) and RNA in situ hybridization (RISH). Expression data from The Cancer Genome Atlas (TCGA) and published studies were compared with clinical outcome. Multiplexed immunophenotyping was used to identify PD-L1+ cell populations in post-treatment glioblastoma. Using a 5% cut-off, PD-L1 expression was significantly associated with a poor prognosis in both histologically defined (n = 125, log-rank P < .001) and recurrent isocitrate dehydrogenase (IDH)-wildtype glioblastoma (n = 60, log-rank P = .015). PD-L1 remained a significant negative prognosticator in Cox regression analysis (hazard ratio: 1.96, P = .021). Analysis of TCGA data confirmed decreased overall survival in recurrent non–glioma CpG island methylator phenotype (G-CIMP) glioblastoma (n = 12, log-rank P = .023), but not in glioblastoma as a group (n = 444, log-rank P = .135). PD-L1 RISH

PD-L1 IHC (SP263) N(%)				
	Total n = 183a	≥1% n = 54 (29.5)	≥5% n = 43 (23.4)	≥25% n = 28 (15.3)
Age (yr)				
Median (range)	48 (4-75)	52 (4-74)	53 (18-74)	53 (23-74)
Sex				
Female	57 (31)	16 (30)	14 (33)	6 (21)
Male	124 (69)	38 (70)	29 (67)	22 (79)
Presentation				
Primaryb	46 (25)	13 (24)	11 (26)	9 (3)
Recurrent/post-therapy	137 (75)	41 (76)	32 (74)	19 (68)
Diagnosis (WHO 2016)				
LGG	6 (3.3)	0 (0)	0 (0)	0 (0)
AA, IDHmut	21 (11.6)	1 (1.8)	0 (0)	0 (0)
AO, IDH-mut/1p19q codeleted	5 (2.7)	2 (3.7)	1 (2.3)	1 (3.6)
Glioblastoma, IDHwt	81 (44.2)	37 (68.5)	30 (69.7)	20 (71.4)
Glioblastoma, IDHmut	13 (7.1)	2 (3.7)	1 (2.3)	0 (0)
Glioblastoma, NOS	31 (16.9)	12 (22.2)	11 (25.6)	7 (25)
DMG, H3K27Mmut	16 (8.8)	0 (0)	0 (0)	0 (0)

TABLE:

LGG, low-grade (diffuse) glioma (WHO grade II); AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; DMG, diffuse midline glioma.

an = 5 cases did not have available clinical information.

bPrior to chemotherapy or radiotherapy.

Glioblastoma, NOS: WHO grade IV diffuse gliomas with negative IDH R132H staining and an alternative IDH1 or IDH2 mutation probability between 11 and 89%.

Not shown: AANOS (not otherwise specified, n = 2); AAIDHwt (n = 1); AOANOS (anaplastic oligoastrocytoma, n = 1); AONOS (n = 1); DMG non-H3K27M (n = 5).

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showed a significant correlation with IHC ($P < .0001$). PD-L1 was observed in the proliferating perivascular stem cell and immune niche of post-treatment glioblastoma. A 5% PD-L1 expression cut-off identified a subset of glioblastoma that is associated with a worse clinical outcome. This association remained significant within the newly defined IDH-wildtype classification. These findings could have implications for patient stratification in future clinical trials of PD-1/PD-L1 blockade.

REFERENCES:

1. Nduom EK, Weller M, Heimberger AB. Immunosuppressive Mechanisms in Glioblastoma. Neuro Oncol. 2015;17(suppl 7):vii9-vii14.
2. Dong H, Strome SE, Salomao DR et al. Erratum: Tumor-associated B7-H1 Promotes T-cell Apoptosis: A Potential Mechanism of Immune Evasion. Nat Med. 2002;8(8):793-800.
3. Wintterle S, Schreiner B, Mitsdoerffer M et al. Expression of the B7-Related Molecule B7-H1 by Glioma Cells: A Potential Mechanism of Immune Paralysis. Cancer Res. 2003;63(21):7462-7467.
4. Berghoff AS, Kiesel B, Widhalm G et al. Programmed Death Ligand 1 Expression and Tumor-Infiltrating Lymphocytes in Glioblastoma. Neuro Oncol. 2015;17(8):1064-1075.
5. Nduom EK, Wei J, Yaghi NK et al. PD-L1 Expression and Prognostic Impact in Glioblastoma. Neuro Oncol . 2016;18(2):195-205.

Molecular Examination of Hidradenitis Suppurativa in Clinical Samples: Towards Understanding Mechanisms and Exploring Therapeutic Targets

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Hidradenitis suppurativa (HS) is a chronic inflammatory disease of the skin which classically presents as recurrent painful and exudative lesions most often found in intertriginous areas with high densities of apocrine glands. HS prevalence is estimated between 0.05% and 4.10% of the population; however, its morbidity can be significant due to the debilitating nature of the lesions and the social stigma associated with purulent and malodorous discharge.[1] While the exact pathophysiology of HS is not known, the NOTCH signaling pathway involved in embryologic development, cell differentiation, and molecular signaling has previously been implicated without identification of exact genes.[2,3] This study aims to evaluate the cytoarchitecture, cell surface markers, and molecular signaling pathways present in HS patients to further understand the disease and identify potential therapeutic targets to ultimately provide an alternative to surgical excision.

Samples were obtained from 11 patients with HS who underwent surgical excision and compared to 11 samples of normal skin (NS). Histopathology of HS and NS samples were compared via hematoxylin and eosin (H&E) staining and imaging at 10x magnification.

Anti-CD3, a T-cell marker, and anti-CD31, a PECAM and vascular T-cell specific marker, were used for immunohistochemical (IHC) analysis. IHC was conducted in four HS and four healthy skin specimens with six regions of interest (three epidermal and three dermal) per sample. Cellular quantification was performed at 40x magnification and compared with Student's t-test.

Finally, RNA was extracted and quantified, and cDNA was generated via reverse transcriptase PCR from 3 NS and 4 HS biopsies. A NOTCH signaling PCR array was used to identify the degree of gene regulation. Candidate genes were identified if their fold change was greater than or less than 2.5 when compared to normal skin in at least three of the HS samples.

Histopathologic examination showed that HS skin had wider epidermal layers, extending into and engulfing the dermis, as well as extensive dermal cellular infiltration and aggregation. IHC analysis revealed that, at the dermal level, HS lesions

had a significantly greater quantity of CD3+ (324.29±139.28 vs. 14.93±16.32, p<0.0001), and CD31+ (322.15±155.46 vs. 2.84±5.56, p<0.0001) cells/mm2 than healthy skin samples (Table).

NOTCH array analysis identified three genes in HS with a 2.5-fold upregulation compared to NS: KRT1 (keratin family), UbD (ubiquitin D), and CCNE1 (cyclin-dependent kinase). Ten genes were down-regulated in HS more than 2.5-fold compared to NS: CCND1 (cyclin D1 regulator of G1/S transition), Hey1 (involved in somite development), MFNG (demarcates boundaries during embryological development), MMP7 (metalloproteinase involved in tissue remodeling), Notch 4 (cell proliferation and differentiation), DLL4 (encodes for NOTCH ligand), LFNG (defines boundaries during embryological development), PPAR-g (adipocyte differentiation), and LMO2 (yolk sac erythropoiesis).

Epidermal and dermal cytoarchitecture of HS lesions differ in comparison to healthy skin with HS lesions demonstrating a significantly greater dermal lymphocytic infiltrate compared to healthy skin. Additionally, genes involved in embryological development as well as skin and adipocyte differentiation are dysregulated in HS. Work is ongoing to correlate the identified candidate genes with their respective protein levels by IHC with the goal of identifying molecular targets for treatment of HS.

Immunohistochemical Identification and Quantification of Cells in Hidradenitis Suppurativa vs. Normal Skin

Skin Type	CD3		CD31	
	Epidermis (n=12)	Dermis (n=12)	Epidermis (n=12)	Dermis (n=12)
"Hidradenitis Suppurativa (cells/mm2±SD)"	2.28±5.46	324.29±139.28	4.27±7.72	322.15±155.46
"Normal (cells/mm2±SD)"	6.40±10.37	14.93±16.32	2.84±4.20	2.84±5.56
p-Value	0.2123	<0.0001	0.5806	<0.0001

TABLE:

REFERENCES

1. Saunte DML, Jemec GBE. Hidradenitis Suppurativa: Advances in Diagnosis and Treatment. JAMA 2017;318(20):2019–2032.
2. Hoffman L, Ghias M, Lowes, M. Patho-Physiology of Hidradenitis Suppurativa. Seminars in Cutaneous Medicine and Surgery 2017;36:46-54.
3. Blok JL, Li K, Brodmerkel C, Jonkman MF, Horváth B. Gene Expression Profiling of Skin and Blood in Hidradenitis Suppurativa. Br J Dermatol 2015;174(6):1392-1394.

The Effect of TET Mutations on DNA Methyltransferase Cytotoxicity and ERV Induction in B- and T-cell Malignancies

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Cancers exhibit altered DNA methylation compared to normal cells, including decreased methylation at regions normally silenced for genome stability and increased methylation at promoter regions of tumor suppressors. 5-azacytidine (Aza) is a DNA methyltransferase inhibitor (DNMTi) that removes DNA methylation and is FDA approved for the treatment of myelodysplastic syndrome. We have shown that DNMTi upregulate the interferon response, tumor antigens, and antigen presentation in solid tumors, contributing to an overall upregulation in immune signaling.[1] DNMTi activate a canonical interferon signaling pathway through upregulated expression of dsRNA, specifically hypermethylated endogenous retroviruses (ERVs) that activate dsRNA sensors. The interferon response activated by ERV signaling recruits immune cells, promoting tumor clearance and sensitization to immune therapy.[2]

We sought to determine whether DNMTis cause a similar ERV activation and cell killing in B- and T-cell malignancies, focusing first on mantle cell lymphoma (MCL). We further hypothesized that mutations in ten-eleven translocation methylcytosine dioxygenases (TET), enzymes that normally demethylate DNA, might lead to a hypermethylated state making cells more resistant to direct cytotoxicity from DNMTis. We also sought to test the effects of TET mutation on ERV induction and immunogenicity. For 5-Aza to inactivate DNMTis it must be incorporated into the DNA of actively dividing cells. Since Aza is only stable in the body for <30 minutes and MCL cells have slow doubling times, only a fraction of cells divide during that 30 minute window. To improve response in these cancers, extended drug availability is necessary.

We used three different treatment regimens to demonstrate that extending the availability of active drug shows a better response, significantly more so than increasing

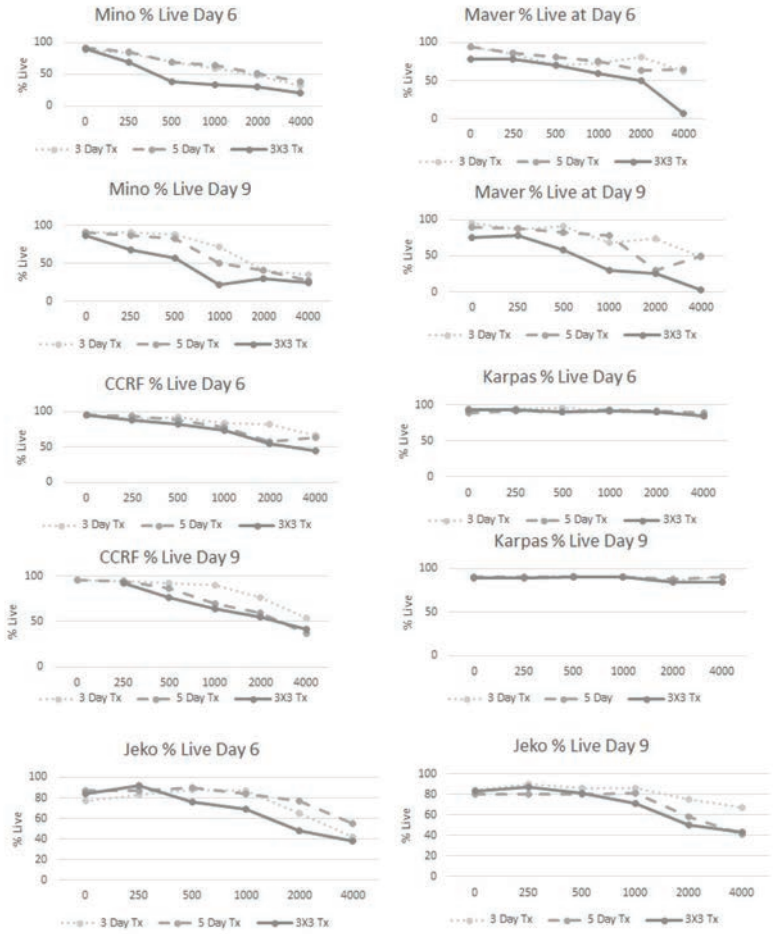

FIGURE 1: Dose response curve for AZA treatment.

the drug dose or treating for a longer period of time. The treatments included: one dose a day for 3 days (3 Day Tx; blue), one dose a day for 5 days (5 Day Tx; red), and three doses a day for three days (3X3 Tx; green). This was done for a dose response curve of 250, 500, 1000, 2000, and 4000 ηg/mL, compared to PBS control (0). Figure 1 shows the percentage of live cells at two time points, day 6 and 9 after starting treatment. In the TET wild type MCL cells (Maver, Mino, Jeko) the 3X3 group showed better killing when compared to 3- and 5-day Tx. The differences between the 3X3 treatment and the single dose per day was more pronounced in the slowest growing cell lines. Mino, which showed the largest difference in killing, doubles between 50-72hrs, whereas Jeko (doubling time 26-33hrs) shows a less drastic difference between the 3 dose versus 1 dose per day groups. This supports the hypothesis that slower growing tumors benefit from sustained drug delivery over increased drug dose. TET mutated leukemia cells (Karpas299, CCRF-CEM), were less Aza sensitive. This supports our hypothesis that TET mutations lead to a hypermethylated state more resistant to DNMTi. The resistance correlates with the extent of TET mutations. Karpas, the most resistant, has mutations in TET1 and TET2, whereas CCRF only has TET3 mutated. In vivo responses by these tumors to DNMTi is likely due to increased immunogenicity, rather than just direct cytotoxicity, which is supported by the increase in ERV expression shown in Figure 2 (ERVs profiled: Syncytin-1, ervFC2, and envW2). CCRF showed increased ERV expression in both the five-day treatment and the 3X3 treatment.

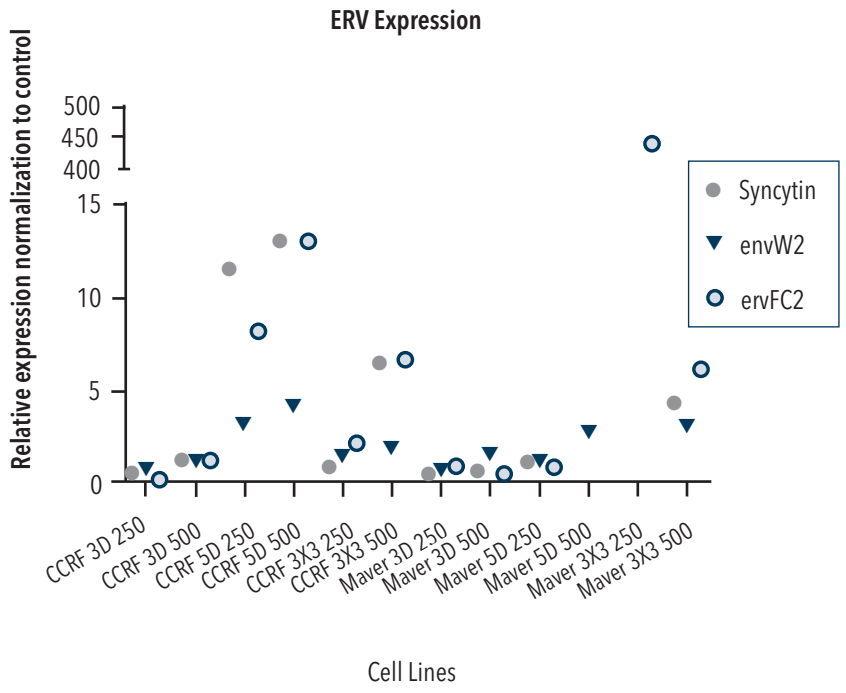

FIGURE 2: ERV expression of CCRF and Maver Cell Line.

REFERECES

1. Chiappinelli, K. B., Strissel, P. L., Desrichard, A., Li, H., Henke, C., Akman, B., ... & Makarov, V. (2015). Inhibiting DNA methylation causes an interferon response in cancer via dsRNA including endogenous retroviruses. Cell, 162(5), 974-986.

2. Stone, M. L., Chiappinelli, K. B., Li, H., Murphy, L. M., Travers, M. E., Topper, M. J., ... & Hung, C. F. (2017). Epigenetic therapy activates type I interferon signaling in murine ovarian cancer to reduce immunosuppression and tumor burden. Proceedings of the National Academy of Sciences, 114(51), E10981-E10990.

A Developmental Profile of Glucose Transport and Utilization in Mice

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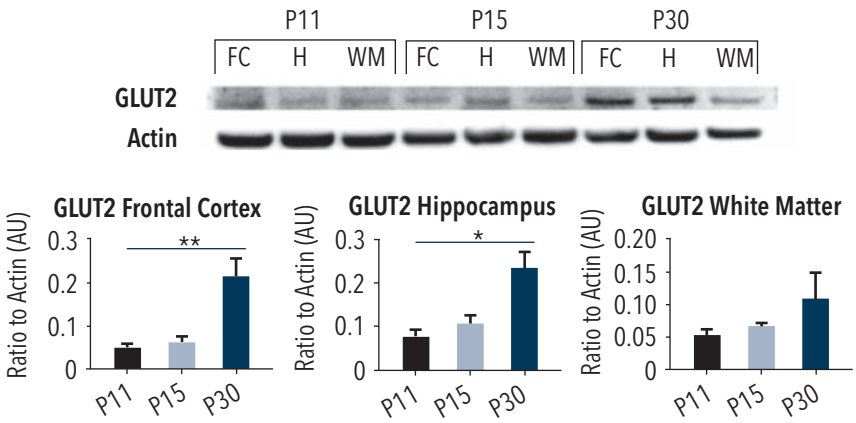

FIGURE 1: Western Blot of GLUT2 levels in the frontal cortex (FC), hippocampus (H), and white matter (WM) of P11, P15, and P30 mouse brains (top). Western Blot quantification of GLUT2 levels, expressed as ratio to actin in the frontal cortex, hippocampus, and white matter of P11, P15, and P30 mouse brains (N = 3) (bottom).

The brain uses glucose and related substrates for energy (Berg et al., 2002) but how the brain transports and uses glucose during development has not been completely characterized. The aim of this project is to compile an age, region- and cell-specific profile of glucose transport and utilization during development using a mouse model. This profile can be used to better understand metabolic changes in pediatric diseases such as perinatal hypoxia, epilepsy, hyperglycemia, and mitochondrial disease.

Brains of C57Bl/6 mice were harvested and dissected at ages P11, P15, and P30, corresponding to neonates, young children, and adolescents respectively. The frontal cortex, hippocampus, and white matter were isolated by microdissection, each having different cell populations important in learning, memory, and cognition during early development. Glucose transporters (GLUT) 1, 2, 3, 4, and 8 were selected as measures of brain glucose transport (Gomez et al., 2010) and quantified via Western Blotting.

GLUT1 is in the blood-brain barrier endothelium (Sankar et al., 2002). As vascularization is established early, we did not expect significant changes in GLUT1 expression during development, and no significant difference between ages in the frontal cortex was seen (Figure 1).

GLUT2 is preferentially expressed in astrocytes (Qutub and Hunt, 2005). As the number of astrocytes increases with age (Reemst et al., 2016), we expected an increase in GLUT2 expression during development, especially in the hippocampus (Oberheim et al., 2012). GLUT2 expression was significantly increased by P30 in the frontal cortex and (Figure 2) and there was no significant difference in GLUT2 expression in white matter.

GLUT3 is the main glucose transporter in neurons (Gomez et al., 2010), but there is little temporal and no region-specific data for GLUT3 expression. Results showed no statistically significant changes in GLUT3 expression in the hippocampus and white matter and a mild statistically insignificant upward trend in the frontal cortex. The lack of statistical significance may be that the peak expression occurs between the ages we analyzed and was not detected.

GLUT4 and GLUT8 are both insulin-dependent glucose transporters (Sankar et al., 2002). An upward trend in GLUT4 expression by P30 was seen, but no significance in the frontal cortex, hippocampus, or white matter was seen. There was very high variability among individuals. GLUT 8 expression, however, showed an extremely significant increase in P30 mice in the frontal cortex, hippocampus, and white matter, which can be explained by the feeding pattern of mice. From birth until P15, the mice are in their suckling phase and receive mostly proteins and fatty acids through their mothers' milk (McKenna et al., 2015) but by P30 mice feed on their own and have a diet rich in carbohydrates, which stimulates insulin release. Since GLUT8 is an insulin-dependent glucose transporter, the increase in insulin should cause an increase in expression of GLUT8 in the brain.

Although GLUT4 should have shown similar patterns in expression, it is only weakly expressed in the brain. Therefore, GLUT8 may be the key insulin-dependent glucose transporter in the brain. Links to metabolic changes in diabetic pediatric patients should be further explored.

Future studies include immunostaining human brain from a neonate, a young child, and an adolescent. Mice subjected to perinatal hypoxia will be compared to this profile to better understand the metabolic changes that occur in this common pediatric form of brain injury.

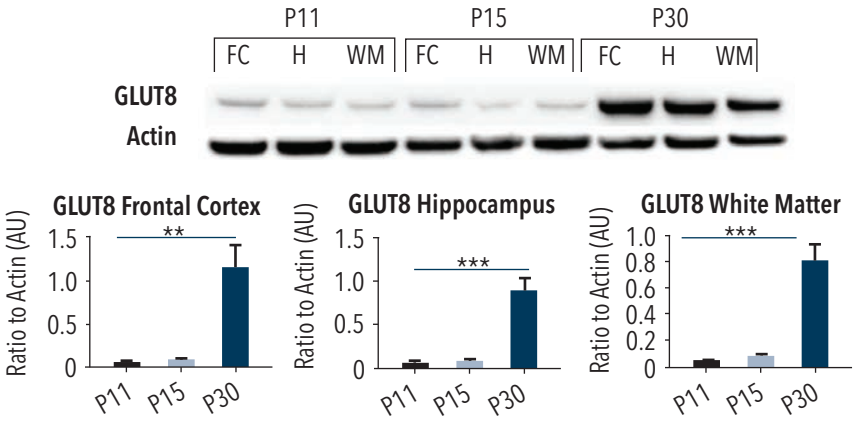

FIGURE 2: Western Blot of GLUT8 levels in the frontal cortex (FC), hippocampus (H), and white matter (WM) of P11, P15, and P30 mouse brains (top). Western Blot quantification of GLUT8 levels, expressed as ratio to actin in the frontal cortex, hippocampus, and white matter of P11, P15, and P30 mouse brains (N = 3) (bottom).

REFERENCES:

1. Berg, J. M., Tymoczko, J. L., & Stryer, L. (2002). Each Organ Has a Unique Metabolic Profile. Biochemistry. 5th Edition. Retrieved from https://www-ncbi-nlm-nih-gov.proxygw.wrlc.org/books/NBK22436/

2. Gómez, O., Ballester-Lurbe, B., Poch, E., Mesonero, J. E., & Terrado, J. (2010). Developmental Regulation of Glucose Transporters GLUT3, GLUT4 and GLUT8 in the Mouse Cerebellar Cortex: GLUTs in the Cerebellum. Journal of Anatomy, 217(5), 616–623. https://doi.org/10.1111/j.1469-7580.2010.01291.x

3. McKenna, M. C., Scafidi, S., & Robertson, C. L. (2015). Metabolic Alterations in Developing Brain After Injury: Knowns and Unknowns. Neurochemical Research, 40(12), 2527–2543. https://doi.org/10.1007/s11064-015-1600-7

4. Oberheim, N. A., Goldman, S. A., & Nedergaard, M. (2012). Heterogeneity of Astrocytic Form and Function. Methods in Molecular Biology (Clifton, N.J.), 814, 23–45. https://doi.org/10.1007/978-1-61779-452-0_3

5. Qutub, A. A., & Hunt, C. A. (2005). Glucose Transport to the Brain: A Systems Model. Brain Research Reviews, 49(3), 595–617. https://doi.org/10.1016/j.brainresrev.2005.03.002

6. Reemst, K., Noctor, S. C., Lucassen, P. J., & Hol, E. M. (2016). The Indispensable Roles of Microglia and Astrocytes during Brain Development. Frontiers in Human Neuroscience, 10. https://doi.org/10.3389/fnhum.2016.00566

7. Sankar, R., Thamotharan, S., Shin, D., Moley, K. H., & Devaskar, S. U. (2002). Insulin-Responsive Glucose Transporters — GLUT8 and GLUT4 Are Expressed in the Developing Mammalian Brain. Molecular Brain Research, 107(2), 157–165.

The Association of Polymorphism rs3736228 Within the LRP5 Gene with Bone Mineral Density in a Cohort of Caucasian Young Adults

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Characteristic	Females		Males	
	N	Mean ± SD	N	Mean ± SD
Age (years)	96	22.3 ± 4.4	93	23.7 ± 4.2
Weight (kg)	96	62.4 ± 9.2	93	78.0 ± 12.3
Height (cm)	96	165.9 ± 5.8	93	178.8 ± 7.7
BMI	96	22.7 ± 2.9	93	24.3 ± 3.1

TABLE: AIMMY Demographics

Osteoporosis is a significant burden for our aging population. Developing a better understanding of the genetic underpinnings of poor bone quality may assist in the future development of prevention strategies. A study by Correa-Rodriguez (2016) identified a group of single nucleotide polymorphisms (SNPs) that were associated with both body composition and bone mineral density (BMD) in a population of Caucasian young adults.[1] In particular, they found rs3736228 in the low-density lipoprotein receptor related protein 5 (LRP5) gene particularly influenced BMD. SNP rs3736228 has been demonstrated to have a pleiotropic effect on phenotypes, demonstrating associations in other studies with body composition, circulating nutrient levels, and obesity.[2,3,4] The aim of this study is to expand existing knowledge of LRP5 and explore the association of rs3736228 polymorphisms and BMD to replicate the findings of previous studies regarding the association of SNP rs3736228 with BMD in a cohort of healthy young adults.

Participants from the University of Calgary cohort from the Assessing Inherited Metabolic Syndrome Markers in the Young (UC AIMMY) study included 209 healthy young adults and mixed ethnicity, predominantly Caucasian (81%) young adults (male n=102, female n =107, average age=23 years). Phenotypes were height, weight, body mass index (BMI), and total BMD. Genotyping: Allelic discrimination was determined using Applied Biosystems Taqman and Applied Biosystems 7900HT Realtime PCR. Statistical Analysis, after being tested for Hardy-Weinberg equilibrium (HWE), the data was stratified by sex and analyzed using analysis of covariance (ANCOVA) and a dominant model. Where the f-tests were significant, post hoc pairwise comparisons were performed and the resulting p-values were adjusted for multiple comparison using the Sidak method.

The genotype distribution for rs3736228 in the AIMMY cohort was found to be in HWE. Using a dominant model, we found that females with one or more copies of the risk T allele had a significant lower mean total BMD (CC: n=69, 1.129 ± 0.010; CT/TT n=29, 1.089 ± 0.016; p = 0.0347). However, a similar association was not seen in males. No significant associations were observed with height, weight, or BMI.

The rs3736228 polymorphism within the LRP5 gene was found to be negatively associated with BMD in females in the UC AIMMY cohort. It is known that the polymorphism rs3736228 alters the codon in position 1330. The more common C allele encodes alanine, while the minor T allele encodes valine. This mutation downregulates the LRP5 cell surface receptor function, which plays a pivotal role in bone formation. The LRP5 gene has now been shown in multiple studies to be associated with bone quality measures like calcaneal Quantitative Ultrasound (QUS) and BMD. Our study has expanded these findings and suggests that rs3736228 also influences BMD in healthy young females. This supports the work of a recent study which found that when stratifying by sex, females only

showed a trend towards significance in calcaneal QUS measures.[1] While the development of BMD is polygenic, our work suggests that focus on LRP5 polymorphisms may be particularly helpful in defining genetic risk for low BMD. In addition, given that this polymorphism has shown to have pleiotropic effects, we plan to investigate the associations of rs3736228 with other anthropomorphic phenotypes.

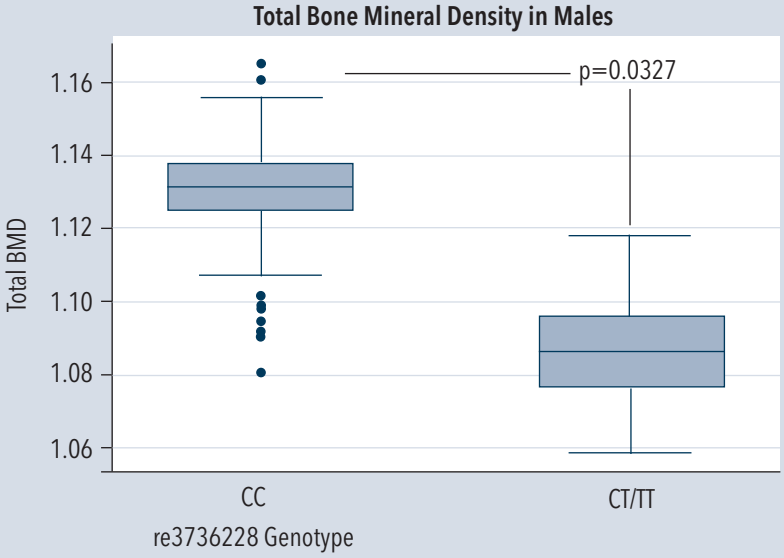

FIGURE: Plot of SNP Variations vs. Total BMD

REFERENCES:

1. Correa-Rodriguez M, Schmidt-RioValle J, & Rueda-Medina, B. The rs3736228 polymorphism in the LRP5 gene is associated with calcaneal ultrasound parameter but not with body composition in a cohort of young caucasian adults. Journal of Bone and Mineral Metabolism 2016:1-7.
2. Falcon-Ramirez E, Casas-Avila L, Cerda-Flores RM, Castro-Hernandez C, Rubio-Lightbourn J, Velazquez-Cruz R, et al. Association of LRP5 haplotypes with osteoporosis in mexican women. Molecular Biology Reports 2013:40(3):2705-2710.
3. Funakoshi Y, Omori H, Yada H, & Katoh T. A1330V polymorphism of the low-density lipoprotein receptor-related protein 5 gene and bone mineral density in japanese male workers. Environmental Health and Preventive Medicine 2011:16(2):106-112.
4. Jiang XY, Chen HH, Cao FF, Li L, Lin RY, Wen H, et al. A polymorphism near osteoprotegerin gene confer risk of obesity in Uyghurs. Endocrine 2010:37(3):383-388.

Association of GREB1 Polymorphisms with Bone and Muscle Health Phenotypes

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Previous studies have identified specific genetic loci associated with variation in bone mineral density (BMD) in genome-wide association analyses.[1,2] One region in chromosome 2p22-25 was found to have significant association with BMD variations in the lumbar spine.[3] Growth Regulation by Estrogen in Breast Cancer 1 gene (GREB1), a gene within this loci, was recently shown to be upregulated during osteoblast and chondroblast differentiation.[4] In one study, genetic variants of GREB1 were found to be associated with BMD variations at the lumbar spine and femoral neck in Caucasian adults, establishing GREB1 as a target gene for future BMD genetic studies.[5] The current study sought to elucidate the role of genetic variation in GREB1, specifically polymorphisms rs5020877 and rs10929757, on BMD measures in a cohort of African American children as well as two cohorts of Caucasian young adults.

The relationship between GREB1 polymorphisms and phenotype were assessed in three distinct cohorts: Bone Health, 73 healthy African American children previously recruited to assess fracture risk factors; Functional Single Nucleotide Polymorphism Associated with Human Muscle Size and Strength (FAMuSS), 368 healthy Caucasian young adults previously recruited as part of a 3-month long program to evaluate

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genetic influences on strengthening muscles in the non-dominant arm; and Assessing Inherited Markers of Metabolic Syndrome in the Young (AIMMY), 153 health Caucasian young adults and 75 healthy African American young adults previously recruited to identify significant risk factors for metabolic syndrome. The Applied Biosystems Taqman Allelic Discrimination Assays and Real-Time PCR system were used to genotype the DNA samples from the cohorts. All statistical analyses were performed separately by gender, and Hardy-Weinberg Equilibrium (HWE) was assessed for each SNP in race-specific cohorts. Analysis of covariance (ANCOVA) tests were utilized, where the respective covariates were entered into the model. Post hoc pair-wise comparisons were performed, and the resulting p-values adjusted for multiple comparisons using the Sidak method.

Both SNPs were found to be in HWE in the FAMuSS and AIMMY cohorts, but neither was in HWE in the Bone-Health cohort. In the Bone Health cohort, rs5020877 was significantly correlated with Dual X-Ray Absorptiometry (DEXA) bone mineral content (BMC) in the lumbar spine in males (p=0.036) as well as left hip (p=0.036); whereas, in females, rs10929757 was significantly associated with total body BMD (p=0.016), as well as BMD for the left hip (p<0.001), lumbar spine (p=0.036), and total body (p=0.035). In the FAMuSS cohort, rs5020877 genotype was significantly associated with baseline one-rep maximum (1-RM) strength in the non-dominant (ND) arms of females (p=0.021). In the AIMMY cohort, neither SNP showed a significant association with any phenotype. There were no significant SNP-SNP interactions between rs5020877 and rs10929757 for any of the phenotypes analyzed.

	Covariates used	Sex	rs5020877	rs10929757
Baseline isometric strength – Non-dominant arm	Age, weight	F	0.14	0.13
		M	0.35	0.41
Baseline isometric strength – Dominant arm	Age, weight	F	0.15	0.3
		M	0.32	0.33
Baseline 1-RM strength – Non-dominant arm	Age, weight	F	0.021	0.43
		M	0.19	0.61
Baseline 1-RM strength – Dominant arm	Age, weight	F	0.11	0.48
		M	0.29	0.42
Baseline bone (+ marrow) volume – Dominant arm	Age, weight	F	0.32	0.48
		M	0.66	0.7
Baseline bone (+ marrow) volume – Non-dominant arm	Age, weight	F	0.11	0.44
		M	0.71	0.87
Baseline cortical bone volume – Dominant arm	Age, weight	F	0.44	0.26
		M	0.61	0.94
Baseline cortical bone volume – Non-dominant arm	Age, weight	F	0.051	0.33
		M	0.69	0.53

TABLE: Phenotype Analysis for GREB1 Polymorphisms in the FAMuSS Cohort

The results from this study demonstrate that variations in the GREB1 gene, originally known for its role in tumor progression and more recently for its role in osteoblast/chondroblast differentiation, may contribute to differences in baseline 1-RM strength of dominant and non-dominant arms, lumbar spine BMC as well as total body, left hip, and lumbar spine BMD. These findings suggest independent effects of these two loci and that patients with rare allele for rs5020877 are stronger but have decreased bone quality while those with the rare allele for rs10929757 have an increased bone quality. However, given the small number of patients having the rare allele for either SNP, further study is needed to verify these findings.

REFERENCES:

1. Richards JB, Kavvoura FK, Rivadeneira F, et al. Collaborative meta-analysis: associations of 150 candidate genes with osteoporosis and osteoporotic fracture. Ann Intern Med. 2009;151:528-537.
2. Rivadeneira F, Styrkarsdottir U, Estrada K, et al. Twenty bone-mineral density loci identified by large-scale meta-analysis of genome-wide association studies. Nat Genet. 2009;41:1199-1206.
3. Wynne F, Drummond FJ, Daly M, et al. Suggestive linkage of 2p22-25 and 11q12-13 with low bone mineral density at the lumbar spine in the Irish population. Calci Tissue Int. 2003;72:651-658.
4. Liu T, Gao Y, Sakamoto K, et al. BMP-2 promotes differentiation of osteoblasts and chondroblasts in Runx2-deficient cell lines. J Cell Physiol. 2007;211:728-735.
5. Hegarty KG, Drummond FJ, Daly M, et al. GREB1 genetic variants are associated with bone mineral density in Caucasians. J Bone Miner Metab. 2018;36(2):189-199.

Evaluating Racial Disparities in Breast Cancer Referrals for Hereditary Risk Assessment

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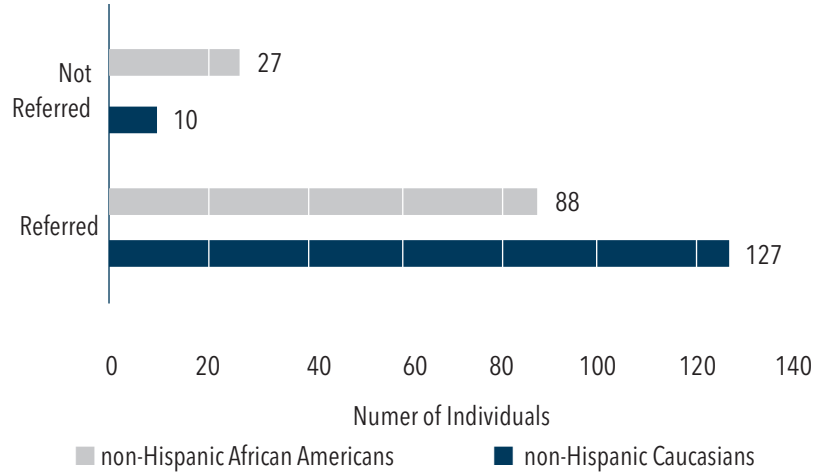

FIGURE 1: Physician referral rates for breast cancer patients treated at GW Cancer Center between 2014 and 2018 meeting NCCN criteria differed significantly by race (BNH 76.5%, N = 88 and WNH 92.7%, N=127; χ^2=13.0642, p-value=0.00030 (p-value significance <0.05).

There is a pronounced onco-racial disparity in Washington, D.C., which had the highest national incidence of breast cancer in African American patients between 2010 and 2015 and the worst outcomes.[1] Recent data indicates a higher incidence of deleterious BRCA1 [and BRCA2] mutations and variants of uncertain significance (VUS) in African American patients compared to Caucasian patients when controlling for patients of Ashkenazi Jewish (AJ) descent.[2] Despite this, African American women meeting National Comprehensive Cancer Network (NCCN) criteria for genetic testing are less likely to complete such testing compared to Caucasian women nationally.[3] Studies have investigated psycho-social drivers of minority patient aversion to genetic testing. We hypothesize that lack of physician referral for cancer genetic counseling and testing for African American women contributes to this disparity.

A total of 1,180 patients (non-Hispanic African Americans (BNH) N=502; non-Hispanic whites (WNH) N=435) were treated at the George Washington Cancer Center (GW Cancer Center) for breast cancer (both in situ and invasive carcinoma), between 2014 and 2018. Utilizing GW Cancer Center's Cancer Registry, we identified women by race (BNH N =115; WNH N =137) who met select NCCN criteria for referral for genetic evaluation including breast cancer diagnosis under age 50, triple negative breast cancer (TNBC) under age 60, and two primary breast cancers. Excluded patients were those who were not BNH or WNH or who did not meet the select NCCN criteria listed above. Patients were then stratified by race according to who underwent genetic evaluation (BNH N= 81, WNH N= 116), whether at our Ruth Paul Cancer Genetics and Prevention Service (RPCGPS) or elsewhere, by reviewing GW Cancer Center records, RPCGPS records, and patient clinic records for genetic screening results from outside institutions. Patient charts were used to identify individuals who received a physician referral during the course of their cancer care.

Twenty-one percent of breast cancer patients seen in GW Cancer Center between 2014–18 met study criteria for referral for genetic evaluation (n = 252; BNH N=115, WNH N=137), including breast cancer diagnosis under age 50 (BNH N = 76; WNH N = 108), TNBC under age 60 (BNH N =14; WNH N =5), and two primary breast cancers (BNH N = 18, WNH N = 16). Several patients

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identified met two or more criteria for referral (BNH N = 7, WNH N = 8). Physician referral rates differed significantly by race (BNH 76.5%, N = 88 and WNH 92.7%; N= 127; $\chi 2$ =13.0642, p-value=0.00030) (Figure 1). There was no significant difference in adherence by race for patients referred to genetic counseling (BNH 92%, N=83; WNH= 91%, N=123, $\chi 2$ =0.00178, p-value=0.894) (Figure 2).

Low genetic testing rates for African American breast cancer patients are an impediment to resolving the prominent onco-racial breast cancer disparities, particularly in young, TNBC, and patients with multiple primary tumors. This study identified a race-based phenomenon in physician referral rates. Possible reasons for this discrepancy may include lag in physician education on the topic of hereditary risk, barriers in physician-patient communication and/or implicit bias. Future work is needed to clarify the root cause in order to increase the identification of at-risk women in the African American community.

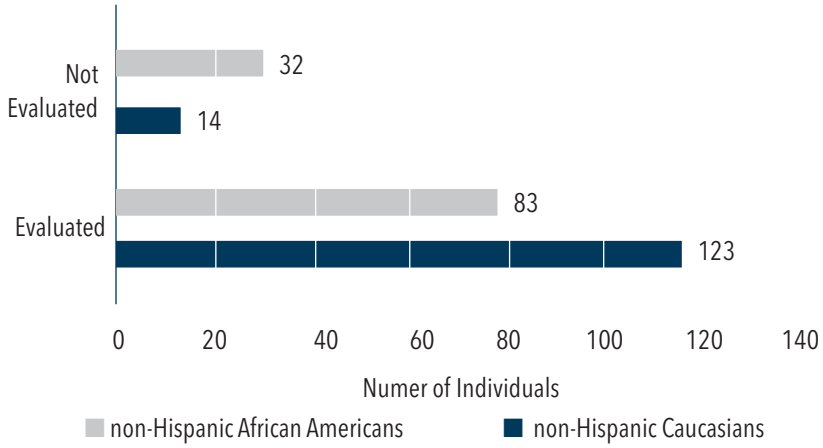

FIGURE 2: Individual patients treated for breast cancer at GW Cancer Center between 2014 and 2018 who met NCCN criteria for referral and were referred by their physician for genetic counseling stratified by race and referral adherence.

REFERENCES:

1. Cancer Facts and Figures: 2018. Atlanta, GA: The American Cancer Society, 2018. (Accessed July 4, 2018, at https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2018/cancer-facts-and-figures-2018.pdf.)
2. Hall MJ, Reid JE, Burbidge LA, et al. BRCA1 and BRCA2 mutations in women of different ethnicities undergoing testing for hereditary breast-ovarian cancer. Cancer 2009; 115(10):2222-23.
3. Jones T, McCarthy AM, Kim Y, et al. Predictors of BRCA1/2 genetic testing among black women with breast cancer: a population study. Cancer Med. 2017; 6(7):1787-1789.

Utilization of Ancillary Services for Voice and Swallowing Outcomes Following Cardiothoracic Surgery

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We sought to determine the utilization of otolaryngology and speech-language pathology services among infants following open cardiothoracic procedures. Given their susceptibility to iatrogenic-induced vocal cord dysfunction, the proper use of ancillary services is critical in limiting long-term swallowing and speech complications.

A fourteen-month retrospective review of the Society of Thoracic Surgeons database at a single tertiary children's hospital was performed to include all infants undergoing open cardiothoracic surgery. Demographic, operative, and outcome variables were identified.

Our results showed, 67 patients were identified (36M/31F; mean [SD] gestational age 36.5 [4.5] weeks) whose surgeries included patent ductus arteriosus ligation (n=20), aortic operation (n=10), Tetralogy of Fallot repair (n=8), and Norwood procedure (n=6). Post-operatively, 20/67 patients (30%) had documented weak cry or weak cough; of these, 10 were evaluated by otolaryngology, and seven were found to have vocal cord paralysis. 30/67 patients (45%) had post-operative feeding difficulties, and 13 had documented aspiration. Of the 13 with aspiration, five underwent a modified barium swallow with speech-language pathology, and five had otolaryngology consultations. On discharge, 13/67 patients (19%) necessitated gastrostomy tube placement, and 8/67 patients (12%) required supplemental oxygen or continued ventilation. Following discharge, 8/67 patients (12%) were followed by otolaryngology for vocal cord paralysis (n=7), persistent dysphagia (n=3), or stridor (n=2).

Open cardiothoracic surgery in infancy is associated with high rates of voice and swallowing dysfunction. Inpatient ancillary services are underutilized in managing this population, and outpatient follow-up is poor, suggesting the need for prospective protocols to ensure adequate care.

Depth-Camera Measured Biomechanics of the Lower Extremity Reveal Movement Abnormalities and Targets for Prevention in ACL Reconstructed Patients

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The anterior cruciate ligament (ACL) is one of the most commonly injured knee ligaments which requires extensive rehabilitation. Athletes who injure their ACL risk long-term disability compromising general function, including increased rates of osteoarthritis. Roughly 80% of ACL injuries have a noncontact origin, suggesting that ACL injury risk is greatly influenced by patterns of motion.[1] The potential benefit of prospectively identifying differences in landing strategies has prompted the development of ACL injury-prevention programs utilizing biomechanical interventions, which correct hazardous movement abnormalities and reduce rates of injury.[2] Studies have identified specific movements, such as excessive internal rotation angles at the hip and knee, as placing individuals at greater risks for ACL injury. To detect these abnormalities, motion capture technologies are

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employed, but they are often expensive, labor-intensive, and impractical screening tools in clinic.[3] The depth camera in the Microsoft Kinect 2 system has emerged to be a promising low-cost assessment system that utilizes surface mapping technology to generate high-quality human kinematic profiles. It maps surfaces by sending out bursts of infrared light, which provide real-time tracking of human limbs.[4] This study seeks to evaluate knee kinematics in the controls, ipsilateral, and contralateral leg of ACL reconstructed patients to assess ACL injury risk.

A cross-sectional study of ACL reconstructed patients at 6 months post-operation (n=15) and controls (n=12) was performed. Participants performed three single leg squats for each leg with arms spread out and opposite leg forward, with movement captured via the Microsoft Kinect 2 camera placed 1-2 meters in front of the participant. Custom MATLAB software was utilized to analyze raw data, which included hip internal rotation (IR), adduction, and knee flexion at the highest point of flexion. Negative values for hip IR corresponded to hip external rotation. Statistics comparing control, contralateral, and ipsilateral legs were conducted using one-way analysis of variance and student T-test, with significance set at $p < 0.05$.

The mean age in years was 27.7 and 31.2 for the control and ACL reconstructed groups, respectively. The percentage of females was 17% and 51% for control and ACL reconstructed groups, respectively. For degree of hip IR, the contralateral group (7.74) was significantly higher than those of the ipsilateral (-2.06; $p<0.05$) and control group (-1.94; $p<0.05$). There was no significant difference in hip adduction among all groups. Comparing knee flexion, controls (85.5) had significantly higher degrees of flexion compared to reconstructed (55.4; $p<0.01$) knees.

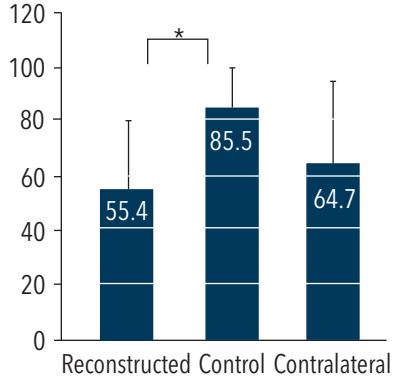

FIGURE 1: Bar graph comparing knee flexion across all groups

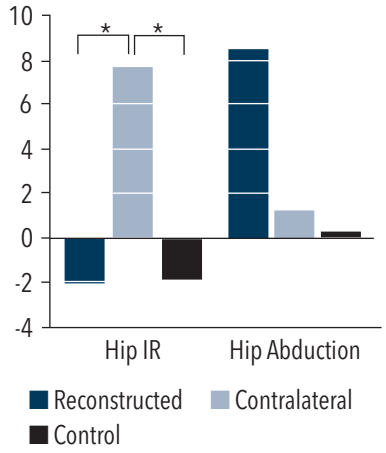

FIGURE 2: Bar graph comparing hip IR, hip abduction across all groups. Negative values for hip IR correspond to external rotation.

At the greatest point of flexion, the contralateral leg in the ACL group demonstrated significantly more internal rotation during a single leg squat, thereby indicating that the knee control in the contralateral leg is abnormal. Rehabilitation may have improved deficiencies in the reconstructed knee while abnormal mechanics on the contralateral side may further predispose patients to injury on that side. While not significant, ipsilateral knees had higher hip adduction than that of contralateral and control knees. This could also be used as a motion analysis marker for abnormal reinjury biomechanics. The depth camera accurately quantified that ACL patients cannot reach the same knee flexion as controls in both the ipsilateral and contralateral leg; instead, ACL patients performed abnormal compensatory behaviors that further increased risk of reinjury. Therefore, this prevention-focused motion analysis system has emerged as a fast, low-cost, and non-invasive method to assess lower extremity biomechanics with great research and clinical utility.

REFERENCES

1. Renstrom P, Ljungqvist A, Arendt E, et al. Non-contact ACL injuries in female athletes: an International Olympic Committee current concepts statement. British J Sports Med 2008;42(6):394-412.

2. Padua DA, DiStefano LJ, Hewett TE, et al. National Athletic Trainers' Association Position Statement: Prevention of Anterior Cruciate Ligament Injury. J of Athl Training 2018;53(1):5.

3. Carlson VR, Sheehan FT, and Boden BP. Video Analysis of Anterior Cruciate Ligament (ACL) Injuries: A Systematic Review. J of Bone and Joint Surgery Rev 2016;4(11):e5.

4. Gray AD, Willis BW, Skubic M, et al. Development and validation of a portable and inexpensive tool to measure the drop vertical jump using the Microsoft Kinect V2. Sports Health 2017;9(6):537-544.

Trends in Treatment Strategies and Comparison of Outcomes in Lymph Node Positive Bladder Cancer

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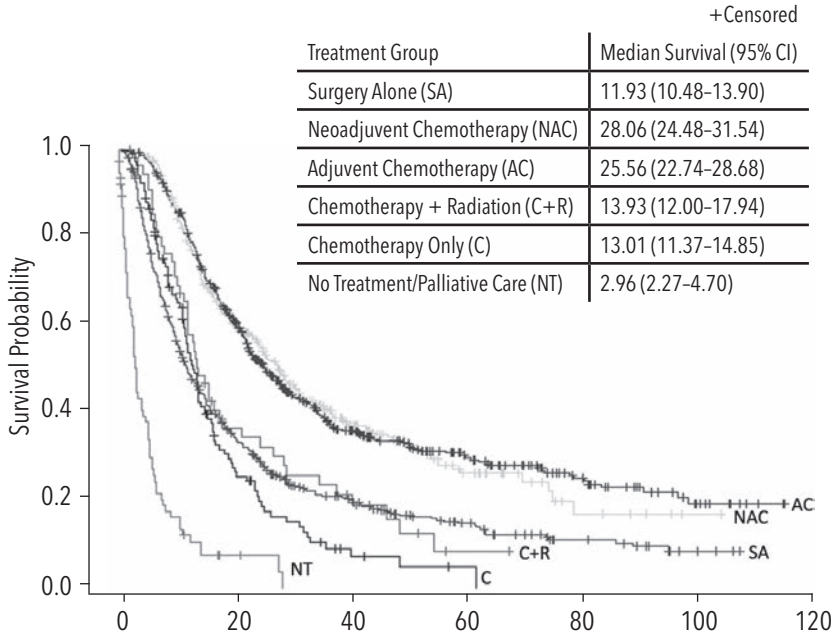

While extensive research has assessed treatment outcomes in muscle invasive bladder cancer, lymph node positive disease (LN+) has been excluded from randomized studies or grouped with metastatic disease.[1-3] Although node positivity confers reduced 5-year cancer specific and overall survival, it is potentially curable if treated before systemic metastasis.[4] Treatment is not standardized, however, especially with lack of radiographic response to induction chemotherapy.[5] This study seeks to compare outcomes and demonstrate trends in treatment for LN+ bladder cancer.

We performed a retrospective cohort study using the National Cancer Database (2006-2014) and identified 1869 cT2-4N1-3M0 bladder cancer patients treated with (1) radical cystectomy (RC), (2) neoadjuvant chemotherapy (NAC) + RC, (3) adjuvant chemotherapy (AC) + RC, (4) radiation + chemotherapy, (5) chemotherapy alone, or (6) no treatment/palliative care only. The primary outcome was survival by treatment, analyzed using Kaplan-Meier and multivariable Cox-proportional hazards regression. Secondary outcomes included pathologic down-staging, analyzed using univariable/multivariable logistic regression models. A univariable logistic regression model of treatment by year was used to identify treatment trends over time. Multivariable models were adjusted for confounding demographic, facility-level, and clinicopathologic variables.

Among 1869 patients (cN1, 48%; cN2, 44%; cN3 8%), 567 underwent RC, 418 underwent NAC, 591 underwent AC, 61 underwent radiation + chemotherapy, 136 underwent chemotherapy alone, and 96 had no definitive treatment. Overall survival did not differ between NAC and AC, but both had improved survival compared to RC alone. All other treatment groups had worse survival outcomes in comparison to NAC. When comparing NAC to RC alone, down-staging to pT0 (adjusted odds ratio [aOR]=26.39) and pN0 (aOR=6.88) was higher for NAC. Overall, utilization of NAC and no treatment has increased, use of AC and RC alone has declined, and use of chemotherapy and radiation without surgery has not changed significantly.

Combined chemotherapy and RC is associated with improved outcomes for LN+ bladder cancer compared to RC or chemotherapy alone, although there is no significant difference between NAC and AC. Use of radiation and chemotherapy without RC has stayed consistent and is associated with worse oncologic outcomes compared to RC with perioperative chemotherapy, but there is no significant difference when compared to RC or chemotherapy alone.

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REFERENCES

1. Grossman HB, Natale RB, Tangen CM, et al. Neoadjuvant Chemotherapy plus Cystectomy Compared with Cystectomy Alone for Locally Advanced Bladder Cancer. N Engl J Med. 2003;349(9):859-866. doi:10.1056/NEJMoa022148
2. Stein BJP, Lieskovsky G, Cote R, et al. Radical Cystectomy in the Treatment of Invasive Bladder Cancer : Long-Term Results in 1,054 Patients. J Clin Oncol. 2011;19(3):666-675.
3. Vale CL. Neoadjuvant Chemotherapy in Invasive Bladder Cancer: Update of a Systematic Review and Meta-Analysis of Individual Patient Data. Eur Urol. 2005;48(2):202-205. doi:10.1016/j.eururo.2005.04.006.
4. Herr HW, Donat SM. Outcome of Patients With Grossly Node Positive Bladder Cancer After Pelvic Lymph Node Dissection and Radical Cystectomy. J Urol. 2001;165(1):62-64. doi:10.1097/00005392-200101000-00015.
5. Milowsky MI, Bryan Rumble R, Booth CM, et al. Guideline on Muscle-Invasive and Metastatic Bladder Cancer (European Association of Urology guideline): American Society of Clinical Oncology clinical practice guideline endorsement. J Clin Oncol. 2016;34(16):1945-1952. doi:10.1200/JCO.2015.65.9797.

Impact of Smoking on Outcomes Following Knee and Shoulder Arthroscopy

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With well over a million procedures performed annually,[1] arthroscopy of the knee and shoulder are two of the most commonly performed orthopaedic surgeries. Optimization of patient outcomes by minimizing modifiable risk factors is crucial, and one well-established modifiable risk factor is smoking. Approximately 15.5% of Americans smoke,[2] and the prevalence of smoking is highest in males ages 25-64,[3] a group which also encompasses the majority of patients undergoing arthroscopic procedures.[1]

The first step towards mitigating the risk associated with smoking is to define its impact on postoperative outcomes. The purpose of this study was to evaluate any association between preoperative smoking and perioperative and early postoperative complications in a large population following shoulder and knee arthroscopic surgery for sports injury.

The National Surgical Quality Improvement Program (NSQIP) database was queried retrospectively for patients who underwent knee or shoulder arthroscopic surgery for sports injury between 2010-2016. These patients were identified using the current procedural terminology (CPT) codes. This database provides an invaluable source of data on a large sample of patients collected in a reliable, systematic way from centers across the country.

Deaths and complications recorded in the first 30 days postoperatively were included and categorized as cardiac, renal, wound (including all surgical site infections), sepsis, thromboembolic, or pulmonary. A composite outcome was formed and

Procedure	aOR for smokers vs non-smokers	Lower 95% CI	Upper 95% CI	p
KAM	1.228	0.979	1.541	0.08
SHADEC	1.462	1.03	2.075	0.033
KAC	1.058	0.703	1.592	0.79
KAA	1.213	0.859	1.713	0.27
KAB	1.969	1.407	2.757	<.0001
DCSA	1.137	0.686	1.883	0.62
SHADEB	1.933	1.211	3.084	0.006
SHACUR	1.297	0.874	1.925	0.2

aOR: adjusted odds ratio; CI: confidence interval. KAM: Knee arthroscopy with meniscectomy (medial or lateral); SHADEC: shoulder arthroscopy with decompression; KAC: knee arthroscopy

TABLE: Adjusted odds of reaching the composite outcome* for smokers, by procedure.

**Composite outcome: Any cardiac, renal, wound (including all surgical site infections), sepsis, thromboembolic, or pulmonary complication as recorded in the NSQIP database.*

defined as positive if a patient experienced any of the above complications. Univariate and multivariate analyses were performed to determine whether preoperative smoking was an independent risk factor for any of these postoperative complications individually or for the composite outcome.

The study included 134,822 cases. In univariate analysis, smoking was associated with increased rates of complication in knee arthroscopy with the following: ACL reconstruction or medial and lateral meniscectomy, and shoulder arthroscopy with the following: debridement, decompression, or rotator cuff repair. Significant associations then underwent multivariate analysis, which found that smoking was an independent risk factor for any complication/mortality event in shoulder arthroscopy with decompression (OR=1.46; 95% CI: 1.030-2.075), shoulder arthroscopy with debridement (OR=1.933; 95% CI: 1.211-3.084) and knee arthroscopy with medial and lateral meniscectomy (OR=1.97, 95% CI:1.407-2.757).

Preoperative smoking was found to be an independent risk factor for complications for several arthroscopic procedures, though with variability between types of procedure. In our study, patients who smoked were significantly younger, and, presumably, healthier. This is a potential confounder, which may account for some of the variability in the impact smoking had on outcomes between different procedures. Strengths of this study include the use of a nationally validated database, with a large sample size and robust clinical characteristics. Limitations include the retrospective nature of the study, lack of data on surgical technique and simultaneous procedures, 30-day follow up window, and reliance of self-reporting of smoking status.

Overall, our data highlights that even in generally low-risk arthroscopic procedures, preoperative smoking may increase the risk of potentially serious perioperative and early-postoperative complications. Our work adds to the breadth of literature demonstrating that smoking is detrimental to patient outcomes in orthopaedic surgery in both the short- and long-term.

REFERENCES:

1. Shah N V., Solow M, Kelly JJ, et al. Demographics and rates of surgical arthroscopy and postoperative rehabilitative preferences of arthroscopists from the Arthroscopy Association of North America (AANA). J Orthop. 2018;15(2):591-595. doi:10.1016/j.jor.2018.05.033.

2. National Center for Chronic Disease Prevention and Health Promotion (US), Health O on S and H. The Health Consequences of Smoking—50 Years of Progress. Centers for Disease Control and Prevention (US); 2014. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24455788. Accessed June 28, 2018.

3. Jamal A, Phillips E, Gentzke AS, et al. Current Cigarette Smoking Among Adults — United States, 2016. MMWR Morb Mortal Wkly Rep. 2018;67(2):53-59. doi:10.15585/mmwr.mm6702a1.

Comparison Between Medical Therapy and Endovascular Treatment of the Extracranial Atherosclerotic Vertebral Artery Disease: A Systematic Review

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Atherosclerosis is a common cause of vertebral artery stenosis leading to vertebrobasilar or posterior circulation strokes and transient ischemic attacks (TIA). About 20 % of ischemic strokes occur in the vertebrobasilar circulation.[1] Vertebrobasilar circulation perfuses the medulla, cerebellum, pons, midbrain, thalamus, and occipital cortex. Therefore, atherosclerotic disease in these vessels can result in significant multisystem disability and death. There are two portions of the vertebral artery, extracranial (V1 – V3) and intracranial (V4). Though the extracranial vertebral artery disease is generally regarded to have more benign outcomes as compared to intracranial, 50% of these patients may have a stroke at initial presentation while another 26% may present with TIAs.[2] For decades, the primary treatment in these patients has consisted of antiplatelet and anticoagulation drugs such as aspirin and warfarin. However, advances in endovascular technology with balloon angioplasty or angioplasty with stent placement have become effective alternatives. Recent trials such as the VIST trial have shown that interventional techniques are feasible, safe, and effective.[3] However, more evidence is required to reach a consensus. The aim of the present

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study is to compare best medical treatment and the endovascular approach in terms of stroke and death rates at 1 month and at last follow-up.

We identified ECVA studies published between January 1966 and December 2017 using a search on PubMed. The rates of stroke and stroke and/or death were estimated for best medical treatment and endovascular treatment at 1 month and at last follow-up. A random effects model was used to calculate pooled proportions (PP) across studies and 95% confidence intervals. A total of 57 reports were included in the analysis, eight studies reported outcomes in patients receiving medical treatment (362 patients) and forty-nine studies reported upon patients treated with endovascular approach (2142 patients). The mean age of patients in the medical group was 65.6 years (range 61.3 - 69.0 years) and 64.1 years (range 53.5 - 72 years) in the endovascular group. The 30-day incidence of stroke was 26 (7.2%) in the medical treatment group compared to 18 (0.84%) in the endovascular group, resulting in a higher risk for patients in the best medical treatment when compared to endovascular treatment (p-value = 0.0001). Similarly, at follow-up, 33 (12.3%) strokes were observed in the medical group compared to 51(2.4%) in the endovascular group (p-value = 0.0001). There was also statistically significant difference in stroke related death in the medical group versus endovascular group 12 (4.5 %) vs 1 (0.04%) (p-value =0.001). There was no statistically significant difference in rates of TIA or death to other causes between the two groups.

	Medical	Endovascular
No. of Studies	8	49
No. of Subjects	362	2142
Mean Age	65.6	64.1
Females	84	580

TABLE 1: Baseline Characteristics

Our analysis demonstrated that endovascular treatment significantly reduced the risk of stroke and death when compared to best medical treatment alone at 30 days and at follow-up. However, because of the paucity of long-term outcomes of patients on standard medical therapy and large variability observed between endovascular case series, a prospective randomized trial is warranted in order to better understand the safety and efficacy of the endovascular treatment compared to the standard medical therapy.

REFERENCES

1. Merwick A, Werring D. Posterior circulation ischaemic stroke. BMJ 2014;348:3175
2. Caplan L, Wityk R, et al. New England medical center posterior circulation registry. Annals of Neurology 2004;56:389-398
3. Markus HS, Larsson SC, et al. Stenting for symptomatic vertebral artery stenosis: The Vertebral Artery Ischaemia Stenting Trial. Neurology 2017;89:1229-1236

	Medical	Endovascular	Significance
# of Peri-Op Strokes	26 (7.2%)	18 (0.84%)	p = <0.0001
# of Strokes at Follow-Up	33 (12.3%)	51 (2.4%)	p = <0.0001
# of Stroke Related Deaths at Follow-Up	12 (4.5%)	1 (0.04%)	p = 0.001

**Peri-Op = within 30 days

TABLE 2: Outcomes

The Impact of Insulin Dependence on Post-Operative Complications in Diabetic Patients Undergoing Anatomic Pulmonary Resections.

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The prevalence of diabetes mellitus (DM) has been rapidly increasing, contributing greatly to the burden of disease in patients and increasing economic costs for the healthcare system in the United States.[1,2,3] Impact on quality of life after surgical treatment is a significant concern for many diabetic patients.[4] While the impact of DM on various surgical procedures has been studied, its impact on patient outcomes following anatomic pulmonary resections remains to be established. Therefore, we conducted this retrospective analysis to investigate the impact of insulin dependence on DM patients undergoing anatomic pulmonary resections.

The National Surgical Quality Improvement Program surgical registry maintained by the American College of Surgeons was queried to identify patients who underwent pulmonary resections from 2005 to 2016 by Current Terminology Procedure (CPT) codes. Ultimately, 18,246 patients were included in the analyses after excluding patients with missing data. Patients were stratified into three cohorts by their diabetes mellitus status — non-diabetics (Non-DM), non-insulin dependent diabetes mellitus (NIDDM), and insulin-dependent diabetes mellitus (IDDM). These cohorts were compared for differences in their demographics, preoperative comorbidities, and complication rates using one-way analysis of variance (ANOVA) for continuous variables and Pearson's Chi-squared tests or Fischer's exact tests (expected cell count < 5) for categorical variables. Multivariate logistic regression models were generated with NIDDM and IDDM as independent risk factors to establish risk associations between diabetes mellitus and specific adverse events in these patients.

Upon analyzing the preoperative comorbidities between the three cohorts, NIDDM and IDDM patients had significantly higher rates of dyspnea ($p<0.001$), chronic obstructive pulmonary disease ($p=0.014$), congestive heart failure ($p=0.001$), hypertension requiring medication management ($p<0.001$), acute renal failure ($p=0.048$), dialysis dependence ($p<0.001$), open wound/wound infections ($p<0.001$), hematologic disorders ($p<0.001$), and functional dependence ($p<0.001$) compared to non-diabetic patients. The non-diabetic cohort had a significantly larger proportion of smokers (35.51%) than the NIDDM (30.34%) patients and

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DEMOGRAPHICS	Non-DM	%	NIDDM	%	IDDM	%	P-value
	15,433		2,017		796		
Sex							<0.001
Female	8447	54.73%	897	44.47%	340	42.71%	
Male	6986	45.27%	1120	55.53%	456	57.29%	
Race							<0.001
American Indian/Alaska Native	101	0.65%	14	0.69%	4	0.50%	
Asian or Pacific Islander	576	3.73%	104	5.16%	20	2.51%	
Black or African American	1007	6.52%	173	8.58%	95	11.93%	
Hispanic	21	0.14%	1	0.05%	1	0.13%	
White	13728	88.95%	1725	85.52%	676	84.92%	

TABLE 1A:

PRE-OPERATIVE COMORBIDITIES							
Smoke	5480	35.51%	612	30.34%	245	30.78%	<0.001
Dyspnea							<0.001
No Dyspnea	11934	77.33%	1487	73.72%	567	71.23%	
At Rest	265	1.72%	35	1.74%	21	2.64%	
Moderate Exertion	3234	20.96%	495	24.54%	208	26.13%	
Ventilator Dependence	29	0.19%	0	0.00%	5	0.63%	0.002
COPD	3756	24.34%	541	26.82%	216	27.14%	0.014
Ascites	10	0.06%	1	0.05%	4	0.50%	<0.001
Congestive Heart Failure	75	0.49%	16	0.79%	11	1.38%	0.001
Hypertension	8291	53.72%	1675	83.04%	672	84.42%	<0.001
Acute Renal Failure	15	0.10%	4	0.20%	3	0.38%	0.048
Dialysis Dependent	44	0.29%	14	0.69%	16	2.01%	<0.001
Disseminated Cancer	1130	7.32%	111	5.50%	55	6.91%	0.011
Wound Infection	93	0.60%	23	1.14%	15	1.88%	<0.001
Steroid Use	709	4.59%	82	4.07%	59	7.41%	<0.001
Weight Loss	453	2.94%	57	2.83%	26	3.27%	0.823
Bleeding Disorder	431	2.79%	83	4.12%	51	6.41%	<0.001
Blood Transfusions	46	0.30%	2	0.10%	5	0.63%	0.057
Functional Status							<0.001
Independent	15293	99.09%	1981	98.22%	781	98.12%	
Partially Dependent	123	0.80%	33	1.64%	14	1.76%	
Totally Dependent	17	0.11%	3	0.15%	1	0.13%	
OPERATIVE VARIABLES							
ASA Classification							<0.001
1-No Disturb	58	0.38%	0	0.00%	1	0.13%	
2-Mild Disturb	3146	20.38%	174	8.63%	32	4.02%	
3-Severe Disturb	10942	70.90%	1575	78.09%	604	75.88%	
4-Life Threat	1284	8.32%	268	13.29%	159	19.97%	
5- Moribund	3	0.02%	0	0.00%	0	0.00%	
Anesthesia Administered							0.065
Epidural	20	0.13%	3	0.15%	0	0.00%	
General	15385	99.69%	2014	99.85%	793	99.62%	
MAC/IV Sedation	5	0.03%	0	0.00%	2	0.25%	
Other	5	0.03%	0	0.00%	1	0.13%	
Regional	9	0.06%	0	0.00%	0	0.00%	
Spinal	9	0.06%	0	0.00%	0	0.00%	

aValues expressed as Mean ± Standard Deviation.

All other values expressed as (%) and N.

TABLE 1B:

Continued from p. 35

IDDM patients (30.78%; p<0.001), as well as a history of disseminated cancer (7.32%) compared to either diabetic cohort (p=0.011; Table 1a-b). From the multivariate regression models, NIDDM patients were found to be at a significantly higher risk for reoperation (OR 1.349, 95% CI 1.063-1.712, p=0.014) following pulmonary resections when compared to non-diabetics (Table 2). IDDM patients were at a significantly higher risk for experiencing pneumonia (OR 1.342, 95% CI 1.033-1.748, p=0.027), unplanned intubation (OR 1.449, 95% CI 1.063-1.976, p=0.019), ventilator dependence for greater than 48 hours (OR 1.792, 95% CI 1.281-2.507, p=0.001), acute renal failure (OR 2.888, 95% CI 1.548-5.389, p=0.001), strokes (OR 2.353, 95% CI 1.184-4.675, p=0.015), cardiac arrest (OR 1.94, 95% CI 1.086-3.465, p=0.025), and an extended length of hospital day of at least 10 days (OR 1.357, 95% CI 1.120-1.644, p=0.002; Table 2).

Discussion: Diabetes mellitus patients had greater risk for numerous complications than non-diabetics. By establishing risk associations between diabetes mellitus and these adverse outcomes, surgeons are better prepared to handle these potential complications. Targeting glucose levels to <200 mg/dL in diabetics may lower the risk for these serious complications. IDDM patients were at an increased risk for pneumonia, intubation, ventilator dependence, renal failure, cardiac arrest, and extended length of stay, demonstrating the impact of insulin dependence on surgical complications. Thus, surgeons can better preoperatively plan for these adverse events in diabetic patients undergoing pulmonary resections.

	IDDM		NIDDM	
Complication	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value
Superficial Incisional SSI	0.699 (0.361-1.354)	0.288	0.835 (0.513-1.360)	0.47
Deep Incisional SSI	1.338 (0.294-6.093)	0.707	0.868 (0.251-2.996)	0.822
Organ/Space SSI	0.615 (0.305-1.240)	0.174	0.90 (0.516-1.570)	0.711
Wound Dehiscence	1.137 (0.140-9.222)	0.905	-	-
Pneumonia	1.342 (1.033-1.748)	0.027	0.965 (0.797-1.168)	0.712
Unplanned Intubation	1.449 (1.063-1.976)	0.019	1.005 (0.791-1.277)	0.966
Pulmonary Embolism	1.004 (0.461-2.184)	0.993	0.825 (0.516-1.321)	0.424
Ventilator Dependence (48 hours)	1.792 (1.281-2.507)	0.001	0.824 (0.632-1.075)	0.154
Progressive Renal Insufficiency	0.485 (0.233-1.01)	0.053	0.741 (0.399-1.376)	0.342
Acute Renal Failure	2.888 (1.548-5.389)	0.001	1.219 (0.666-2.229)	0.521
Urinary Tract Infection	0.788 (0.481-1.291)	0.343	0.968 (0.675-1.388)	0.86
CVA/Stroke	2.353 (1.184-4.675)	0.015	1.141 (0.561-2.319)	0.716
Cardiac Arrest	1.94 (1.086-3.465)	0.025	1.148 (0.696-1.894)	0.588
Deep Venous Thromboembolism	1.532 (0.879-2.671)	0.132	0.964 (0.609-1.526)	0.875
Myocardial Infarction	1.949 (0.961-3.952)	0.064	1.467 (0.867-2.483)	0.153
Post-Operative Blood Transfusions	0.974 (0.733-1.296)	0.859	0.966 (0.797-1.171)	0.725
Systemic Sepsis	1.353 (0.729-2.513)	0.338	0.977 (0.684-1.394)	0.897
Septic Shock	0.697 (0.410-1.184)	0.182	1.111 (0.717-1.721)	0.639
Return to OR	0.809 (0.602-1.086)	0.158	1.349 (1.063-1.712)	0.014
Extended Length of Stay (≥ 10 days)	1.357 (1.120-1.644)	0.002	1.089 (0.944-1.258)	0.243

TABLE 2:

REFERENCES:

1. NCD Risk Factor Collaboration (NCD-RisC). Worldwide Trends in Diabetes Since 1980: A Pooled Analysis of 751 Population-Based Studies with 4.4 Million Participants. Lancet. 2016;387(10027):1513-30.
2. Gregg EW, Zhuo X, Cheng YJ, et al. Trends in Lifetime Risk and Years of Life Lost Due to Diabetes in the USA, 1985-2011: A Modelling Study. Lancet Diabetes Endocrinol. 2014;2(11):867-74.
3. Boyle JP, Thompson TJ, Gregg EW, et al. Projection of the Year 2050 Burden of Diabetes in the U.S. Adult Population: Dynamic Modeling of Incidence, Mortality, and Prediabetes Prevalence. Popul Health Metr. 2010;8:29.
4. Cykert S. Risk Acceptance and Risk Aversion: Patients' Perspectives on Lung Surgery. Thorac. Surg. Clin. 2004;14:287-293.

Intubation Related Vocal Cord Paresis: Outcomes from a Patient Cohort

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	Complete Resolution	No Complete Resolution	T-test
Average Glottal Function Index (GFI)	2.89	7.66	p= 6.30E-4
Average Physical Exam Score	0	1.52	p= 5.16E-09

TABLE: Differences in Average Post-Treatment GFI and Physical Exam Scores for Patients With and Without Complete Resolution of Vocal Cord Weakness

Vocal cord paresis and paralysis are recognized complications of endotracheal intubation; the effects of such complications can range from quality of life changes such as effortful speaking, vocal fatigue, and loss of high register, to more severe outcomes like aspiration pneumonia and complete voice loss.[1]

We conducted a retrospective study, approved by the institutional review board of The George Washington University Medical Faculty Associates. The charts of 62 patients were reviewed for this study. Each of these subjects was diagnosed with vocal fold paresis/paralysis based on five assessments: patient history, subjective rating scales, laryngeal examination, laryngeal electromyography (EMG), and acoustic/aerodynamic measures.

Subjects were classified into four groups based on EMG interpretations of reinnervation or denervation: mixed, reinnervation, denervation, and none. Multiple studies have shown that signs of reinnervation/denervation on EMG are indicative of patient prognosis; however, the duration of time between the symptom onset and EMG test date has also been shown to be correlated with prognosis.[2,3] Thus, the above groupings, plus the duration of time between the intubation date and EMG date, were combined to create a new definition of prognosis of each subject's vocal cord paresis/paralysis. Using these criteria, a score was determined for each patient—a score closer to 2 indicated a good prognosis while a score closer to 0 indicated a poor prognosis. Data analysis was conducted on 3 tiers of patients.

Our analyses of all patients showed that subjects less than or equal to 60 years of age had an average prognosis score of 1.21 while subjects above the age of 60 had a score of 0.79; the difference between these prognosis values were statistically significant at $p < 0.05$. Our results found that hypertension was the most common comorbidity seen in patients who were diagnosed with a post-intubation vocal cord paresis/paralysis. Results from the second tier of analysis showed that of all 38 patients from whom we had post-treatment data for, only 9 patients (24%) achieved complete resolution of their vocal cord paresis/paralysis (defined as a Glottal Function Index (GFI) of 7 or below and a physical exam score of 0). The average age of subjects that achieved complete resolution was 54.44 years and the average age of those that did not completely recover had an average age of 59.94. A chi-squared analysis assessing the relationship between age at intubation and outcomes showed no statistically significant differences ($p = 0.05$). We also found that patients with complete resolution of their vocal cord dysfunction had a post-treatment average GFI of 2.89, which was 4.77 points lower than the average GFI for patients without complete resolution. This difference was statistically significant at $p < 0.05$.

This study is one of the first to use a larger sample of these patients to analyze trends—using the compiled data we were able to show how various cofactors can be used to determine both the initial prognosis and outcomes of these patients. Our results should help guide physicians to better prevent, diagnose, and treat patients with vocal cord injury due to intubation.

REFERENCES

1. Nazal CH, et al. Vocal Cord Paralysis After Endotracheal Intubation: An Uncommon Complication of General Anesthesia. Brazilian J of Anesthesiology (English Edition) 2018;68(6):637-640.

2. Parnes SM and Satya-Murti S. Predictive Value of Laryngeal Electromyography in Patients with Vocal Cord Paralysis of Neurogenic Origin. Laryngoscope 1985;95(11):1323-1326.

3. Munin MC, Murry T, and Rosen CA. Laryngeal Electromyography: Diagnostic and Prognostic Applications. Otolaryngol Clin of North Am 2000;33(4):759-770.

Provider Differences in Management of Normal Second Stage

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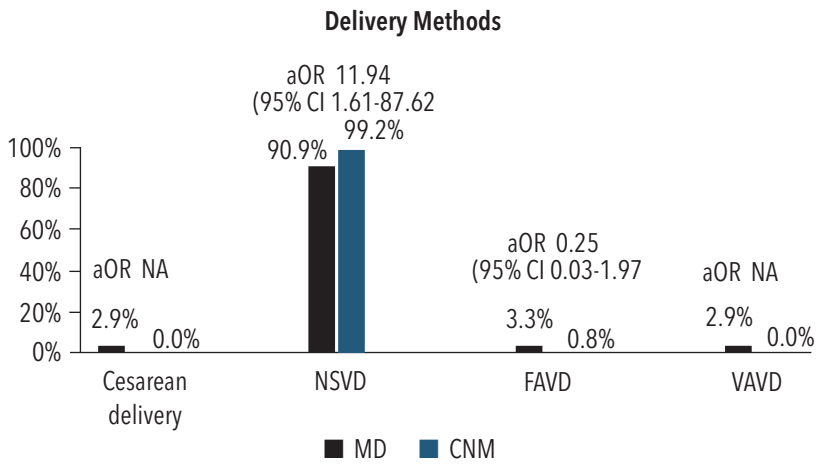

FIGURE: Delivery Methods. aOR- adjusted Odds Ratio, CI-confidence interval, NSVD- normal spontaneous vaginal delivery, FAVD- forceps assisted vaginal delivery, VAVD- vacuum assisted vaginal delivery

This study evaluates whether delivery provider affects maternal and neonatal outcomes in nulliparous women with singleton gestation and a normal second stage of labor.

We performed a retrospective cohort study of term, nulliparous women with singleton gestations and cephalic presentation, who had a second stage of labor <3 hours in duration at a single institution. Exclusion criteria were intrauterine fetal demise, planned cesarean delivery or suspected major fetal anomaly. Outcomes were compared by delivery provider: obstetrician (MD) and certified nurse midwife (CNM). Primary outcome was incidence of cesarean delivery, with maternal and neonatal outcomes compared secondarily.

We evaluated 427 women: 120 with CNM providers and 307 with MD providers. Maternal demographics significantly differed by maternal age at delivery, race, hypertension, BMI, epidural use, induction, and gestational age at delivery. There was a significant difference in rates of normal spontaneous vaginal deliveries (NSVD): 99.2% in CNM patients and 90.9% in MD patients. Incidence of cesarean delivery was 0% in the CNM group and 2.93% in the MD group. The CNM group had a lower incidence of composite maternal morbidity: 9.17% vs 19.54% in the MD group. There were no significant differences in maternal or neonatal outcomes.

In term nulliparous women with singleton gestations who had a normal second stage, delivery provider did not significantly affect the incidence of cesarean delivery but significantly affected the incidence of normal spontaneous vaginal delivery. Most women delivered vaginally, with 97.07% of women in the MD group and 100% in the CNM group. Maternal demographics significantly differed between the two groups, with older patients and decreased epidural use in the CNM group. MD patients had higher rates of hypertension and induction, as well as higher BMI scores. MD and CNM groups had similar maternal and neonatal outcomes when controlling for confounders.

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	MD (n=307)	CNM (n=120)	p-value	OR (95% CI)	p-value	aOR (95% CI)	p-value
Delivery Subtype	9 (2.93%)	0 (0%)	0.0668 (*)				
CD	279	119 (99.17%)	0.0022*	NA	-	NA	-
NSVD	(90.88%)	1 (0.83%)	0.3049	11.94 (1.61 – 88.79)	0.0154*	11.94 (1.61 – 87.62)	0.0154*
FAVD	10 (3.26%)	0 (0%)	0.0668 (*)	0.25 (0.03 – 1.97)	0.1881	0.25 (0.03 – 1.97)	0.1881
VAVD	9 (2.93%)			NA	-	NA	-
Chorioamnionitis	20 (6.51%)	3 (2.50%)	0.0986 (*)	0.37 (0.11 – 1.26)	0.1119	0.31 (0.07 – 1.38)	0.1247
Endometritis	1 (0.33%)	0 (0%)	0.9999	NA	-	-	-
Postpartum hemorrhage	20 (6.51%)	6 (5.00%)	0.5563	0.76 (0.30 – 1.93)	0.5576	0.63 (0.23 – 1.73)	0.3715
Transfusion	0 (0%)	0 (0%)	NA	NA	-	-	-
3rd degree lac.	12 (3.91%)	2 (1.67%)	0.3669	0.42 (0.09 – 1.89)	0.2565	0.42 (0.09 – 1.92)	0.2641
4th degree lac.	1 (0.33%)	0 (0%)	0.9999	NA	-	-	-
At least 1 negative maternal outcome	60 (19.54%)	11 (9.17%)	0.0096*	0.42 (0.21 – 0.82)	0.0155*	0.38 (0.19 – 0.77)	0.0072*
NICU admission	10 (3.26%)	7 (5.83%)	0.2697	1.84 (0.68 – 4.95)	0.2274	1.87 (0.69 – 5.03)	0.2167
CPAP	13 (4.23%)	8 (6.67%)	0.2962	1.62 (0.65 – 4.00)	0.3002	1.64 (0.66 – 4.06)	0.2859
Sepsis	2 (0.65%)	1 (0.83%)	0.9999	1.28 (0.12 – 14.27)	0.8400	1.30 (0.12 – 14.46)	0.8314
Seizure	0 (0%)	1 (0.83%)	0.2810	NA	-	-	-
HIE	0 (0%)	2 (1.67%)	0.0785(*)	NA	-	-	-
Shoulder dystocia	6 (1.95%)	1 (0.83%)	0.6787	0.42 (0.05 – 3.54)	0.4262	0.43 (0.05 – 3.59)	0.4336
Death	0 (0%)	1 (0.83%)	0.2810	NA	-	-	-
At least 1 negative neonatal outcome	21 (6.84%)	10 (8.33%)	0.5930	1.24 (0.57 – 2.71)	0.5936	1.26 (0.57 – 2.76)	0.5683

MD- medical doctor, CNM- certified nurse midwife, OR- odds ratio, aOR- adjusted odds ratio, NA: not enough occurrence for accurate result; CD- cesarean delivery, NSVD- normal spontaneous vaginal delivery; FAVD- forceps assisted vaginal delivery; VAVD- vacuum assisted vaginal delivery; CPAP- continuous positive airway pressure; NICU- neonatal intensive care unit; HIE- hypoxic ischemic encephalopathy

Data are in mean (%)

TABLE: Perinatal Outcomes

Screening For Vocal Cord Paralysis In High Risk Premature Infants After Patent Ductus Arteriosus (PDA) Ligation

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Patent Ductus Arteriosus (PDA) is a congenital heart abnormality, affecting up to 80% of all Extremely Low Birth Weight (ELBW) infants.[1] Surgical repair of this cardiac anomaly renders the Recurrent Laryngeal Nerve vulnerable to iatrogenic damage, ultimately inducing vocal cord paralysis.[2] Our objectives were to determine the clinical compliance with postoperative screening for vocal cord paralysis and associated comorbidities in premature infants following PDA ligation. Given that vocal cord paralysis is a known complication of PDA ligation, an understanding of the current screening methodology is needed for improvement of care.

A three-year retrospective chart review was performed for all premature infants who underwent PDA ligation at the Children's National Health System pediatric hospital. Vocal cord palsy, otolaryngology postoperative consultations, and comorbidities (voice abnormalities, feeding difficulties, gastrostomy tube placement, and supplemental oxygen at discharge) were extracted from the charts. Standard statistical methodology was applied.

Seventy-four patients were identified (33M/41F; mean [SD] gestational age 25.6 [3.5] weeks). Of these patients, 17 infants (23%) were diagnosed with vocal cord paralysis; 14 patients (19%) experienced left-sided vocal cord paralysis, 2 patients experienced bilateral vocal cord paralysis, and 1 patient experienced right-sided vocal cord paralysis. Despite 33 patients (45%) suffering from post-operative voice abnormalities, defined as a hoarse or weak cry, and 48 patients (55%) with documented feeding difficulties, only 27 patients (36%) were evaluated by otolaryngology for possible vocal cord paralysis. Patients with known vocal cord paralysis had a higher rate of gastrostomy tube placement (53% vs. 12%, p= 0.0003), supplemental oxygen (64% vs. 36%, p= 0.0417), and need for chronic ventilation at discharge (18% vs. 0.09%, p=0.0013) compared to those who did not.

It's important to note, not all patients who post-operatively presented with symptoms concerning for vocal cord paralysis were sufficiently evaluated. Additionally, the reported prevalence of vocal cord paralysis may be underestimated due to the lack of otolaryngology evaluation after PDA ligation, highlighting the need for a better screening protocol. Premature infants with known vocal cord paralysis after PDA ligation have greater feeding and respiratory comorbidities. Therefore, timely diagnosis may improve discharge planning and patient/parent counseling.

[T]he reported prevalence of vocal cord paralysis may be underestimated due to the lack of otolaryngology evaluation after PDA ligation, highlighting the need for a better screening protocol.

REFERENCES:

1 Evans N. Diagnosis of patent ductus arteriosus in the preterm newborn. Arch Dis Child. 1993; 68: 58–61.

2 Lee K. Essential Otolaryngology: Head and Neck Surgery, 8th edn. San Francisco, CA: McGraw Hill, 2003.

Rectus Fascia vs. Fascial Lata for Autologous Fascial Pubovaginal Sling: A Single-Center Comparison of Perioperative And Functional Outcomes

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Our objective in this study was comparing perioperative and functional outcomes of autologous fascia lata vs. rectus fascia pubovaginal sling in female patients with stress urinary incontinence (SUI).

Autologous fascial pubovaginal slings (AFPVS) are the most widely used surgical treatment in patients with complex SUI.[1,2] The majority of series have reported the use of rectus fascia grafts.[3,4] Despite this convention, the use of fascia lata has been described as an alternative with the benefits of minimizing postoperative pain and theoretically lowering the risk of abdominal complications such as wound infections. Furthermore, fascia lata harvest been used in patients with significant central obesity, poor quality abdominal fascia or with an extensive history of abdominal surgery.[5] Despite potential benefits, fascial lata sling harvest is associated with its own set of complication related to morbidity from a second incision site and concerns with long term durability.[3] To our knowledge, fascia lata slings have never been assessed in direct comparison with rectus fascial slings. The aim of the present series is to compare perioperative and functional outcomes of autologous fascia lata vs. rectus fascia pubovaginal sling in female patients with SUI.

	Fascia lata pubovaginal sling N=21	Rectus fascia pubovaginal sling N=84	p-value
Operative time (min)	84 (±29.1)	81.9 (±28.9)	0.68
Length of stay (hours)	25 (±9.8)	33.7 (±24.3)	0.15
Estimated blood loss (ml)	91.7 (±90.3)	141.6 (±104.2)	0.04
Postoperative complications	11 (52.4%)	41 (48.9%)	0.81
Clavien grade 1	11	25	
Clavien grade 2	1	14	
Clavien grade 3	0	3	
Clavien grade 4 or 5	0	0	
Readmission	1 (4.8%)	3 (3.6%)	0.99
Wound complications	0 (0%)	12 (14.3%)	0.12
Seroma	0	9*	
Wound infections	0	4*	

*: one patient had both a seroma and a wound infection

TABLE 1: Perioperative Outcomes

Materials and methods: Charts of all female patients who underwent AFPVS for SUI from 2012 to 2017 at a single academic center were retrospectively reviewed. Patients were divided into two groups: those with the autologous sling harvested from the fascia lata (FL group) and those with the autologous sling harvested from the rectus fascia (RF group). Peri-operative and functional outcomes were compared.

Results: Between 2012 and 2017, 105 women underwent pubovaginal slings: 21 using FL and 84 using RF. Operative time did not differ significantly between the FL and RF groups (84 vs. 81.9 min; p=0.68). There were more wound complications in the RF group, but this difference did not reach statistical significance (0% vs. 14.3%; p=0.12). The overall complications rates were comparable in the FL and RF groups (52.4% vs. 48.9%; p=0.81), but the proportion of postoperative complications Clavien grade ≥ 2 tended to be higher in the RF group (4.8% vs. 20.2%; p=0.11). Overall, wound complications accounted for 29.3% of post-operative

complications in the RF group (12/41). The functional outcomes were comparable between FL and RF group, with similar rates of patients cured of SUI symptoms at 1 month (82.4% vs. 76.4%; p=0.74), 1 year (55.6% vs. 63.8%; p=0.76), and the latest follow-up (66.7% vs. 65.8%; p=0.87).

When compared to rectus fascia harvest for pubovaginal sling, fascia lata harvest may decrease perioperative morbidity, especially wound complications, without compromising functional outcomes.

REFERENCES

1. AUA/SUFU Guideline. Surgical Treatment of Female Stress Urinary Incontinence (SUI): AUA/SUFU Guideline. 2017
2. Syan R, Brucker BM. Guideline of guidelines: urinary incontinence. BJU Int. 2016 117(1):20-33.
3. Zoorob D, Karram M. Role of autologous bladder-neck slings: a urogynecology perspective. Urol Clin North Am. 2012;39(3):311-6.
4. Bayrak Ö, Osborn D, Reynolds WS, Dmochowski RR. Pubovaginal sling materials and their outcomes. Turk J Urol. 2014;40(4):233-9.
5. Flynn BJ, Yap WT. Pubovaginal sling using allograft fascia lata versus autograft fascia for all types of stress urinary incontinence: 2-year minimum followup. J Urol. 2002;167(2 Pt 1):608-12.

	Fascia lata pubovaginal sling N=21	Rectus fascia pubovaginal sling N=84	p-value
1-month SUI outcomes Cured Improved Unchanged Worsened Lost to follow-up	 14 (82.4%) 1 (5.9%) 2 (11.8%) 0 4	 55 (76.4%) 9 (12.5%) 6 (8.3%) 2 (2.8%) 12	0.74
1-month de novo self-catheterization	3 (17.7%)	21 (28.8%)	0.54
Change in post-void residual at 1 month (ml)	+ 50.9 (±51.5)	+ 20.5 (±104.8)	0.06
1-year SUI outcomes Cured Improved Unchanged Worsened Lost to follow-up	 5 (55.6%) 3 (33.3%) 1 (11.1%) 0 13	 30 (63.8%) 9 (19.2%) 6 (12.8%) 2 (4.3%) 37	0.76
1-year de novo self-catheterization	1 (11.1%)	3 (6.4%)	0.51
Change in post-void residual at 1 year (ml)	+ 43.6 (±66.5)	- 7 (±77.8)	0.18
Last follow-up SUI outcomes Cured Improved Unchanged Worsened NA	 12 (66.7%) 5 (27.8%) 1 (14.3%) 0 3	 48 (65.8%) 17 (23.3%) 6 (8.2%) 2 (2.7%) 11	0.87
Last follow-up de novo self-catheterization	3 (16.7%)	7 (9.6%)	0.41
Last follow-up change in post-void residual at 1 year (ml)	+ 46 (±55.4)	+27.4 (±113.5)	0.10
Mean follow-up (months)	8.5 (±9.5)	14.1 (±15.4)	0.11

SUI: stress urinary incontinence

TABLE 2: Functional Outcomes

Cortical Thickness Asymmetries in MRI-Abnormal Pediatric Epilepsy Patients: A Potential Metric for Surgery Outcome

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Neuroanatomical morphometric analysis in childhood temporal and extratemporal epilepsy reveals patterns of widespread cortical thinning.[1,2] The degree of neocortical thinning both ipsilateral and contralateral to the focus in epilepsy is associated with poor epilepsy surgery outcome for MRI-normal adult epilepsy as well as MRI-abnormal childhood epilepsy.[3,4] This project extends upon previous investigations by looking at cortical thickness (CT) differences in a pre-surgical pediatric epilepsy population with MRI abnormalities.

Sagittal T1-weighted MPRAGE structural MRI scans were performed on 25 pediatric epilepsy patients (age 7–17 years) with abnormalities on MRI. All surface-based morphometric processing and analyses were conducted using FreeSurfer (FS) 6.0. A repeated measures ANOVA was used to examine the effects of focus (temporal or extratemporal), side (ipsilateral or contralateral), and lobe (frontal, temporal, parietal, occipital) on cortical thickness.

Repeated measures ANOVA revealed a significant effect of lobe ($p<.01$) such that Temporal CT> Frontal CT> Occipital CT> Parietal CT. There was also a trend towards significance for the side of focus (ipsilateral vs. contralateral) and a significant interaction between side and lobe (repeated measures ANOVA, $p=.055$; $p<.05$), indicating that CT by lobe varied according to whether the patient had a temporal or extratemporal epilepsy focus. On the ipsilateral side, the order of greatest CT by lobe was: Temporal> Frontal> Parietal> Occipital. On the contralateral side, the occipital lobe was thickest but the other lobes were consistent in their order. There were no significant CT differences related to having a temporal versus extratemporal focus.

Across both temporal and extratemporal epilepsy groups, we found that the temporal lobe was thickest on the ipsilateral side. This may be because the MRI abnormality which was largely dysplasia contributed to a thicker CT. Seizure onset and duration was associated with thinning of the ipsilateral parietal lobe suggesting that perhaps the parietal lobe is vulnerable to long-term effects of ongoing seizure activity.

Seizure onset and duration was associated with thinning of the ipsilateral parietal lobe suggesting that perhaps the parietal lobe is vulnerable to long-term effects of ongoing seizure activity.

Additionally, the majority of this sample (84%) were noted to have successful surgery outcomes, as quantified by an Engel Class 1 or 2 designation post-surgery, a standard metric indicating seizure freedom or significant reduction. Thus, our results are in accordance with previous research, suggesting that thicker ipsilateral CT is associated with a good surgery outcome. Here, we observed thinner CT on the contralateral side in patients that largely experienced surgical success. Future research with larger sample sizes could investigate the relationship between CT and different types of MRI abnormality and surgery outcomes.

REFERENCES:

1. Widjaja, E., Mahmoodabadi, S. Z., Snead, O. C., Almehdar, A., & Smith, M. L. (2011). Widespread Cortical Thinning in Children with Frontal Lobe Epilepsy. Epilepsia, 52(9), 1685-1691.

2. Mueller, S. G., Laxer, K. D., Barakos, J., Cheong, I., Garcia, P., & Weiner, M. W. (2009). Widespread Neocortical Abnormalities in Temporal Lobe Epilepsy with and Without Mesial Sclerosis. Neuroimage, 46(2), 353-359.

3. Bernhardt, B. C., Bernasconi, N., Concha, L., & Bernasconi, A. (2010). Cortical Thickness Analysis in Temporal Lobe Epilepsy. Reproducibility and Relation to Outcome. Neurology, 74(22), 1776-1784.

4. Kamson, D. O., Pilli, V. K., Asano, E., Jeong, J. W., Sood, S., Juhász, C., & Chugani, H. T. (2016). Cortical Thickness Asymmetries and Surgical Outcome in Neocortical Epilepsy. Journal of the Neurological Sciences, 368, 97-103.

Perioperative Complications Associated with Congestive Heart Failure in Elderly Patients Following Primary Hip Hemiarthroplasty

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Although there are reports of the impact of congestive heart failure (CHF) on total knee arthroplasty (TKA) and total hip arthroplasty (THA), there is a lack of literature analyzing CHF in hip hemiarthroplasty (HHA) procedures. The main objective of this study was to evaluate the effect of CHF on risks for complications following HHA.

The American College of Surgeons National Surgery Quality Improvement Program (ACS-NSQIP) is a multi-institutional national database tracking 30-day patient outcomes and complications for surgical procedures. The ACS-NSQIP database was queried for all patients who had undergone HHA from 2005 to 2016. Pearson's chi-squared tests and Fischer's exact tests were utilized to compare differences in demographics, comorbidities, and complication rates. Multivariate logistic regression analyses were used to assess the impact of CHF as an independent risk factor for postoperative complications following HHA.

Six hundred HHA patients (4.08%) had CHF, and this patient cohort was older (p<0.001) and had a larger proportion of males (p<0.001). CHF was found to be a significant independent risk factor for pneumonia (OR 1.55, 95% CI 1.146-2.097, p=0.004), progressive renal insufficiency (OR 3.277, 95% CI 1.681-6.387, p<0.001), pulmonary embolisms (OR 2.728, 95% CI 1.256-5.926, p=0.011), cardiac arrest (OR 3.582, 95% CI 2.128-6.031, p<0.001), extended length of stay (≥5 days) (OR 1.447, 95% CI 1.218-1.720, p<0.001), readmission (OR 1.294, 95% CI 1.004-1.669, p=0.047), and mortality (OR 2.189, 95% CI 1.688-2.839, p<0.001). (See Tables 1 and 2.)

Operative Variables	Control	%	CHF	%	P-value
	14095		600		
Days to Operation from Admission	1.607 ± 7.256		2.998 ± 4.676		<0.001
Total Operating Time (Minutes)	79.96 ± 43.440		79.00 ± 40.680		0.594
Total Hospital Stay Length (Days)	7.66 ± 7.912		11.21 ± 11.560		<0.001
Days from Operation to Death	13.57 ± 8.879		10.74 ± 8.848		0.004
Days from Operation to Discharge	6.22 ± 7.026		8.24 ± 9.621		<0.001
Discharge Destination					<0.001
Home	2821	20.01%	103	17.17%	
Other than Home	7803	55.36%	391	65.17%	
Not Reported	3471	24.63%	106	17.67%	
Care Type					0.561
Inpatient	14024	99.50%	598	99.67%	
Outpatient	71	0.50%	2	0.33%	

TABLE 1: Surgery Related Variables of HHA in Control vs CHF patients

This data illustrates that a known diagnosis of CHF in patients undergoing hip hemiarthroplasty puts patients at a significantly higher risk of longer hospital stays following their procedures. Increased length of stay (LOS) in orthopedic procedures is associated with increased postoperative morbidity (i.e. deep venous thromboembolisms1) and increases the cost burden to the health care system.[2]

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Beyond LOS, this study found that CHF is an independent risk factor for developing pneumonia following HHA. A lower threshold of suspicion for pneumonia development in the postoperative period should be maintained for these patients; more aggressive antibiotic prophylaxis may be considered for CHF patients in HHA.

Additionally, this study found CHF to be an independent risk factor for cardiac arrest. The compensatory beta-adrenoreceptor stimulation of the heart to increase stroke volume in the setting of CHF is associated with increased risk of arrhythmias and sudden cardiac death (SCD).[3] Heart failure was also found, unsurprisingly, in this study to be a significant risk factor for post-surgical mortality in HHA. In the setting of HHA, we recommend monitoring patients with a history of CHF with telemetry/EKG for evidence of ventricular arrhythmias or other risk factors for SCD prior to discharge and in follow-up visits. Cardiac function testing prior to orthopedic procedures such as HHA may also be warranted.

The current study also found an increased risk of progressive renal insufficiency due to CHF prior to HHA. CHF and kidney disease are tightly associated.[4] Furthermore, there is evidence to suggest that arthroplasty is associated with decreased kidney function.[5] Therefore, this finding raises the concern for the need for careful review of the use of perioperative and postoperative medication usage in HHA patients with history of CHF.

Further studies evaluating the timing of medical optimization of CHF before undergoing HHA is warranted. More specific precautionary measures must be taken to preemptively address potential complications in order to optimize favorable outcomes.

Postoperative Complications	Odds Ratio	95% Confidence Interval		P Value
Superficial Incisional SSI	1.725	0.625	4.758	0.292
Deep Incisional SSI	1.014	0.358	2.873	0.979
Organ/Space SSI	1.091	0.258	4.608	0.905
Wound Disruption	4.205	0.874	20.234	0.073
Pneumonia	1.55	1.146	2.097	0.004
Unplanned Intubation	1.305	0.751	2.267	0.346
Pulmonary Embolism	2.728	1.256	5.926	0.011
Ventilator Dependence (>48 hours)	1.032	0.451	2.364	0.94
Progressive Renal Insufficiency	3.277	1.681	6.387	<0.001
Acute Renal Failure	1.653	0.705	3.877	0.248
Urinary Tract Infection	1.064	0.756	1.498	0.722
CVA/Stroke	1.004	0.397	2.539	0.994
Cardiac Arrest	3.582	2.128	6.031	<0.001
Myocardial Infarction	1.622	0.977	2.695	0.062
Blood Transfusions	1.175	0.964	1.431	0.11
Deep Venous Thromboembolism (DVT)	1.305	0.591	2.883	0.511
Systemic Sepsis	1.353	0.798	2.295	0.262
Septic Shock	1.584	0.817	3.071	0.173
Death	2.189	1.688	2.839	<0.001
Return to Operating Room	1.087	0.722	1.637	0.69
Extended Length of Stay (≥ 5 days)	1.447	1.218	1.72	<0.001
Readmission	1.294	1.004	1.669	0.047

TABLE 2: Multivariate Analyses Assessing Congestive Heart Failure as an Independent Risk Factor for Postoperative Complications

REFERENCES

1. Lewis TC, Cortes J, Altshuler D, Papadopoulos J. Venous Thromboembolism Prophylaxis: A Narrative Review with a Focus on the High-Risk Critically Ill Patient. J Intensive Care Med 20.
2. Missios S, Bekelis K. Hospitalization Cost After Spine Surgery in the United States of America. J Clin Neurosci 2015;22:1632-1637.
3. Goyal V, Jassal DS, Dhalla NS. Pathophysiology and Prevention of Sudden Cardiac Death. Can J Physiol Pharmacol 2016;94:237-244.
4. Silverberg D, Wexler D, Blum M, Schwartz D, Iaina A. The Association Between Congestive Heart Failure and Chronic Renal Disease. Curr Opin Nephrol Hypertens 2004;13:163-170.
5. Kimmel LA, Wilson S, Janardan JD, Liew SM, Walker RG. Incidence of Acute Kidney Injury Following Total Joint Arthroplasty: A Retrospective Review by RIFLE Criteria. Clin Kidney J 2014;7:546-551.

Impact of Mesenchymal Stem/Stromal Cell Intra-Arterial Delivery during Pediatric Cardiac Surgery on Neurogenesis in the Porcine Subventricular Zone

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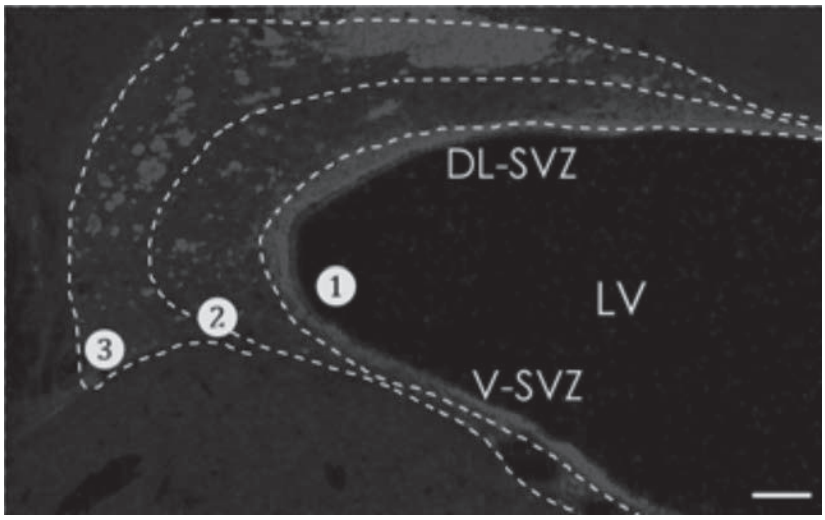

FIGURE 1: DCX+ Staining, scale bar: 200 µm

Congenital heart disease is the leading birth defect, affecting almost 1% of births each year.[1] Moreover, children who undergo cardiac surgery with cardiopulmonary bypass (CPB) show significant cognitive and behavioral impairments.[2] The subventricular zone (SVZ) in the postnatal/adult brain is a very crucial region for neurogenesis and it plays an important role in neocortical growth of the gyrencephalic front lobe during postnatal life. Our preclinical studies have shown that CPB insults can cause a reduction in the neural stem progenitor cell (NSPC) pool.[1] Others have shown that MSCs promote neurogenesis from SVZ NSPCs in early and late rodent models.[2] The aim of this study was to observe the impact of mesenchymal stem/stromal cell (MSC) intra-arterial delivery through cardiopulmonary bypass (CPB) on the proliferation of neural stem/progenitor cells (NSPC) and neuroblast migration in the porcine subventricular zone (SVZ).

The porcine SVZ resembles its human counterpart and therefore served as the animal model for this study. Two-week old piglets (n=12) were randomly assigned to one of three groups: (1) Control, (2) Deep hypothermic circulatory arrest (CPB/DHCA), and (3) CPB/DHCA followed by MSC administration. MSCs (10x10 6 per kg) were delivered intra-arterially through CPB. The piglet brains were fixed three hours post-CPB. NSPC proliferation was determined by SOX2+ and Ki67+ antibodies. Neuroblast and radial-glia like cells were identified by DCX+ and GFAP+ antibodies. The anterior-SVZ was divided into three tiers which were then subdivided into ventral and dorsolateral SVZ. Quantification of the NSPC and neuroblasts in each tier/region was performed, as these parameters reflect neurogenic activity.

CPB/DHCA increased early proliferation of NSPCs. MSC delivery did not alter CPB-induced increased early proliferation of NSPCs. However, MSC delivery reduced the length of GFAP+ processes of radial-glia like cells and the amount of DCX+ neuroblasts in tier 1 and increased the density of DCX+ cells in tiers 2 and 3 where neuroblasts migrate tangentially toward the frontal lobe. These findings suggest that MSC delivery changes neuroblast distribution within the SVZ and promotes neuronal migration toward the frontal lobe.

Our data shows that CPB insults cause proliferation of SVZ neural stem/progenitor cells. Moreover, our preliminary results suggest that MSC delivery has the potential to affect migratory stream of young neurons in SVZ in the acute phase. To determine the ameliorative effect of MSC delivery on neurogenesis, we will need to further investigate the SVZ in long term studies.

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REFERENCES

1. Nobuyuki I, Scafidi J, Murada A, et al. White matter projection in congenital heart disease. American Heart Association Circulation 2012; 125:859-871.

2. Morton PD, Korotcova L, Lewis BK, et al. Abnormal neurogenesis and cortical growth in congenital heart disease. Sci Transl Med. 2017 January 25; 9(374).

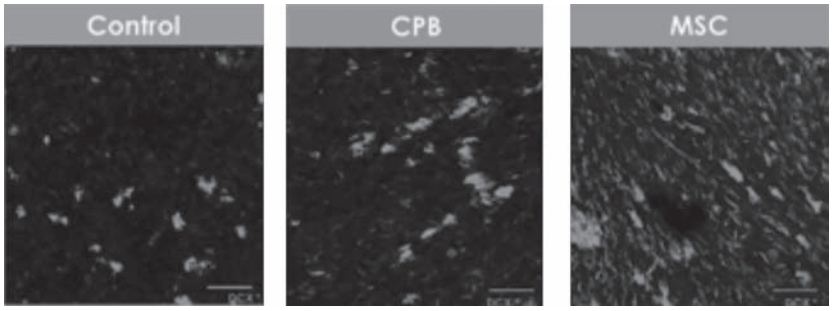

FIGURE 2: DCX+ Dorsolateral Zone Tier 2+3, scale bar 50 µm.

Long-Term Changes in Flow-Mediated Dilation Among Post-Operative Abdominal Aortic Aneurysm Patients

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Ruptured abdominal aortic aneurysms (AAA) cause around 175,000 deaths globally per year.[1] In the U.K., AAA rupture comprises 1% of causes of death for men over 65 years.[2] Several studies have explored flow-mediated dilation (FMD) as a biomarker for endothelial dysfunction, which plays a key role in the pathophysiology of aneurysm development. A previous study from this group (OxAAA), in fact, found that decreased FMD of the right brachial artery was correlated with AAA progression; in addition, soon after surgical repair of the aneurysm, FMD among participants improved.[3] No previous study has explored whether this improvement persists long-term, however. The purpose of this study, therefore, was to evaluate the long-term changes in endothelial function of a subset of participants in the previous study.

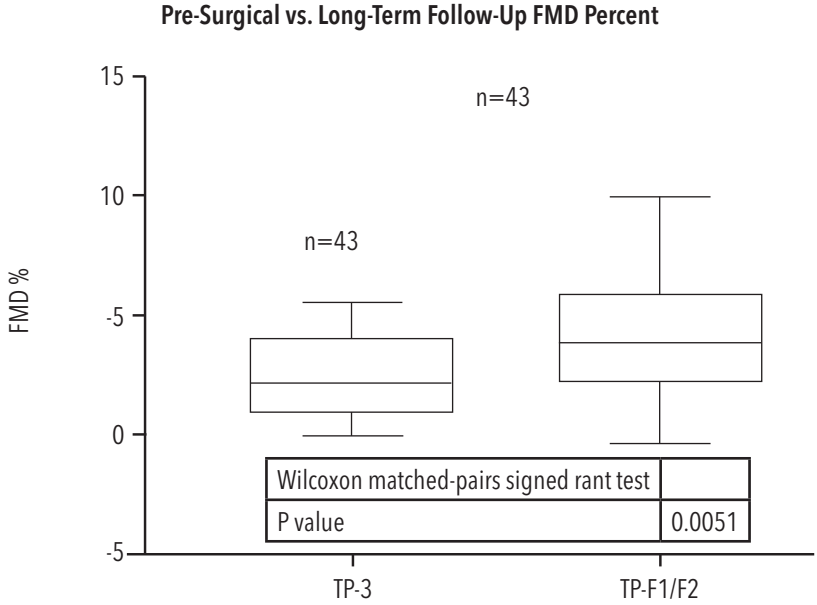

FIGURE 1: Pre-surgical FMD values (TP3) vs. Post-surgical long-term follow up FMD values (1 year and 2–4 year follow up combined, TP-F1/F2). Analyzed using Wilcoxon Sign Rank Test.

The study was conducted using the OxAAA database of participants who had received either an endovascular (EVAR) or an open AAA repair at The John Radcliffe Hospital in Oxford, U.K. since 2013. Participants who had received at least a preoperative and postoperative FMD evaluation were included in the study (N=43). Participants were recalled to the hospital from May-August 2018 for follow-up collection of blood samples, and FMD measurement using high-frequency ultrasound of the brachial artery. Arterial diameter was measured at baseline, during four minutes of artery occlusion, and immediately

after release of occlusion to measure FMD. The data were analyzed using the software "Brachial Analyzer," and results were compared against participants' previous FMD values. The Wilcoxon Sign Rank Test was used to compare patients' FMD values at two time points (pre-surgical and 1–4-year follow up), while ANOVA was used to analyze patients' changes in FMD over multiple points of follow up (pre-surgical, post-surgical, 1-year, and 2–4-year follow up).

Demographic information about this cohort and FMD protocol has previously been published;[3] this subgroup, however, was 97% male and 100% white, consistent with the UK epidemiology of AAAs. The average number of days from surgery to follow up for participants was 996.7. Analysis showed a statistically significant increase in FMD among all 43 patients between 1–4 years after AAA repair (p=0.0061) (Figure 1). The following figure (Figure 2) demonstrates an increase in FMD at each point of follow up after surgery for the 13 participants for which all data points were available; however, the small sample size limits any interpretation of statistical significance .

This study demonstrated improved FMD several years after AAA repair among patients. These findings support the hypothesis that AAAs are systemic vascular diseases, and not simply local, abdominal pathology. The exact mechanism is unknown at this time. Current vascular research findings present the aortic thrombus as a possible source of systemic inflammation; another U.K. cohort study of AAAs that found that even small, sub-surgical AAAs substantially increase the risk of MI

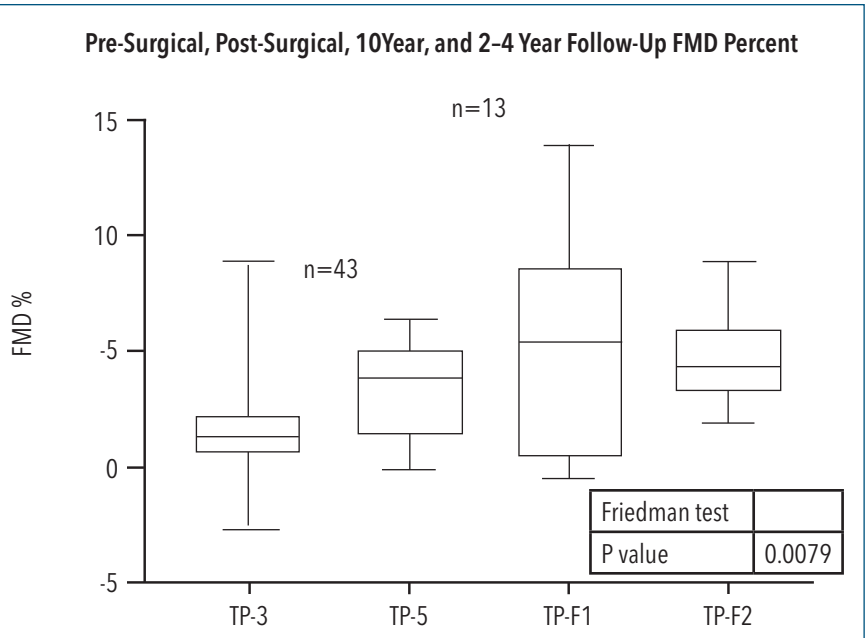

FIGURE 2: Pre-surgical FMD values (TP-3), Post-surgical (TP-5), 1-Year Follow-Up (TP-F1), and 2–4 Year Follow-Up (TP-F2) FMD % ANOVA Analysis.

or stroke among patients.[4] If AAA is indeed a systemic disease, perhaps the risk of rupture is not the only consideration for timing of surgical intervention. FMD could therefore not only be used as a cost-effective, reproducible, and non-invasive biomarker to aid in determinations of surgical interventions, but may serve as a biomarker for increased cardiovascular disease risk. Though the power of this study is limited, it provides the first evidence for long-term improvement in endothelial function after AAA surgery; further research should build on these findings.

REFERENCES

1. Howard, D. P., Banerjee, A., Fairhead, J. F., Handa, A., Silver, L. E., Rothwell, P. M., Oxford Vascular Study. Age-Specific Incidence, Risk Factors and outcome of Acute Abdominal Aortic Aneurysms in a Defined Population. The British Journal of Surgery 2015; 102(8);907-15.
2. Thompson SG, Brown LC, Sweeting MJ, et al.; the RESCAN collaborators. Systematic Review and Meta-Analysis of the Growth and Rupture Rates of Small Abdominal Aortic Aneurysms: Implications for Surveillance Intervals and Their Cost-Effectiveness. Southampton (U.K.): NIHR Journals Library; 2013 Sep. (Health Technology Assessment, No. 17.41.) Chapter 1, Background. Available from: https://www.ncbi.nlm.nih.gov/books/NBK261036/
3. Lee, R., Bellamkonda, K., Jones, A., Killough, N., Woodgate, F., Williams, M., ... & Channon, K. M. Flow Mediated Dilatation and Progression of Abdominal Aortic Aneurysms. European Journal of Vascular and Endovascular Surgery 2017; 53(6), 820-829.
4. Bath, M. F., Saratzis, A., Saedon, M., Sidloff, D., Sayers, R., Bown, M. J., ... & Quarmby, J. Patients with Small Abdominal Aortic Aneurysm Are at Significant Risk of Cardiovascular Events and This Risk Is Not Addressed Sufficiently. European Journal of Vascular and Endovascular Surgery 2017; 53(2), 255-260.

Racial Disparities in Late-Stage Prostate Cancer: A Seer Database Analysis 2005–15

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Incidence of metastatic prostate cancer in the United States has increased over the past ten years, but it is unknown how this trend varies over time in different racial and ethnic populations.[1] Racial and ethnic disparities in prostate cancer incidence and mortality are well documented.[2,3] Further examination of differences in initial rates of prostate cancer diagnosis by race/ethnicity is needed to distinguish factors precipitating disparity.

We identified all men first diagnosed with prostate cancer from 2005–15 in the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute, which monitors 18 population-based cancer registries. Yearly cancer diagnosis frequency from 2005 to 2015 was categorized and analyzed by stage (in situ/localized, regional, and distant), race/ethnicity [White, Asian American/Pacific Islander (AAPI), Black], and age group (45–54, 55–69, 70-75). Chi-squared tests and multivariable logistic regression models were used for data analysis with $p<0.05$ considered significant.

In the 10-year study period, the proportion of regional-stage prostate

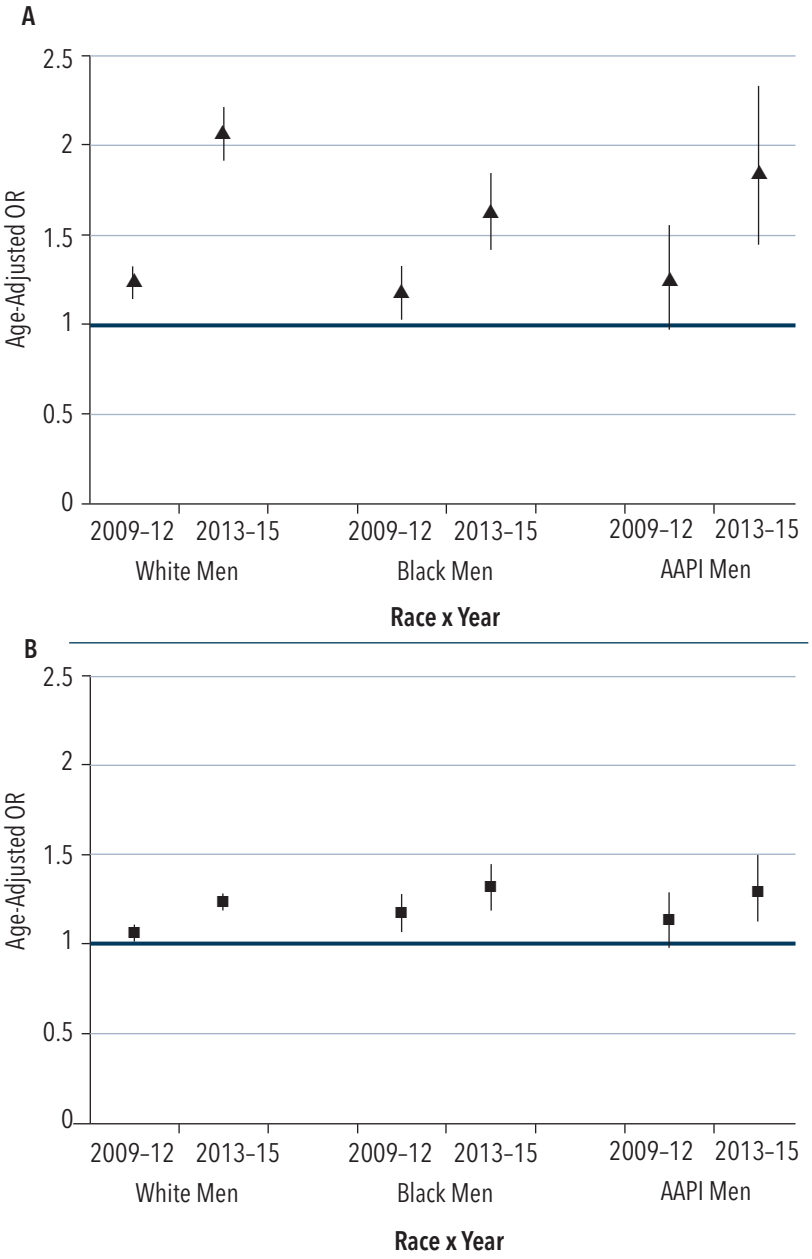

FIGURE 1: Adjusted OR (with 95% confidence interval) for prostate cancer in 2009-12 and 2013–15 versus 2005–08, stratified by race. Adjusted for age. A. Distant vs in situ/localized. B. Regional vs in situ/localized. Horizontal black line shows the odds for the reference group. White, Asian, and Black men's odds of having an initial diagnosis of distant prostate cancer, versus in situ/localized cancer, increased by 106%, 84%, and 62%, respectively (p=.001), between the early and late years of the study. Their odds of having an initial diagnosis of regional prostate cancer versus in situ/localized cancer, increased by 24%, 30%, and 31%, respectively (p=.001).

cancer increased from 14.2% to 16.6% of cases (p<.0001) and distant-stage increased from 3.3% to 5.8% (p<.0001). The odds of being diagnosed with regional-stage prostate cancer in 2013–2015 compared to 2005–08 were 1.3 times higher for Black men (95% CI: 1.2-1.5), 1.3 times higher for AAPI men (95% CI: 1.1-1.5), and 1.2 times higher for White men (95% CI.2-1.3) (Figure 1a). The odds of being diagnosed with distant-stage prostate cancer in 2013–15 compared to 2005–08 were 1.6 times higher for Black men (95% CI: 1.4-1.9), 1.8 times higher for AAPI men (95% CI: 1.5-2.3), and 2.1 times higher for White men (95% CI: 1.9-2.2) (Figure 1b). In 2005-2008, 2009-2012, and 2013-2015 respectively, the odds of being diagnosed with distant-stage prostate cancer were 1.8 times higher, 1.7 times higher, and 1.4 times higher for Black men compared to White men (all respective p<.0001) (Figure 2a), and 1.5 times higher, 1.5 times higher, and 1.4 times higher for AAPI men compared to White men (all respective p<.001) (Figure 2b).

The incidence of late-stage prostate cancer has increased significantly in all U.S. males despite race and ethnicity. Men from minority groups experienced higher rates of newly-diagnosed distant-stage prostate cancer within each year group when compared to White men, with rates declining over time. Regional-stage prostate cancer increased the most over time in AAPI and Black men, while newly diagnosed distant-stage prostate cancer increased the most over time in White men. Genetic variation in disease progression, differences in socioeconomic status, and healthcare access have been posited as theories for disparities in prostate

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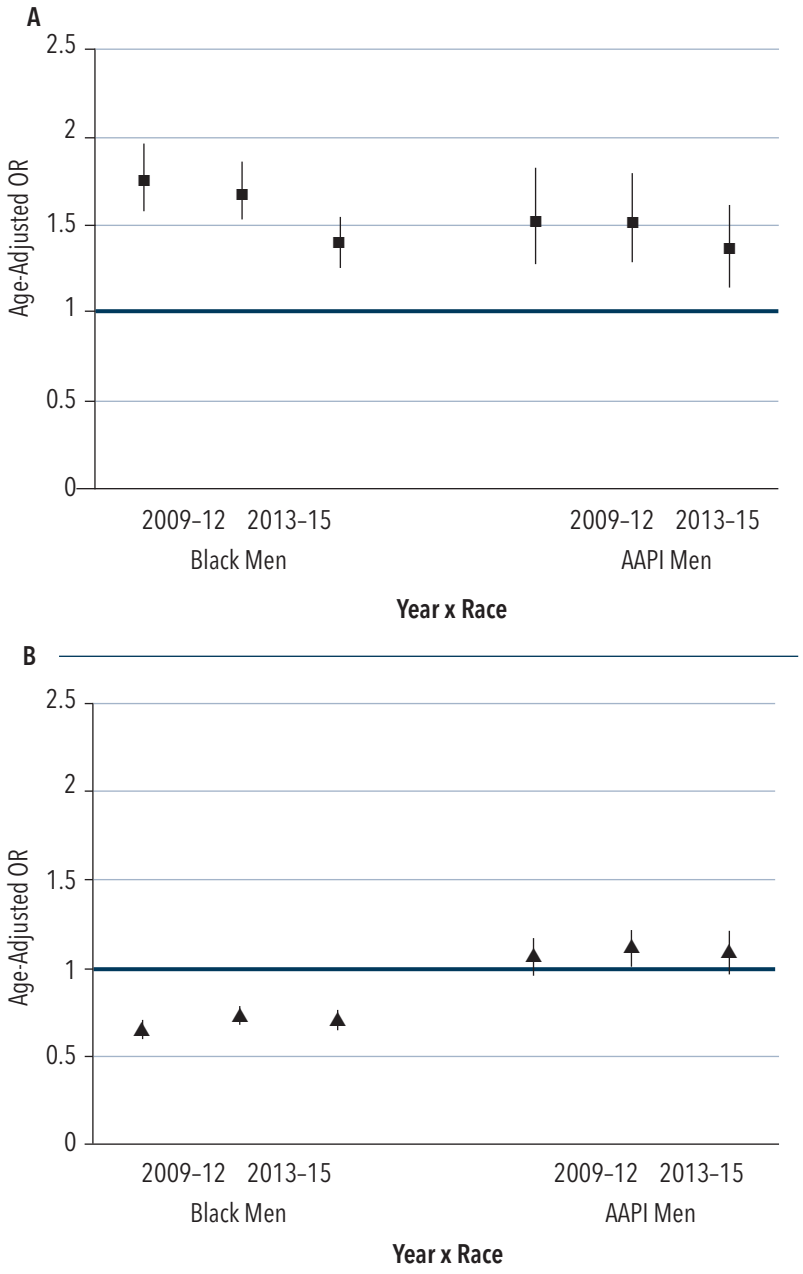

FIGURE 2: Adjusted OR (with 95% confidence interval) for prostate cancer in Black and AAPI men when compared to White men, stratified by year. Adjusted for age. A. Distant vs in situ/localized. B. Regional vs in situ/localized. Horizontal black line shows the odds for the reference group. In 2005–08, 2009–12, and 2013–15, respectively, the odds of having an initial diagnosis of distant prostate cancer, versus in situ/localized, were 76%, 68%, and 40% higher for Blacks than Whites, and 53%, 52%, and 37% higher for AAPI than Whites (p<.001). The odds of initial diagnosis of regional prostate cancer, versus in situ/localized, were 36%, 28%, and 30% lower for Blacks than Whites (p<.001), for the respective year groups, while 2009-2012 showed the only significantly higher odds of regional diagnosis in AAPI compared to whites to be 11% higher (p<.05).

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cancer care.[2,4,5] Changes in guidelines for PSA-based screening may be responsible for this increase, although this was not directly investigated. Based on our findings and previous studies, we suggest considering racial and ethnic disparities when developing PSA-based screening guidelines in an effort to reduce them.[2,3]

REFERENCES

1. Jemal A, Fedewa SA, Ma J, et al. "Prostate Cancer Incidence and PSA Testing Patterns in Relation to USPSTF Screening Recommendations." JAMA 2015 Nov 17; 314(19): 2054-61.
2. Shenoy D, Packianathan S, Chen AM, et al. "Do African-American men need separate prostate cancer screening guidelines?" BMC Urol 2016 May 10; 16(1): 19.
3. Chao GF, Krishna N, Aizer AA, et al. "Asian Americans and prostate cancer: A nationwide population-based analysis." Urol Oncol 2016 May; 34(5): 233.e7-15.
4. Kelly SP, Anderson WF, Rosenberg PS, et al. "Past, Current, and Future Incidence Rates and Burden of Metastatic Prostate Cancer in the United States." Eur Urol Focus 2018 Jan; 4(1): 121-127.
5. Powell IJ, Bock CH, Ruterbusch JJ, et al. "Evidence supports a faster growth rate and/or earlier transformation to clinically significant prostate cancer in black than in white American men, and influences racial progression and mortality disparity." J Urol 2010 May; 183(5): 1792-6.

Developing a Method to Objectively Assess Sensory Nerve Fiber Sensitivity: A Pilot Study

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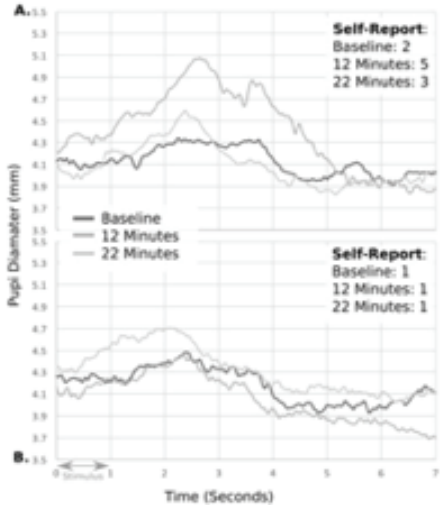

FIGURE: n-PRDs from an individual with tourniquet-induced ischemia. The tourniquet was removed at 20 min and effects of reperfusion were noted at 22 min. A) 5 Hz (at 1.5mA) n-PRDs; nPRD AUCs were 0.690, 1.900, and 1.04 for baseline, 12 min, and 22 min, respectively. B) n-PRDs from the 2000 Hz (at 1.5mA) stimulus; nPRD AUC values were 0.505, 0.699, and 0.824 for baseline, 12 min, and 22 min, respectively.

The rate of chronic pain in the United States is greater than the combined rates of diabetes, heart disease, and cancer, with associated healthcare costs ranging from $560-$635 billion per year.[1,2] It is imperative that an objective assessment tool be developed to ensure adequate pain evaluation and appropriate analgesic intervention is provided to patients experiencing chronic pain.

Pupillary reflex dilation (PRD) occurs when an alerting stimulus activates peripheral nociceptive fibers and elicits pupillary dilation. Preliminary data indicates that a unique PRD (nPRD) can be produced by depolarizing sensory nerves (Aβ, Aδ, and C fibers) via non-noxious neurospecific electrical stimuli at particular frequencies. The amplitude of the nPRD correlates with pain self-report and the area under the curve (AUC) of the nPRD reflects nerve fiber sensitivity. These findings suggest that the AUC could function as a metric of nerve fiber sensitivity and that the nPRD has potential utility in objective assessment of pain characteristics, including type and intensity.

In this pilot study of healthy adult subjects, infrared pupillometry was used to evaluate whether the nPRD was reflective of known differences in nerve fiber physiology under tourniquet-induced ischemic conditions. Changes in sensory nerve fiber activity occur in ischemic environments due to factors such as degree of myelination.[3] At a baseline state, activated, myelinated Aβ touch fibers inhibit transmission of presynaptic pain signals by unmyelinated, nociceptive C-fibers to diminish pain sensation.[5] However, in an ischemic

environment, Aβ fibers are not activated. Thus, there is no suppression of C-fibers, resulting in stable or increased pain. It was hypothesized that this phenomenon could be quantified by the nPRD AUC for each fiber type and that tourniquet application would cause decreased Aβ sensitivity and increased C-fiber sensitivity (see Table).

A baseline electrical stimulus for each subject was determined by their perception threshold. For baseline nPRD measurement, each fiber type was assessed using perception intensity at a specific activating stimulation frequency (C fiber at 5 Hz, Aδ at 250 Hz, and Aβ at 2000 Hz). A tourniquet was then placed on the subject's upper arm to induce ischemia, and the same nPRD measurements were repeated at 5-minute intervals for each fiber type. The tourniquet was removed at 20 minutes, and final measurements were taken during reperfusion.

In this pilot study, five subjects demonstrated the hypothesized outcome. As demonstrated in the Figure, at 12 minutes after tourniquet placement the Aβ nPRD AUC was diminished while the C-fiber nPRD AUC was significantly increased from baseline. This suggests that ischemia resulted in removal of Aβ regulation and disinhibition of the C-fiber. During the reperfusion period, the C-fiber nPRD AUC decreased towards baseline indicating a return of Aβ suppression. During this reperfusion period, the Aβ nPRD AUC increased to reflect heightened sensitivity. For all five subjects, there was a significant difference between the Aβ nPRD AUC values (p=0.024) and a difference trending towards significance between the C-fiber nPRD AUC values (p=0.091) at the three time points.

These results indicate that the nPRD has potential to detect modulation of nerve fiber sensitivity. The data from subsequent trials will help determine whether infrared pupillometry in conjunction with selective neurostimulation can be used to objectively measure and monitor pain.

Time point	ΔAUC of C-fiber (5Hz)	ΔAUC of Aβ fiber (2000Hz)
Baseline	-	-
Before Tourniquet Removal	⬆	⬇
After Tourniquet Removal	⬇	⬆

TABLE: Expected Change in nPRD AUC for Each Nerve Fiber Type at Different Time Point

REFERENCES

1. AAPM Facts and Figures on Pain. Chicago, IL: American Association of Pain Medicine. (Accessed November 4, 2018, at http://www.painmed.org/patientcenter/facts_on_pain.aspx#incidence)
2. Institute of Medicine Report from the Committee on Advancing Pain Research, Care, and Education: Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research. Mil Med. May 2016;181(5):397-399.
3. Kumar K, Railton C, Tawfic Q. Tourniquet Application During Anesthesia: "What We Need to Know?" J Anaesthesiol Clin Pharmacol 2016;32(4):424-430.
4. MacIver MB, Tanelian DL. Activation of C Fibers by Metabolic Perturbations Associated with Tourniquet Ischemia. Anesthesiology 1992;76(4):617-623.
5. Melzack R, Wall PD. Pain Mechanisms: A New Theory. Science 1965;150(3699):971-979.

Laparoscopic Hand-Assisted Resection of a Rare Intra-Adrenal Schwannoma

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Schwannomas are benign tumors of the peripheral nerve sheath. They frequently occur in the head, neck, and extremities.[1] Schwannomas within adrenal glands are extremely rare, with only 42 reports. The majority of cases have been discovered incidentally and do not demonstrate increased incidence in any age group or gender.[2] Importantly, despite their association with the adrenal medulla all previous cases except for one report no clinical or biochemical evidence of hormonal activity.[3] Differentiation of schwannomas from other nonfunctional adrenal masses (adrenal adenoma, adrenocortical carcinoma, adrenal metastasis, adrenal myolipoma, and neuroblastomas) is challenging and imaging alone is insufficient.[4] Current guidelines state that the management of adrenal incidentalomas should consider the tumor size and associated symptoms, where masses larger than 6 cm or those causing symptoms should be resected while masses smaller than 4 cm should be closely followed with imaging.[5]

A previously healthy 37-year-old male with no significant past medical history initially presented to his primary care physician with new onset mild abdominal discomfort for which he used omeprazole with only modest relief. An abdominal ultrasound was obtained and demonstrated a mass in the right-upper quadrant. A subsequent abdominal/pelvis CT study demonstrated a heterogenous 4.3 cm mass adjacent to the right kidney and abutting the inferior vena cava and porta hepatis with no evidence of invasion into any structures. An ultrasound-guided transabdominal biopsy was obtained and demonstrated a spindle cell neoplasm that was consistent with schwannoma. During his pre-surgical evaluation, the patient denied headaches, palpitations, or history of hypertension. He had no surgical history; he was only taking intermittent omeprazole; he was a former smoker; and his family history was remarkable for leukemia, diabetes mellitus, and hypertension. His physical exam was benign, with only a mildly elevated systolic blood pressure and no significant findings on the abdominal exam. Pre-surgical labs were within normal limits. Therefore, surgical resection was discussed for definitive diagnosis and treatment.

While biochemically and hormonally inert, these adrenal tumors cannot be definitively diagnosed or differentiated from other adrenal masses without surgical resection and histopathologic studies.

The patient was consented for a laparoscopic hand-assisted resection of the retroperitoneal mass. Upon entering the abdominal cavity, the mass was easily identified as it protruded anteriorly adjacent to the hepatoduodenal ligament. A Kocher maneuver was performed to reflect and retract the duodenum and gain access into the retroperitoneal space. The mass was dissected free and removed, which required dividing a portion of the right adrenal gland. Hemostasis was achieved, incisions were repaired, and the patient recovered well and was discharged home two days later without any complications.

Gross examination of the resected specimen demonstrated an encapsulated 48 g mass with a heterogenous tan-white-orange and partially-cystic interior. Histopathologic examination demonstrated a spindle cell neoplasm with neural differentiation and areas of high density (Antoni A) and low density (Antoni B) cellularity surrounded by adrenal medulla tissue. Immunohistochemical staining demonstrated a mass that was diffusely positive for S-100 and SOX10; and negative for CD117/c-kit and HMB-45. Together, these data support the diagnosis of intra-adrenal schwannoma.

Adrenal schwannomas are a rare entity that have only been reported 42 times in the medical literature. While biochemically and hormonally inert,

these adrenal tumors cannot be definitively diagnosed or differentiated from other adrenal masses without surgical resection and histopathologic studies. Therefore, adrenal incidentalomas pose a clinical challenge as their discovery requires maintaining a broad differential diagnosis which includes benign schwannomas and malignant adrenal neoplasms.

REFERENCES:

1. Goh BK, Tan YM, Chung YF, et al. Retroperitoneal Schwannoma. Am J Surg 2006;192(1):14-8.
2. Mohiuddin Y, Gilliland MG. Adrenal Schwannoma: A Rare Type of Adrenal Incidentaloma. Arch Pathol Lab Med 2013;137(7):1009-14.
3. Xiao C, Xu B, Ye H, et al. Experience with Adrenal Schwannoma in a Chinese Population of Six Patients. J Endocrinol Invest 2011;34(6):417-21.
4. Strauss DC, Qureshi YA, Hayes AJ, et al. Management of Benign Retroperitoneal Schwannomas: A Single-Center Experience. Am J Surg 2011;202(2):194-8.
5. NIH State-of-the-Science Statement on Management of the Clinically Inapparent Adrenal Mass "Incidentaloma." NIH Consens State Sci Statements 2002;19(2):1-25.

Risk Factors for Amputation Following Lower Extremity Free Tissue Transfer in a Chronic Wound Population

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Microsurgical reconstruction via free tissue transfer (FTT) is the last option for closure of nonhealing, lower extremity (LE) wounds.[1–3] Unfortunately, amputation may be required even if FTT is successful. Thus, assessing amputation risk before reconstruction would help surgeons profile patient risk and direct salvage efforts with outcome-expectations. However, there is a paucity of literature regarding microsurgical outcomes in chronic wound patients. The purpose of this study was to evaluate risk factors for major amputation following LE FTT for closure in a chronic wound population.

Between April 2011 and January 2018, 135 LE FTT procedures were performed by the corresponding author for soft tissue coverage of nonhealing wounds. We studied the relationship of patient demographics, wound characteristics, and perioperative traits with limb-salvage and ambulation rates.

Overall microsurgical success was 96.3% (130/135) and limb salvage rate was 86.7% (117/135). Comorbidities significant for amputation were diabetes (p=0.009), COPD (p=0.002), ESRD (p=0.007), and PVD (0.02). Only hindfoot wound-location was significant for amputation (p=0.006). Significant perioperative traits included elevated platelet count on day of closure (332.8 vs 257.8, p=0.01) gracilis flap-type (p=0.03). Infectious complication was the only postoperative complication predictive of amputation (p=0.007).

By highlighting patient and perioperative traits that increase risk of amputation, these data help clinicians foreshadow the trajectory of wound closure and limb salvage. Furthermore, these results are a first-step to creating protocolized risk-stratification recommendations for patients undergoing LE FTT for complex, nonhealing wounds. To this end, future work will include risk analysis for individual risk factors.

REFERENCES:

1. Falola RA, Lakhiani C, Green J, et al. Assessment of Function After Free Tissue Transfer to the Lower Extremity for Chronic Wounds Using the Lower Extremity Functional Scale. J Reconstr Microsurg. 2018;34(5):327-333. doi:10.1055/s-0037-1621736
2. Lu J, DeFazio M, Lakhiani C, et al. Limb Salvage and Functional Outcomes Following Free Tissue Transfer for the Treatment of Recalcitrant Diabetic Foot Ulcers. J Reconstr Microsurg. 2018;1(212). doi:10.1055/s-0038-1667363
3. Evans KK, Attinger CE, Al-Attar A, et al. The Importance of Limb Preservation in the Diabetic Population. J Diabetes Complications. 2011. doi:10.1016/j.jdiacomp.2011.02.001

Sustainable Development Goals and Mental Health Knowledge Among First Year Medical Students

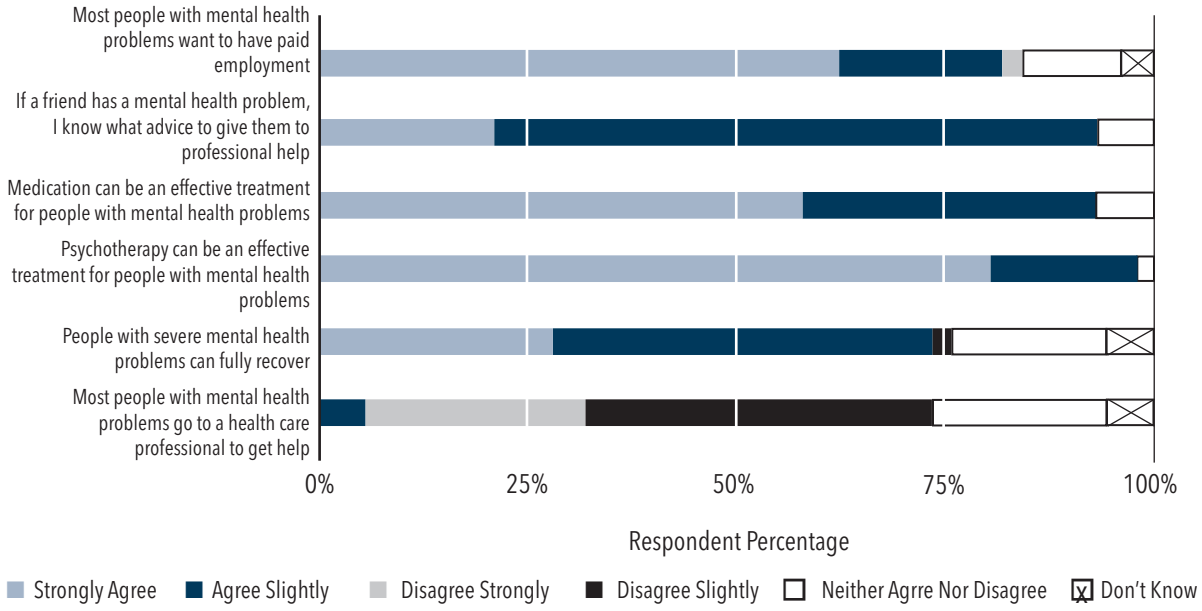

FIGURE 1: MAKS Results of First-Year MD Student Baseline Knowledge of Mental Health Biopsychosocial Factors

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The United Nations Sustainable Development Goals for 2030 highlights the impact of mental health illnesses by including goals for the prevention and treatment of behavioral, developmental, and neurological disorders.[1] One way to measure the progress of these goals is to monitor a foundational indicator such as mental health literacy among medical students. This study aims to look specifically at first year medical students (MS1s) to determine their mental health knowledge, as well as their attitudes towards mental health biopsychosocial factors.

Fifty-nine first-year medical students at the George Washington University School of Medicine and Helath Sciences were selected to participant in this study prior to the onset of the behavioral sciences section of the curriculum, allowing the assessment of baseline knowledge. The first-years underwent a baseline assessment, an intervention to introduce negative mental health outcomes, as well as a post intervention assessment. The baseline assessments included the Mental Health Knowledge Schedule (MAKS) and the Attitudes Towards Seeking Professional Psychological Help Scale (ATSPPH). MAKS is designed to assess confidence of one's knowledge of the biopsychosocial factors of mental illness, as well as the ability to identify terms that are defined as mental illnesses under the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5).[2] ATSPPH assesses perceptions towards mental health biopsychosocial factors, also making it ideal as a post intervention assessment.[3] The program intervention itself aimed to introduce students to negative mental health outcomes such as demoralization, burnout, depression, and dependency; as well as the different strategies that can be applied to address the different negative mental health outcomes.

While this investigation looks at many factors, there are three main findings that merit the greatest attention. The first is that there is great discordance to the statement "Most people with mental health problems

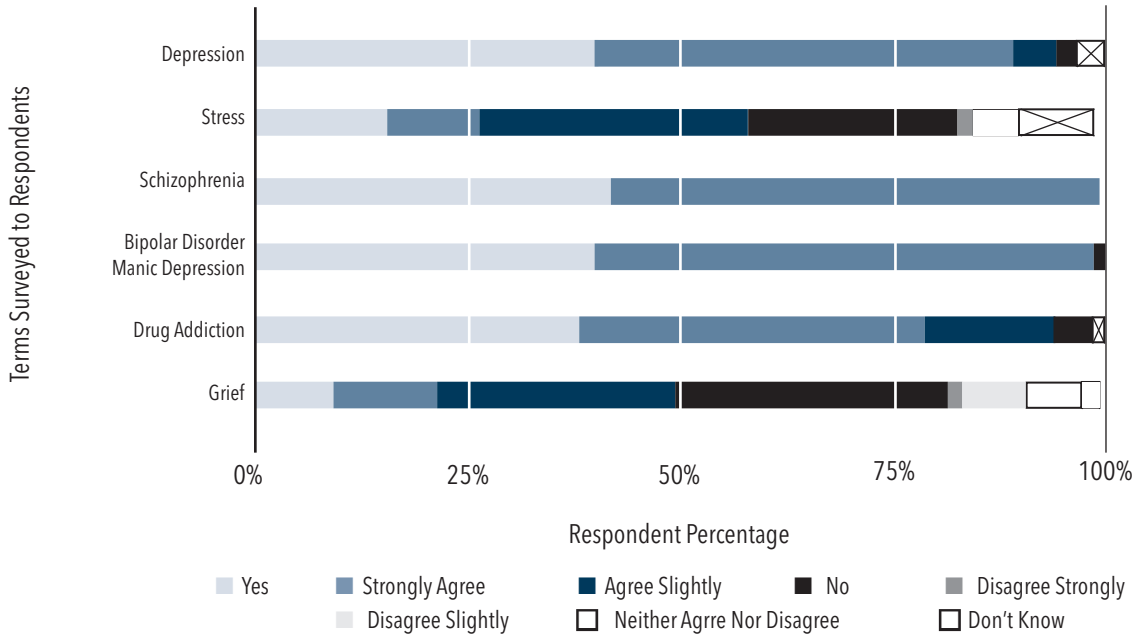

FIGURE 2: MAKS Results of First-Year MD Student Baseline Identification of Mental Health Disorders

go to a healthcare professional to get help." This implies that among MSIs there is either a gap in knowledge or misinformation about the epidemiology of mental health care services. The second finding is that drug addiction and depression responses lack the unanimous agreement to be identified as a mental illness. This is concerning because these are classic DSM-5 definitions, which points to the possibility of biases that students may hold. The third finding is that the intervention showed mixed results in shifting students' attitudes towards positive statements about mental health biopsychosocial factors. This implies that the intervention must be modified in order to address why some students had their attitudes shifted towards the negative.

In conclusion, the combination of the MAKS survey, ATSPPH survey, and the program intervention demonstrates a mix in baseline knowledge and attitudes in mental health knowledge among MSIs. These results identify the knowledge gaps that should be filled in mental health curriculums, particularly about which disorders fall under the DSM-5, as well as the epidemiology of mental health services.

REFERENCES

1. United Nations. (2015). Transforming our World: The 2030 Agenda for Sustainable Development .:. Sustainable Development Knowledge Platform. Retrieved March 1, 2018, from https://sustainabledevelopment.un.org/post2015/transformingourworld/publication

2. Evans-Lacko, S. (2010). Development and Psychometric Properties of the Mental Health Knowledge Schedule. The Canadian Journal of Psychiatry, 55(7), 440-448. doi:10.1177/070674371005500707

3. Elhai, J. D. (2008). Reliability and validity of the Attitudes Toward Seeking Professional Psychological Help Scale-Short Form. Psychiatry Research, 159(3), 320-329. doi:10.1016/j.psychres.2007.04.02

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