It is my pleasure to welcome you to the third annual UConn Health/Jackson Laboratory Postdoc Research Day. I am so happy that this event has taken hold here and has become a new annual tradition. On this day we get to hear about some of the fantastic research being performed by our postdocs at UConn Health and the Jackson Laboratory for Genomic Medicine as well as from the Storrs campus. At the same time, we get to join institutions around the country in celebrating National Postdoc Appreciation Week as we collectively recognize the extremely important contributions that postdocs make to our nation’s research enterprise. There are many people to thank for making this day possible. In particular, I would like to thank the postdocs who volunteered to help plan this day. This includes:

   Sherli Koshy-Chenthittayil, UConn Health  
   Luis Sordo Vieira, The Jackson Laboratory  
   Giulia Vigone, UConn Health

I would also like to thank Dr. Bruce Liang, Dean of the UConn School of Medicine, for his support as well as Sarah Wojiski, the Director of STEM and Undergraduate Education at the Jackson Laboratory in Farmington. In addition, I would like to acknowledge Fran Hernandez, Stephanie Holden and Dawn Lindauer for their work in making this day come together, as well as the Health Center Research Advisory Committee Distinguished Speaker fund which allowed us to invite Dr. Steven N. Austad to join us today as our keynote speaker. I also want to thank the UCH/Jax Postdoc Association for the work they do throughout the year to support the postdoc community here in Farmington. For all postdocs who have not yet gotten involved in the PDA, I encourage you to do so.

Sincerely,

Christopher D. Heinen, Ph.D.  
Director of Postdoctoral Affairs  
UConn Health

Cover photo credits: (Clockwise) Hakimeh Ebrahimi Nik, Sherli Koshy-Chenthittayil, Ju Chen, Jenny Suarez-Ramirez and Bruno Lemos.
The Third
Postdoc Research Day
Tuesday, September 17th, 2019

1:00  Opening Remarks  Academic Rotunda
1:05  Speak4Science Part I  Academic Rotunda
2:05  Coffee Break  Rotunda Lobby
2:20  Speak4Science Part II  Academic Rotunda
3:20  Keynote Address:
Steven N. Austad, Ph.D.
Distinguished Professor and Chair
Department of Biology
University of Alabama at Birmingham

“Busting the Bestiary: Why Worms, Flies, and Mice Are Not Enough for Aging Research”

4:30  Poster Session and Reception  Rotunda Lobby
4:40  Poster Presentations I (Odd numbers)  Rotunda Lobby
5:20  Poster Presentations II (Even numbers)  Rotunda Lobby
6:00  End
Our Speak4Science event will feature a series of 4-minute talks by our postdocs. Each speaker will use one slide to broadly introduce their area of research and why it excites them. To learn more about the details of their research, I encourage you to visit their posters later in the day. The speaker roster and their corresponding poster numbers are listed below.

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Keynote Presentation

Dr. Steven N. Austad

*Busting the Bestiary: Why Worms, Flies, and Mice Are Not Enough for Aging Research*

Steven N. Austad is Distinguished Professor and Chair of the Department of Biology at the University of Alabama at Birmingham and also Scientific Director of the American Federation for Aging Research. With an undergraduate degree in English literature and experience ranging from New York City taxi driver to lion trainer for the film industry, he received his PhD degree in evolutionary ecology from Purdue University in 1981. He had faculty positions at Harvard University, the University of Idaho, and the Barshop Institute for Longevity & Aging Studies at the University of Texas Health Science Center San Antonio in San Antonio, Texas before moving to his current position in 2014. His research encompasses many aspects of the biology of aging from the molecular to the population level. His research specialty is the identification and study of nontraditional species – particularly exceptionally long-lived species – for insight into processes of slow aging. Dr. Austad’s research has won multiple awards, including the Geron Corporation-Samuel Goldstein Distinguished Publication Award, the Nathan A. Shock Award, the Robert W. Kleemeier Award, the Purdue Outstanding Alumnus Award, the Fondation IPSEN Longevity Prize, and the Irving S. Wright Award of Distinction. He maintains a keen interest in communicating science to the general public and in that capacity has served on the Science Advisory Board of National Public Radio and has been a consultant to the Oregon Museum of Science and Industry, the Perot Museum of Nature and Science, and the American Museum of Natural History in New York City. He has written popular science articles for numerous publications including *Natural History* magazine, *Scientific American*, *National Wildlife*, and *International Wildlife*. His trade book, *Why We Age* (1997), has been translated into nine languages. He has also written more than 150 newspaper columns on science and aging. His current book, *Methuselah’s Zoo: the Natural History of Exceptional Longevity* (MIT Press) is due out in fall 2019.
Poster Abstracts

1. Henrique de Assis L Ribeiro – UConn Health

A modular multi-scale agent-based-model of immune response to Aspergillus fumigatus

Henrique de Assis Lopes Ribeiro¹, Lokendra Poudel¹, Bandita Adhikari¹, Luis Sordo Vieira¹, Yu Mei¹, Joseph Masison¹, Borna Mehrad², Reinhard Laubenbacher¹

¹Center for Quantitative Medicine, UConn Health; ²Division of Pulmonary, Critical Care, and Sleep Medicine, University of Florida, Gainesville

Software development and maintenance in academia is a big challenge with some claiming to be impossible. One can envision the problem by noticing that the developers in academia are Ph.D. students and Post-doc fellows that hold temporary positions and have varying software engineering skills. Multi-language is another characteristic of the community so that it is common to see systems written in different languages. Often these systems lack in sound software engineering practices; there may be lots of code repetition, tightly coupling between different parts, and so on. We propose here is a solution for agent-based modeling, with a case study of a simulator of an immune response to Aspergillus fumigatus. Our solution was to create a Dockerized modular architecture where each module is a standalone executable program, running in a Docker container that can read and write in a common in-memory database (Redis). These modules do not communicate directly with each other but only indirectly through the database. In our case study, we have four modules: macrophage, Aspergillus, interact, and diffusion. Macrophage takes care of cell movement and recruitment. Aspergillus deals with fungi growth and internal Boolean-network update. Diffusion computes the diffusion of molecules i.e., transferrin (free and bound to iron), TAF (free and bound to iron) and chemokine. Interact is the critical module, and it does the interaction between the agents, cells, and molecules. We construct this module with an incremental design that allows one to add new agents without having to change old code.

2. Jacqueynn Benjamino – The Jackson Laboratory

Toward the development of a targeted screening panel for mouse microbiome profiling

Jacqueynn Benjamino¹, Daniel Phillips¹, Mark D. Adams¹

¹ The Jackson Laboratory for Genomic Medicine, Farmington

Community profiling of the microbes in an environment is an important step in microbiome research and is frequently accomplished with 16S rRNA gene sequencing based on low cost and ease of sample processing. While 16S rRNA gene sequencing provides information on the bacteria present in a microbiome, it provides no information about gene content and has limited taxonomic resolution, meaning that individual species cannot be distinguished. We take advantage of recent technical advances that have made it possible to construct large pools of custom oligonucleotides as sequence-specific probes at modest cost. Padlock probes (PLPs) designed for selected genomes can provide relative abundance data along with information about targeted gene content present in a sample. As an initial proof of concept, 1800 PLPs were designed to hybridize to three test genomes and synthesized in a multiplex pool. The PLP pool was then amplified and processed to allow for hybridization to genomic DNA (gDNA), followed by ligation and PCR amplification with adapter-tailed primers to create libraries for Illumina sequencing. A computational pipeline was developed to facilitate design of highly specific PLP probes given a set of target genomes and a background database of off-target sequences to avoid. A PLP pool was then designed to target 100 metagenome-derived species that were assembled from metagenome whole-genome shotgun dataset representing the mouse gut microbiome of C57BL/6J and HLB444 mice fed chow and a high-fat diet. Sequencing results showed high specificity for the target genomes of interest, even in the presence of complex eukaryotic and prokaryotic DNA background.
The relative abundance of the three defined genomes in mixed samples as represented by the PLPs was accurate and stable across multiple samples. Subsampling to 20 probes/genome returned similar relative abundances to the entire probe set (~600 per genome), suggesting that fewer probes are needed to accurately identify the abundance of each genome. The mouse microbiome PLP results showed high correlation of the relative abundance profiles with the mWGS sampled data across samples. Overall, the results of this assay show a promising, simple, low-cost option for microbiome profiling and targeted detection of organisms in microbiome samples.

3. Cory Brunson – UConn Health

Topological modeling of personalized outcomes prediction
Cory Brunson1, Amy Peterson2, Evelyn Nitch-Griffin3, Thomas P. Agresta1, Reinhard C. Laubenbacher1

1Center for Quantitative Medicine, UConn Health; 2Department of Mathematics, Colorado State University; 3Department of Mathematics, University of Connecticut

Secondary research use of administrative healthcare data holds new potential and new challenges for outcome prediction and disease subtyping, among other aims. In recent years, machine learning researchers have introduced myriad predictive modeling techniques to these efforts, which can often improve predictive accuracy at the expense of interpretability. In particular, kernel-based models use measures of patient–patient similarity distilled from multitudinous variables to more precisely identify patients with similar prognoses. Regardless of their internal complexity, the external behavior of these models can be used to gain insight into the roles of different predictors with respect to the outcomes associated with specific populations. Because these models rely most strongly on the most similar patient profiles, in contrast to classical geometric methods, topological data analytic (TDA) methods are well-suited to this problem. The goal of this project is to develop a topological framework to explore predictive relationships within large, heterogeneous data. This framework will be rooted in personalized models of patient outcomes, which we aggregate into a coherent population-level exploratory model. Our preliminary work includes a robustness analysis of personalized prediction models and related topological constructions, a study of disparities in predictive accuracy due to smaller populations or lower-quality data, and a stability analysis of the mathematical construction of a _personalized prediction complex_.

4. Ali Foroughi pour – The Jackson Laboratory

Impact of -omics preprocessing pipelines on the accuracy of predictive convolutional neural networks
Ali Foroughi pour1, Javad Noorbakhsh1, Jeffrey Chuang1,2

1 The Jackson Laboratory for Genomic Medicine; 2Genetics and Genome Sciences, UConn Health

Deep learning has recently gained popularity in bioinformatics. In particular, convolutional neural networks (CNNs) are used to analyze biological images as well as -omics data such as gene expression and genetic aberrations. Although pipelines typically perform several preprocessing steps prior to feeding the data into the network, the effect of different filtration techniques, objectives used to rank features, and the policies that determine the place of each feature in the “expression image” have not yet been studied in detail. Here we compare the effect of several preprocessing pipelines on the accuracy of CNNs. In particular, we compare pipelines utilizing different initial feature filtering algorithms and “-omics image construction” policies. Analyses on datasets comparing high-risk versus low-risk breast, colon, and lung cancer patients (all publically available on gene expression omnibus [1] with accession numbers GSE2034, GSE39582, and GSE68465, respectively) suggest the preprocessing may drastically affect the prediction accuracy of a fixed network and optimal training parameters. In particular, the area under curve (AUC) of the CNN differentiating lung cancer
patients with poor and long survivals varied from 56% (optimal Bayesian filter [2] reporting the top 200 genes ordered by their log likelihood ratio) to 97% (optimal Bayesian filter reporting the top 4000 genes randomly ordered in the feature vector). The minimum/maximum AUCs of colon and breast cancer datasets were 51%/70% and 55%/92%, respectively. Overall, CNNs were only mildly sensitive to the order of features in the input, and small feature sets resulted in much lower AUCs than large ones.

5. Asis Jana – UConn Storrs

Investigating cell entry mechanism of non-enveloped viruses using computer simulations

Asis Jana1, Dr. Eric May1

1Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut

The process by which membrane lacking (non-enveloped) viruses enter and infect host cells remain poorly understood. Many non-enveloped virus contain a membrane-active peptide, which is sequestered inside the capsid during much of the virus life cycle but become active under certain cellular conditions such as change in the environmental pH. Flock House Virus (FHV) is an excellent model system for studying non-enveloped viruses. Experimental studies have provided strong evidence that FHV replicate into the living cells through the acid-dependent endocytic pathway, where low pH inside endosome acts as a trigger for γ liberation from capsid interior, which can then disrupt host cell membrane and the externalization and activity of these γ peptides is optimal at pH 6. However, the molecular mechanism that underlie in this process still remain unclear and are particularly challenging to address by using current experimental techniques. All-atom molecular dynamics (MD) simulation often termed as “atomic microscope” have emerged as a powerful tool for studying viral systems. In the current study, we performed a series of atomically detailed simulations sampling over 20 μs to study the mechanism and energetics of the membrane lytic (γ) peptides liberation from the FHV capsid interior to the exterior and the role of pH in this process. Our simulation results qualitatively agree with the experimental finding and will help us to understand the mechanism of host cell membrane breaching by non-enveloped virus in general.

6. Lokendra Poudel – UConn Health

Simulating Aspergillus fumigatus infection and battle for iron with multi-scale agent-based model

Lokendra Poudel1, Henrique de Assis Lopes Ribeiro1, Bandita Adhikari1, Luis Sordo Vieira1, Yu Mei1, Joseph Masison1, Borna Mehrad2, Reinhard Laubenbacher1

1Center for Quantitative Medicine, UConn Health; 2Division of Pulmonary, Critical Care, and Sleep Medicine, University of Florida, Gainesville

Invasive aspergillosis is among the most common fungal infection in immunocompromised hosts and carries a poor outcome. Aspergillus fumigatus, are ubiquitously distributed in the environment. Healthy hosts clear the inhaled spores without developing disease, but individuals with impaired immunity are susceptible to a life-threatening respiratory infection that can then disseminate to other organs. The increasing use of immunosuppressive therapies in transplantation and cancer has dramatically increased suffering and death from this infection, and this trend is expected to continue. A better understanding of the components of host defense in this infection may lead to the development of new treatments against this infection, possibly in combination with antifungal drugs. Iron is essential to all living organisms, and restricting iron availability is a critical mechanism of antimicrobial host defense against many microorganisms; conversely, successful pathogens have evolved potent mechanisms for scavenging iron from the host. These mechanisms have the potential to be harnessed therapeutically, for example with drugs that enhance the host’s iron sequestration mechanisms. The overarching goal of this work is to advance a multi-scale mathematical model that can
serve as a simulation tool of the role of iron in invasive aspergillosis. The model will integrate mechanisms at
the molecular scale with tissue-level events capturing hormone production in the liver.

7. Jennifer VanOudenhove – UConn Health

Comprehensive epigenomic and transcriptomic profiling of human embryonic heart reveals the
regulatory landscape of heart development and implicates noncoding sequences in congenital heart
defects

J. VanOudenhove1, A. Wilderman1,2, T. Yankee1,2, J. Cotney1,3

1 Genetics and Genome Sciences, UConn Health; 2 Graduate Program in Genetics and Developmental
Biology, UConn Health; 3 Institute for System Genomics, UConn

Spatiotemporal regulation of gene expression during development can occur through tissue-specific gene
regulatory sequences known as enhancers. Genome-wide association studies (GWAS) show that a majority
of disease-associated variants are enriched in enhancers and there is growing evidence that enhancer
alterations can result in birth defects or predisposition to disease later in life. Congenital heart defects (CHDs)
are the most common form of birth defect, effecting 1 in 100 live births. Of the 80% of cases of CHDs without
a pathogenic copy number variation, less than 10% have identifiable loss of function mutations in genes.
Therefore, noncoding mutations could be a substantial contributor to the remaining unknown cases. However,
our limited understanding of the language of the noncoding genome and lack of functional annotations from
early developing heart prevent causative assignment of noncoding variation in CHDs. To address this, we
created a comprehensive catalog of chromatin state annotations during critical stages of human heart
development (4 to 8 post conception weeks). We generated genome-wide profiles of seven post-translational
histone modifications (H3K4me1-3, H3K27ac, H3K27me3, H3K9me3, and H3K36me3) for two human
embryonic hearts from each of nine distinct Carnegie stages (CS13-14, CS16-21, and CS23) for a total of
144 primary ChIP-seq datasets. Using imputation followed by segmentation with a 25 state chromatin model
developed by Roadmap Epigenome we identified 177,412 heart enhancers. Of these 34,034 had not been
previously annotated in Roadmap. We identified 92% of all validated heart positive enhancers from the Vista
Enhancer Browser (n=281), a 7.5-fold enrichment versus active enhancers lacking activity in the heart
(p=2.2x10^-16). To explore the impact these chromatin states have on gene expression, we generated bulk
strand-specific RNA-seq data at comparable time points for three embryonic hearts. We find enhancers are
enriched near genes expressed more strongly in the heart than other tissues. Finally, we evaluated the
enrichment of heart trait-associations from the GWAS Catalog in enhancers from our data and found
significant enrichment of SNPs associated with CHDs, electrocardiogram measures, aortic root size, and atrial
fibrillation. Our functional annotations will allow for better interpretation of whole genome sequencing data of
patients with heart related conditions and advance the field of personalized genomic medicine.

8. Samir Amin – The Jackson Laboratory

Comparative molecular life history of spontaneous canine and human gliomas

Samir Kumar B. Amin1, Kevin J. Anderson1, C. Elizabeth Boudreau2, Emmanuel Martinez-Ledesma3,4, Emre
Kocakavuk5,6, Kevin C. Johnson1, Floris P. Barthel7, Frederick S. Varn1, Cynthia Kassab6, Xiaoyang
Ling6, Hoon Kim1, Mary Barter7, Chew Yee Ngan1, Margaret Chapman, Jennifer W. Koehler8, Andrew D.
Miller9, C. Ryan Miller10, Brian F. Porter11, Daniel R. Rissi12, Christina Mazcko13, Amy K. LeBlanc13, Peter J.
Dickinson14, Rebecca A. Packer15, Amanda R. Taylor16, John H. Rossmeisl Jr17, Amy Heimberger6, Jonathan
M. Levine2, Roel G. W. Verhaak1

1 The Jackson Laboratory for Genomic Medicine; 2 Department of Small Animal Clinical Sciences, College of
Veterinary Medicine and Biomedical Sciences, Texas A&M University; 3 Tecnologico de Monterrey,
Sporadic gliomas in companion dogs provide a window on the interaction between tumorigenic mechanisms and host environment. We compared the molecular profiles of canine gliomas with those of human pediatric and adult gliomas to characterize evolutionarily conserved mammalian mutational processes in gliomagenesis. Employing whole genome-, exome-, transcriptome-and methylation-sequencing of 81 canine gliomas, we found alterations shared between canine and human gliomas such as the receptor tyrosine kinases, p53 and cell cycle pathways, and IDH1 R132. Canine gliomas showed high similarity with human pediatric gliomas per robust aneuploidy, mutational rates, relative timing of mutations, and DNA methylation patterns. Our cross-species comparative genomic analysis provides unique insights into glioma etiology and the chronology of glioma-causing somatic alterations. https://www.biorxiv.org/content/10.1101/673822v1

9. Cory Brennick – UConn Health

Un-biased analysis of all possible neoepitopes reveals a new universe of cancer neoepitopes

Cory A. Brennick1*, Mariam M. George1*, Mamar Moussa1, Adam T. Hagymasi1, Tatiana Shcheglova1, Sahar AlSeesi2, Grant L. Keller3, John Sidney4, Alessandro Sette4, Brian M. Baker4, Andrea Schietinger5, Ion I. Mandoiu2 and Pramod K. Srivastava1

*These authors contributed equally.

1Department of Immunology, Carole and Ray Neag Comprehensive Cancer Center, UConn Health, 2Department of Computer Science and Engineering, UConn; 3Department of Chemistry and Biochemistry and Harper Cancer Research Institute, University of Notre Dame, Notre Dame; 4LaJolla Institute of Allergy and Immunology, La Jolla, CA; 5Immunology Program, Memorial Sloan Kettering Cancer Center, New York

Identification of neoepitopes that are effective in cancer therapy is a major challenge in creation of cancer vaccines. Here, using an entirely unbiased approach, we have queried all possible neoepitopes in a cancer model and asked which of those are effective in mediating tumor rejection. This unbiased analysis reveals that our current methods of prediction discard the majority of true anticancer neoepitopes; it uncovers a new universe of effective anticancer neoepitopes which have strikingly different properties from epitopes of viral antigens. We observe that (i) about 2% of all SNVs lead to generation of peptides that can mediate tumor rejection to any significant degree; (ii) each peptide alone elicits a modest protection, and a combination elicits stronger protective immunity, (iii) all the neoepitopes that elicit tumor rejection have poor binding affinity for MHC I, and have positive values for Differential Agretopic Index (iv) even though the protective responses are CD8+ T cell-mediated, there is no correlation between a neoepitope’s ability to elicit a CD8+ T cell response and tumor rejection (v) TILs elicited by these neoepitopes demonstrate a plastic T cell phenotype
shown earlier to be associated with effective responses against viruses and transgenic tumors. Discovery of this abundant source of novel anticancer neoepitopes can be exploited for generation of personalized human cancer vaccines.

10. Hakimeh Ebrahimi-Nik – UConn Health

Mass spectroscopy-defined neoepitopes are a rich source of tumor rejection-mediating neoepitopes in a mouse sarcoma

Hakimeh Ebrahimi-Nik1, Tatiana Shcheglova1, Justine Michaux2, HuiSong Pak2, Elham Sherafat3, Sahar Al Seesi3, Ion Mandoiu2, George Coukos2, Michal Bassani-Sternberg2 and Pramod K Srivastava1

1Carole and Ray Neag Comprehensive Cancer Center, UConn Health; 2Ludwig Centre for Cancer Research, University of Lausanne, Epalinges, Switzerland; 3Department of Computer Science and Engineering, University of Connecticut

Using tandem mass spectrometry (MS), we identified 3646 unique sequences among peptides eluted from purified Kd and Dd MHC I molecules of the BALB/cJ A fibrosarcoma Meth A. These peptides were cross-referenced with the output of neoepitopes predicted for this tumor by our prediction pipeline CCCP (Consensus Caller Cross-Platform). Eleven of the eluted peptides were identified as neoepitopes and eight of eleven neoepitopes were confirmed by targeted MS. Each neoepitope was used to immunize BALB/cJ mice (twice, one week apart, using precise neoepitopes along with bone marrow-derived dendritic cells); mice were challenged with Meth A cells one week after the last immunization, and tumor growth was monitored in individual mice. In parallel, immunized mice were tested for CD8+ T cells to the neoepitopes using tetramer staining and interferon gamma secretion by CD8+ cells. Three of the eight MS-defined neoepitopes elicited highly potent rejection of Meth A fibrosarcoma. Of these three strong neoepitopes, only two elicited a measurable CD8 response. Of the five neoepitopes that did not elicit tumor rejection, two elicited a measurable CD8 response which one of them was the strongest of all CD8 responses detected. These observations indicate that MS-defined neoepitopes can be a rich source of neoepitopes that can mediate tumor rejection. Further, they highlight the fact that CD8 responses are not a good predictive surrogates for tumor rejection.

11. Iman Al-Naggar – UConn Health

Cellular senescence and senolytics in mouse models of aging-related bladder dysfunction.

Al-Naggar I.M.1, Baker D.S.1,4, Robson P.4,5, Xu M.1,5, Smith P.P.1,2,3 and Kuchel G.A.1

1UConn Center on Aging; 2Department of Surgery, University of Connecticut School of Medicine; 3University of Connecticut Institute for Brain and Cognitive Science, Storrs, CT; 4The Jackson Laboratory for Genomic Medicine; 5Department of Genetics and Genome Sciences, University of Connecticut School of Medicine.

Cellular senescence is a process by which cells enter into a permanent proliferative arrest. It can be caused by several stressors including DNA damage, mitochondrial dysfunction, oncogene expression, metabolic stresses, chronic viral infections or other tissue damage signals. Senescent cells are characterized by increases in size and protein production, altered metabolic activity, resistance to apoptosis and sometimes secretion of a battery of inflammatory molecules termed the Senescence Associated Secretory Phenotype (SASP). SASP molecules, along with immune cells attracted and activated by senescent cells, contribute to inflammation and tissue breakdown, stem and progenitor cell dysfunction, and the spread of senescence to nonsenescent cells. They accumulate in many organs with aging and at sites of pathogenesis in several chronic diseases, and are believed to drive the aging phenotype. Senolytics (drugs that can kill senescent cells) have been shown to alleviate several geriatric syndromes and conditions such as frailty and
osteoporosis. We found evidence of senescent cells using markers of senescence in old C57BL/6 mouse bladders. We identified a population of p16 positive myofibroblasts that may be becoming senescent in old bladders using single cell analysis. We used a senescent cell transplantation model to establish a causal role for senescent cells in lower urinary tract aging and aging-related bladder dysfunction. Furthermore, we treated aged mice with a senolytic combination (Dasatinib+Quercetin, D+Q) to test whether we can alleviate the aged bladder phenotype. In all experiments, we used voiding spot assays and cystometric analyses to study changes in bladder function. (Funded by NIA AG054777; AG06352).

12. Yasuyuki Fujii – UConn Health

Induction of cherubism-like jawbone expansion in mice reveals unrecognized contributions of neutrophils to cherubism pathogenesis

Yasuyuki Fujii1, Nelson Monteiro1, Yasuyoshi Ueki3, Ernst J Reichenberger1, I-Ping Chen1

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Cherubism (CBM) is a rare genetic inflammatory bone disorder with characteristic expansile jawbones and an onset of 2 to 5 years of age. CBM is caused by gain-of-function mutations in the Src Homology 3 Domain-Binding Protein 2 (SH3BP2; mouse orthologue Sh3bp2). The activation mechanisms remain unclear despite fast-progressing research in Sh3bp2 knockin (KI, P416R) and knockout mice because those mice do not spontaneously develop jawbone expansion and invasive investigations in children at the time of onset is not feasible. Current research on CBM pathogenesis focuses on the roles of macrophages and hyperactive osteoclasts. Here, we present a new model for inducing CBM-like jawbone lesions in mice and propose that neutrophil-mediated mechanisms play important roles in CBM induction. In this novel approach, we endodontically expose pulp of right mandibular first molars to allow bacterial invasion into alveolar bone of 6-week-old Sh3bp2+/+, Sh3bp2+/KI and Sh3bp2KI/KI male and female littermates (n=6-8 per group). We harvest mandibles at 3, 7, 14 and 21 days after dental drilling. Sh3bp2KI/KI mice but not Sh3bp2+/+ mice develop cherubism-like lesions (9.1% ± 5.0% jawbone expansion) 14 days after drilling shown by µCT. Sh3bp2+/KI mice develop jawbone expansion after 42 days. Immunocytochemistry of periapical lesions of Sh3bp2KI/KI mice (compared to drilled Sh3bp2+/+ mice) showed a high number of interspersed neutrophils starting at day 3 with increased IL-17 (peaked at day 7) as well as citrullinated H3 histone and myeloperoxidase (MPO), signature markers of neutrophil extracellular traps (NETs). In Sh3bp2KI/KI mice, cytokine upregulation after pulp exposure was not limited to the local environment. qPCR showed increased Il1b, Il6 and Tnf expression in left mandibles of treated Sh3bp2KI/KI mice compared to naïve mandibles of untreated Sh3bp2KI/KI mice (fold increases at day 3, 7 and 14 of Il1b = 4.22±0.01, 5.94±0.26, 12.7±0.46; Il6 = 5.8±0.2, 3.5±0.55, 45.8±1.89; and Tnf = 3.4±0.23, 5.6±0.33, 7.3±1.52). These neutrophil-mediated events occur prior to an increase in macrophage and osteoclast numbers and jawbone expansion observed after day 14. Our data suggest that excessive NETs and cytokines released by CBM mutant neutrophils upon bacterial stimulation initiate processes that precipitate the activation of CBM. Investigation of neutrophil-mediated inflammatory response may reveal biomarkers for early stage cherubism and identify therapeutic targets.
Benefits of walnut consumption in colon health

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Inflammatory Bowel Disease (IBD) is a risk factor for colorectal cancer (CRC), the third leading cancer in the United States. Walnuts contain a variety of bioactive compounds that can reduce inflammation, as well as possessing anticancer properties. In this study, we examined the effects of dietary walnuts on dextran sodium sulfate (DSS)-induced colitis and on colon carcinogenesis in animal models. To examine the effect of walnuts on colitis (or IBD) development, male and female C57Bl/6J mice (n=32) were fed a Total Western Diet (TWD) containing 7% walnuts (TWDW) for two weeks and then administered DSS in drinking water for 5 days. Effects of walnuts were evaluated at both the acute (2 days) and recovery (10 days) phases following withdrawal from DSS. For the colon tumor study, male and female A/J mice (n=80) received 6 weekly injections with vehicle or azoxymethane (AO), a widely used model for CRC, and then sacrificed at 10 weeks after the last injection. Mice were fed TWDW throughout the study. Tissue and fecal samples were used for analyses to assess the potential health benefits of walnut consumption, including immunohistochemical markers, metabolomics and microbiome analyses. DSS-induced colonic ulceration was markedly reduced with walnut diet during both the acute (2-fold) and recovery (3-fold) phases. In those mice, dietary walnuts modified the profiles of lipid metabolites present in the fecal samples. In the colon tumor study, a reduction in tumor numbers was observed in male mice fed TWDW (p<0.02). Although a number of CRC immunohistochemical markers were unaffected by walnut consumption, an in-depth microbiome analysis of fecal samples in the carcinogen-treated mice showed that walnut diet increased bacterial richness and diversity, which could be contributing to the anti-tumorigenic effects of walnut. Walnut supplementation provides protection to the colonic mucosa against DSS-induced inflammation. Walnut supplementation also has a significant effect on the community structure of the gut microbiota in mice. We plan to further investigate these beneficial effects of walnuts in a healthy adult population. In this clinical trial, our goal is to determine whether consumption of 2 ounces of walnuts per day for 21 days will positively affect the stool microbiome in those individuals most efficient at producing urolithins from ellagic acid derived from walnuts. Urolithin metabolism will be compared with beneficial anti-inflammatory effects identified within the colonic mucosa following screening colonoscopy.

Calcitonin gene related peptide (CGRP) regulation of periosteal cells

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Sensory nerves and their neuropeptides are involved in pain transmission during injury. Upon tissue injury (i.e. fracture), calcitonin gene related peptide (CGRP) is released from damaged nerve fibers. Our aim was to establish the presence of CGRP+ nerves in the periosteum and determine how CGRP stimulation and CGRP receptor deficiency may regulate periosteal cells using in vitro and in vivo models. Using immunohistochemistry, we established the presence of CGRP+ nerves present in the periosteum of the tibia. We confirmed that components of the CGRP receptor complex, calcitonin-like receptor (CLR) and receptor activity modifying protein-1 (RAMP1), were expressed in periosteal derived stem cells (PDSCs). Similar levels of CLR expression were observed during differentiation of PDSCs on Day 7, 10 and 14. RAMP1 expression increased as PDSCs differentiated. Functionally, CGRP stimulation increased proliferation and mineralization of PDSCs in vitro. Since CGRP is known to activate Gαs signaling, we confirmed that CGRP stimulation increased cAMP levels and downstream signals pCREB and pATF-1 by Western blot. Finally, we have
crossed a Prx1cre mouse with a CLR floxed mouse to generate a new model (PrxCLR) that has CLR deletion in peripheral mesenchymal cells including the periosteum. Periosteal cell cultures from Cre+ PrxCLR mice showed impaired mineralization. Further work is required to evaluate the effect of CGRP signaling on bone mass under basal conditions and during fracture healing in vivo. Altogether, this work identifies a novel axis by which a pain-associated neuropeptide may influence periosteal cell responses.

15. Brandon Goldstein – UConn Health

Stressful events moderate childhood reward processing in predicting adolescent depressive symptoms
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The diathesis stress model posits that disorders develop as a result of a pre-existing vulnerability becoming activated when individuals are exposed to stress. Reward processing deficits have been implicated as a vulnerability marker for developing depression. In particular, blunted reward positivity (RewP), an event-related potential elicited by feedback to monetary gain relative to loss, has previously been found to predict new onsets and increases in depression symptoms. No studies have examined whether stressful life events moderate the effect of the RewP on subsequent depression symptoms. We examined this question during the key developmental transition from childhood to adolescence in a longitudinal community sample (N=369). The RewP was measured when children were 9 years old. Three years later, stressful life events and depression were assessed. We found that stressful life events moderated the effect of the RewP on depression symptoms at follow-up such that a blunted RewP (reduced reward activity) predicted higher depression symptoms in individuals with higher levels of stressful life events. Overall, these findings support a diathesis stress model as reward processing deficits lead to increased depression under high levels of stress.

16. Lourah Kelly – UConn Health

Test of social interpersonal theory and the three step ideation-to-action theory with passive suicidal ideation in college students
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Suicidal ideation (SI) is a significant public health problem among college students that warrants further theoretically-informed research. Passive SI involves thoughts that life is not worthwhile and wishes for death, while active SI includes thoughts to kill oneself. Both forms of SI are associated with significant distress and impairment; however, limited research has distinguished college students without SI from those with passive SI and active SI. Ideation-to-action theories seek to explain this transition from SI to suicidal behavior and increasing suicide risk. Two such theories, Joiner’s Interpersonal-Psychological Theory of Suicide (IPTS) and Klonsky’s Three-Step Ideation-to-Action Theory (3ST), have not yet been applied to passive SI and have limited research with college students. Due to the broader conceptualization of connectedness in 3ST, it was hypothesized that 3ST would be more developmentally sensitive and identify college students with no, passive and active SI, and increasing SI severity relative to IPTS. Results demonstrated mixed support for IPTS and 3ST. Neither theory correctly classified any students with passive SI. Single variables (i.e., burdensomeness for IPTS; pain-connectedness difference scores for 3ST) distinguished students with active SI from no SI.
Students hypothesized to endorse active SI based on both theories did not exclusively endorse active SI. Future directions include adaptations to improve the developmental sensitivity of both theories, refinement of 3ST measures of connectedness, and investigating alternative hypotheses that explain why high risk students did not endorse any SI. Suicide risk assessment and intervention on college campuses should include fostering connection and adaptive thinking patterns.

17. Jamie LoCurto – UConn Health

Transitioning to middle school successfully: Development of a brief intervention to reduce student anxiety
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The transition from elementary to middle school is difficult for most students and specialized transition supports are critical for students with, or at risk of developing, disabilities. Excessive anxiety, included as part of the definition of “emotion disturbance” under IDEA, is the most common form of psychopathology and severely impairs student academic functioning. Currently, no intervention exists to support this high risk group during their transition to middle school. The purpose of this project is to develop and evaluate a brief multi-component intervention, delivered by school clinicians, to reduce anxiety symptoms and improve academic and social functioning. The project proposes an iterative development process (i.e., expert review, two open trials, and pilot RCT) to achieve these goals.

18. Joyshree Biswas – UConn Health

Role of a novel gene C1ql1 in oligodendrocyte progenitor cell maturation
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Oligodendrocyte Progenitor cells (OPCs) are an immature and undifferentiated form of oligodendrocyte, a cell type that produces myelin. Myelin is a lipid rich insulating sheath that protects neuronal axons and improves impulse conduction. Almost 5% of all adult mammalian brain cells are OPCs. The presence of such a large number of OPCs in a mature brain that has presumably finished myelinating its axons is perplexing. A possibility is that new myelination in the adult brain is required for higher order functions such as cognition and learning. Additionally, this pool of OPCs represents a potential source of new oligodendrocytes to replace those lost during injury, inflammation, or in diseases such as multiple sclerosis. Multiple sclerosis is a disease in the CNS characterized by progressive demyelination of axons and subsequent neuronal death. Based upon several RNA seq and immunostaining studies in mice, we demonstrate the expression of the gene complement component 1 q subcomponent-like 1 (C1ql1) in the OPC population across the brain. Our hypothesis is that C1QL1, a secreted OPC-derived protein promotes oligodendrocyte differentiation, by initiating an intercellular signaling pathway through astrocytes. In our study, we aim to characterize C1ql1 and its functional relevance in OPC maturation and proliferation. Our in vitro data suggests C1QL1 is a ligand that binds to a GPCR receptor on astrocytes known as brain specific angiogenesis inhibitor 3 (BAI3), causing the release of tissue inhibitor of metalloproteinases 1 (TIMP1) which is known to cause OPCs to mature into oligodendrocytes. Further, we have developed a conditional knockout mouse of C1ql1 specifically in OPCs and we are proceeding to validate our in vitro findings. Our goal is to understand the molecular mechanisms behind oligodendrocyte maturation via C1ql1 and to use this knowledge for therapeutic gain.
19. Jinyoung Jang – UConn Health

Voltage and calcium signals in dendrites of medium spiny neurons.

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The medium spiny neuron (MSN) is a key neuron in the basal ganglia circuit, involved in several major neurological disorders including Parkinson’s disease, Huntington’s disease and attention deficit hyperactivity disorder (ADHD). MSNs exhibit two neural states. The “Down state” (hyperpolarized membrane potential) alternates with the “UP state” (depolarized membrane potential) in accordance to the temporal availability of glutamatergic synaptic inputs impinging onto their spiny dendrites. To understand the functional roles of MSNs, it is necessary to study dendritic physiological properties. Intrinsic membrane properties of MSN dendrites are poorly understood because glass electrode recordings are not tolerated well by thin dendritic branches. Here, we performed simultaneous somatic whole-cell recording with dendritic imaging to focus on dendritic properties. We injected voltage sensitive dyes (JPW-3028 and JPW-4008) or calcium indicators (OGB1 and Fluo-5N) into MSNs. Dendritic membrane potential changes were induced by backpropagating action potentials (bAPs) or by local application of glutamate on dendrite. Our results reveal an increasing dendritic AP peak latency depending on the distance from the soma. The amplitudes of action potential-induced dendritic calcium influxes decrease with distance from the soma suggesting a distance dependent attenuation of bAPs in dendrites of MSNs. Next, we characterized the voltage waveforms of glutamate evoked plateau potentials in dendrite and cell body simultaneously. Invariably, the dendritic plateaus preceded somatic plateau potentials and the initiation phase of the dendritic UP state (plateau) was steeper than that in the soma, consistent with a local initiation of the dendritic plateau. Detailed characterization of voltage and calcium signals is expected to reveal the intrinsic dendritic properties and bring us closer to the functional roles of MSNs in the basal ganglia circuits.

20. Kevin Johnson – The Jackson Laboratory

Longitudinal molecular trajectories of diffuse glioma in adults

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The evolutionary processes that drive universal therapeutic resistance in adult patients with a diffuse glioma remain unclear. Here, we longitudinally profile 222 patients with glioma by integrating DNA sequencing and clinical annotation datasets. Through mutational and copy number analyses across the three major diffuse glioma subtypes, we observed that driver genes found at initial disease persisted into recurrence and there were few common features of recurrence. Treatment with alkylating-agents resulted in a hypermutator phenotype at different rates across glioma subtypes, and hypermutation was not associated with differences in survival. Acquired aneuploidy was frequently detected in recurrent gliomas characterized by IDH1 mutation but without 1p/19q codeletion and further converged with acquired cell cycle alterations and poor outcomes. We show that clonal structure most often remains stable and evolution classified as neutral at recurrence was associated with more favorable outcomes compared with cases exhibiting subclonal selection. Finally, we found that neoantigens were exposed to stable selective pressures throughout a tumor’s progression. Our
results collectively suggest that the strongest selective pressures occur early during glioma evolution and that current therapies shape this evolution in a largely stochastic manner.

21. Christopher Lee – UConn Health

**Long-lasting sound-evoked afterdischarges in mouse models of tinnitus**

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Neurons in the auditory system typically fire in a stimulus-dependent manner: the spike rate increases at the sound onset and returns to the baseline spike rates after the sound offset. However, a subpopulation of neurons in the inferior colliculus (IC) responds to sounds with a long-lasting sound-evoked afterdischarge (LSA), an increase in spike rate that lasts for seconds to minutes after the offset of the sound. The IC is a major hub of the auditory system, receiving ascending inputs from the auditory brainstem nuclei and descending projections from the auditory thalamus and cortex and providing all ascending auditory input to the thalamus. Due its position in the auditory system, the IC is an essential integration center that shapes the neural signals which lead to the perception of sound. LSA neurons make up an estimated 30% of the neurons in the central nucleus of the IC; however, their role in auditory processing is not yet known. Properties of LSA neurons may be altered with tinnitus. Tinnitus is the perception of a sound in the absence of an external stimulus and affects millions of people. Since tinnitus is considered to be a pathology of the brain, rather than the ear, it is important to understand the neural changes that take place in tinnitus. We are currently studying properties of LSA and the LSA neurons, using multi-channel electrodes to record the spiking activity of dozens of IC neurons in mouse models of tinnitus. If LSA is different in mice with tinnitus, LSA may be used to develop an efficient and reliable test for tinnitus in humans.

22. Raj Luxmi – UConn Health

**Peptidergic signaling through ciliary ectosomes in Chlamydomonas**

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Peptides generated from larger, inactive precursors and released in response to appropriate stimuli regulate multiple physiological and behavioral processes in vertebrates; peptide precursors and essential processing enzymes are also present in animals that lack a nervous system. We previously identified similar genes in the unicellular alga Chlamydomonas reinhardtii, suggesting that peptidergic signaling of this type is ancient. Chlamydomonas produces active peptidylglycine α-amidating monoxygenase (PAM), which converts peptidylglycine substrates into amidated products, but lacks peptide storage granules. CrPAM is largely localized to the Golgi region and cilia. Knockdown/out of PAM expression in Chlamydomonas, planaria, zebrafish and mice revealed its key role in ciliogenesis. Cilia are sensory organelles that release bioactive extracellular vesicles (ectosomes) in response to stimuli. Because the non-ciliary surface of Chlamydomonas is covered by cell wall, we hypothesized that CrPAM might be released in ciliary ectosomes. Since CrPAM expression was higher in resting gametes than in vegetative cells, we focused on a role for CrPAM in mating. An hour after mixing gametes of both mating types, ectosomes and soluble secretome were prepared. CrPAM protein and activity were recovered from mating ectosomes but not the soluble secretome. Although localized to the ciliary membrane of vegetative cells, CrPAM was excluded from vegetative ectosomes. Thus, release of CrPAM into ciliary ectosome is a developmentally regulated process. Time course studies revealed that CrPAM was released in ectosomes early in the mating reaction, suggesting its role in cilium-generated
signaling events involved in mating. Mass spectrometric analysis identified three amidated peptides in mating ectosomes; one amidated peptide acted as a chemoattractant for minus gametes and as a chemo-repellent for plus gametes. Our study in Chlamydomonas suggests that ciliary ectosomes provide a previously unappreciated route for the secretion of amidated bioactive products that may be utilized by motile and primary cilia in vertebrates.

23. Ujwala Gosavi – UConn Health

Rapid block of RNA splicing by chemical inhibition of analog-sensitive CRK9 in Trypanosoma brucei

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Cyclin-dependent kinases (CDKs) are serine/threonine kinases that work along with a cyclin for enzymatic activity and have an ATP binding pocket, a cyclin-binding PSTAIRE-like helix domain, and an activating T-loop. The CDKs mainly function as key regulators of cell cycle progression but also found to have important roles in gene transcription and RNA processing. Gene silencing of the CDK, CRK9 in trypanosomes exhibited a block of spliced leader trans-splicing of nuclear pre-mRNA and a loss of phosphorylation of RPB1, the largest subunit of RNA polymerase II. To decipher CRK9’s role in gene expression, we generated cell lines that exclusively express C-terminally PTP-tagged, ATP analog-sensitive (AS) CRK9 in which the gatekeeper residue M438 was substituted with glycine (CRK9AS1-PTP). Treatment of these cells with 1-NM-PP1, a bulky N6-enlarged ATP analog, inhibited culture growth of CRK9AS1-PTP-expressing cells, causing them to round up and die within 48 hours, similar to but much more rapid than CRK9-silenced cells. The compound was tenfold more effective in CRK9AS1-PTP- (EC50 of 1.5 μM) than in CRK9WT-PTP-expressing cells, indicating that the inhibitor effectively blocked the activity of CRK9AS1-PTP. When treated with 10 μM of 1-NM-PP1, block of trans and cis (intron removal) splicing became apparent after 5 and 60 minutes, respectively, whereas it took ~6 hours before loss of RPB1 phosphorylation was detectable. These results strongly indicate that the RNA processing machinery requires continuous input from CRK9 to remain active. In contrast to our notion that unphosphorylated RPB1 is defective in transcription, RNA pol II-mediated synthesis of selected transcripts continued even at 24 hours of drug treatment, suggesting that most RPB1 phosphorylations are not essential for transcriptional activity per se. Since we found that purified CRK9AS1-PTP but not CRK9WT-PTP accepts bulky phenyl-ethyl ATPγS for thiophosphorylation, we are adopting a pull-down assay using an anti-thiophosphate ester antibody to identify CRK9 direct substrates.

24. Andre Grassmann – UConn Health

Defining molecular mechanisms underlying host-adaptation in pathogenic Leptospira

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Leptospirosis is an important neglected tropical disease with global distribution caused by pathogenic Leptospira. Little is known on how Leptospira regulate gene expression to adapt and succeed in each complex environment encountered during their life cycle. Leptospira species contain up to four Ferric Uptake Regulator (FUR) family protein coding genes, three of them upregulated in host-adapted Leptospira. Transcriptome of three L. interrogans mutant strains with transposon insertions in two FUR members (LIC12034 and LIC11158) were evaluated in in vivo like condition. FURs act as transcriptional regulators of metal homeostasis systems
or peroxide stress responders (PerR). LIC12034 and LIC11158 mutants have increased resistance to oxidative stress in vitro, therefore were named PerR1 and PerR2, respectively. The double knockout is avirulent in the animal models for acute disease and chronic colonization, while the single mutants retain virulence. Transcriptional analysis of mutants for PerR1 and PerR2, resulted in 85 differentially expressed genes in ΔPerR1, 247 in ΔPerR2 and 127 in the ΔPerR1ΔPerR2 strains compared to WT. While a few genes related to oxidative stress response are directly or indirectly repressed by PerR1 and/or PerR2 (such as cytochrome-c peroxidase), the majority of genes in the regulon are not related to oxidative stress response. The expression of a gene cluster related to metal uptake seems to be activated by PerR1 and PerR2. In the double mutant, a reduced expression of Lig proteins was observed, which is directly related to virulence loss. Similarly, the double knockout reduced the expression of an important two component system (LrvA and LrvB), also related to virulence. The large number of transcriptional regulators observed in response to host-like conditions, either controlled by leptospiral PerRs or not, is an evidence of a complex gene network involved in leptospiral adaptation to infect hosts.

25. Yuvabharath Kondaveeti – UConn Health

Dissection of XIST regulatory elements that mark the onset of inactive X erosion in female pluriptotency
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Human induced pluripotent stem cells (hiPSCs) serve as a model system to study genetic disorders in patient specific manner. It has been shown that female hiPSC lines in long-term culture undergo “erosion” of X chromosome inactivation (XCI), characterized by the loss of XIST long non-coding RNA expression and reactivation of silenced genes on the inactive X chromosome. Erosion of XCI compromises the integrity of female hiPSCs, and thereby complicates their use in modeling of genetic disorders in general, and X-linked diseases in particular. Although prior data suggest that maintenance of XCI in human pluripotency requires XIST RNA, the regulatory network controlling XIST is largely unknown, including its cis-acting elements. To address this question, we aim to identify internal XIST CpG islands and dissect their role in XIST expression. We are using the MethylScreen technique to profile the DNA methylation status of XIST internal CpG islands in hESC and differentiated cell lines with differing X inactivation states. To determine which of these CpG islands directly impact XIST expression, we are using CRISPR-Cas9-tagged DNA methyltransferase or DNA demethylase enzymes to alter methylation of CpG islands to assess their role in XIST expression. Using these techniques, we aim to dissect the regulatory mechanisms that control XIST expression, at the onset of inactive X erosion, and ask which may also drive initiation of XCI.

26. Rachael Norris – UConn Health

Complex processing of internalized gap junctions
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Gap junctions connect cells of nearly all tissues in both vertebrates and invertebrates. While they are traditionally thought of as stable, long-lived structures, in some tissues, gap junctions are actually very dynamic, turning over rapidly after they form. The best-described mode of turnover is when one of the two connected cells engulfs the entire gap junction along with cytoplasm and membrane from the neighboring cell to form a double membrane vesicle, commonly referred to as a connexosome. Given the multitude of Connexin43 functions, we are studying the ultrastructure of connexosomes within ovarian granulosa cells,
which have a large number of these vesicles after stimulation with luteinizing hormone. We have categorized and measured 196 internalized structures that contain Cx43 within a defined volume of tissue.

27. Abhijit Rath – UConn Health

Functional interrogation of Lynch syndrome-associated MSH2 missense variants via CRISPR-Cas9 gene editing

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Lynch syndrome (LS) predisposes patients to cancer and is caused by germline mutations in the DNA mismatch repair (MMR) genes. Identification of a frameshift or nonsense mutation resulting in impairment of MMR function is confirmatory for LS diagnosis. However, discovery of a missense variant is often inconclusive, as the effects of these variants of uncertain significance (VUS) on disease pathogenesis are unclear. Unbiased assessment of the impact of VUS on the protein’s function can help determine their significance and thus plays a critical role in the therapeutic management of LS patients. Laboratory based functional studies performed to date have been limited by their artificial nature. To this end, we developed an in cellulo functional assay in which we engineered site-specific MSH2 VUS using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 gene editing in human embryonic stem cells. This approach introduces the variant into the endogenous MSH2 loci, while simultaneously eliminating the wild-type gene. We characterized the impact of the variants on cellular MMR functions including DNA damage response signaling and the repair of DNA microsatellites. We determined that four VUS clearly disrupted MMR function, four VUS did not impact function and two had intermediate effects, providing valuable information for determining their likelihood of being bona fide pathogenic LS variants. This human cell-based assay system for functional testing of MMR gene VUS will facilitate the identification of high risk LS patients.

28. Maria Consuelo Rocha Granados – UConn Health

Impact of commensal bacteria exoproducts on Staphylococcus aureus antibiotic persistence

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Staphylococcus aureus is part of the human commensal microbiota, but when the cutaneous and mucosal barriers are breached, S. aureus can act as an opportunistic pathogen. S. aureus can cause skin and soft tissue infections, and biofilm-associated infections, such as endocarditis. Antibiotic treatment failure of S. aureus infections is not only linked to antibiotic resistance. Bacterial persisters, which are subpopulations of cells in a genetically clonal culture that are able to survive doses of antibiotics lethal to their kin, are thought to be a source of infection relapse. At S. aureus infections sites, other bacteria that are part of the host microbiome can colocalize with the pathogen. These host-colonizing bacteria can secrete metabolites and proteins that affect S. aureus physiology and virulence. The impact of interspecies interactions on S. aureus antibiotic persistence and the nature of the exoproducts involved in crosstalk at infections site remain unknown. In this study, we will first evaluate the impact of secreted products from commensal bacteria on S. aureus antibiotic persistence. Secondly, we will identify the nature of the commensal exoproducts using biochemical and analytical techniques. Our preliminary results suggest that cell-free conditioned media from the skin commensals, Staphylococcus epidermidis and Corynebacterium striatum, can decrease persisters
when the population is treated with levofloxacin, a fluoroquinolone. The results of this study will contribute new knowledge of how secreted metabolites from commensal species can impact S. aureus antibiotic persistence and can potentially lead to the development of new therapeutic strategies to treat chronic and recurrent infections.

29. Shyam Kishor Sah – UConn Health

Functional analysis of novel keloid-associated genetic variants

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Keloids are scars that grow beyond the border of the initial wound in a tumor-like manner. Chronically inflamed keloids are characterized by aberrant activation of fibroblasts and keratinocytes, leading to tissue fibrosis by excessive and prolonged deposition of extracellular matrix. Individuals of African and Asian descent are affected at disproportionately high rates. There is little evidence-based treatment for keloids due to the poorly known pathoetiology. Previous studies provided evidence for genetic predisposition. Through linkage analysis and whole-exome sequencing, we have identified candidate variants in keloid susceptibility genes. We introduced susceptibility variants into human induced pluripotent stem cells (hiPSCs) from a healthy individual using CRISPR/Cas9 genome editing. The isogenic parent and mutant hiPSC lines differ only in the single mutation and therefore reduce effects of genetic variability. We differentiated hiPSC lines with or without mutations into keratinocytes and fibroblasts to study the role of these variants in keloid development by functional assays. We investigate cellular features in cell culture assays and organotypic 3D models whether mutant lines display features that are typical for keloids or tissue fibrosis.

30. Ankita Srivastava – UConn Health

Analyzing the basic function of *Trypanosoma brucei*’s two introns

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In *T. brucei*, all mRNAs are trans-spliced whereas there are only two introns, disrupting the coding regions of poly(A) polymerase 1 (PAP1, Tb927.3.3160) and of the DEAD box RNA helicase DBP2B (Tb927.8.1510). A survey of kinetoplastid genomes revealed that every species harbors orthologous PAP1 and DBP2B genes with introns in the exact same position. Since the introns are also present in Bodo saltans, it appears that they withstood genome streamlining in trypanosomes for approximately 500 million years of evolution. PAP1 is an essential nuclear enzyme, functioning in snoRNA biogenesis (Chikne et al., 2017, J Mol Biol 429:3301-3318). Similarly, DBP2B silencing halted culture growth within 3 days. Given the importance of the two genes and the long-term positional conservation of their introns, we hypothesize that these introns fulfill fundamentally important functions. We began our analysis with PAP1. N-terminal PTP tagging of PAP1 in procyclic trypanosomes allowed us to specifically monitor expression from manipulated PTP-PAP1 alleles. Across different cell lines, intron deletion resulted in a ~2.5 fold increase of both mature mRNA and protein. However, conditional overexpression of PAP1 did not affect growth because trypanosomes were able to store excess enzyme in the cytoplasm (not shown). Our data indicate that trypanosomes harbor a nuclear reservoir of PAP1 RNA that is correctly end-processed but retains the intron, implying that cis splicing of PAP1 RNA occurs later than and is independent from trans splicing. Initial digital droplet PCR experiments revealed that the PAP1-intron RNA population accounts for ~35% of polyadenylated PAP1 RNA in the cell. Interestingly, DBP2B silencing increased the PAP1-intron RNA level to 52%, suggesting that PAP1 expression is regulated by its intron, possibly with DBP2B in a direct role. We have been able to generate three cell lines in which the
PAP1 intron is completely deleted. These cells have moderately increased doubling times and presumably no nuclear reservoir of PAP1 RNA. We will expose these cells to different conditions to see when and to what extent the reservoir provides a selective advantage.

31. Federica Agliano – UConn Health

**IRAK 1/4 inhibition attenuates sterile inflammation induced in a model of auto-inflammatory syndrome**

Federica Agliano¹, Antoine Ménoret¹, Keaton Karlinsey¹, Michael Ragazzi¹, Anthony T Vella¹

¹Department of Immunology, UConn Health

Interleukin-1 receptor-associated kinases (IRAK) 1/4 are serine/threonine kinases involved in the Myddosome formation, a non-redundant pathway necessary to induce a full spectrum of TLR responses. Upon TLR activation, clustering of IRAK4 induces its autophosphorylation, leading to IRAK1 activation and pro-inflammatory gene transcription. In this study we hypothesize that IRAK 1/4 kinases might be a druggable target in the treatment of auto-inflammatory diseases. Mouse macrophages (BMDMs) pre-treated in vitro with IRAK 1/4 inhibitor secrete significantly less pro-inflammatory mediators in response to different TLR agonists. Specifically, this inhibitor significantly reduces the TLR9-dependent ERK-MAP kinase phosphorylation, and NFκB nuclear translocation. Furthermore, BMDMs significantly increase their size when stimulated with the hydrocarbon oil 2, 6, 10, 14-tetramethylpentadecane (TMPD, also known as pristane). Injected in vivo, pristane mimics some features of auto-inflammatory diseases e.g. arthritis and systemic lupus erythematosus (SLE). C57BL/6 female mice treated with IRAK 1/4 inhibitor exhibited a significant reduction of pristane-dependent increase of splenocyte number and spleen weight. Conversely, no difference was observed in males. Using mass spectrometry we found that the urine of pristane-injected mice contained increased levels of putative markers for several inflammatory diseases, which were reduced by IRAK 1/4 inhibition. Pristane-injected mice showed increased serum DNA which was not blocked by the inhibitor, but chemokine release (e.g. CCL2, CCL5 and CCL17) was significantly reduced in IRAK 1/4 inhibitor treated mice. Thus, IRAK 1/4 are two druggable molecules that might represent a new therapeutic target in the recruitment of immune cells during auto-inflammatory diseases in humans, including SLE and arthritis.

32. Karthik Chandiran – UConn Health

**Smad signaling determines the fate of activated CTLs via multiple intersecting signaling pathways**

Karthik Chandiran¹, Jenny Suarez Ramirez¹, Yinghong Hu², Susan M. Kaech³, Linda S. Cauley¹

¹Department of Immunology, UConn Health; ²Emory Vaccine Center, Emory University; ³NOMIS Center for Immunology and Microbial Pathogenesis, Salk Institute for Biological Sciences, La Jolla, CA

Immune responses to an infection are controlled by various factors including antigens, co-stimulatory molecules and cytokines present in the tissue micro-environment. These factors modulate the differentiation of effector CD8 T cells and memory subsets. TGFb plays a pivotal role in fate determination of activated CTLs and Smad proteins are critical for the downstream signaling cascade in TGFb pathway. Using transgenic animal models and pharmacological inhibitors, we investigate the role of TGFb and Smad proteins in fate determination of activated CTLs. We identified that TGFb signaling and Smad proteins play important roles in regulating the expression of several homing receptors on effector CD8 T cells (KLRG1) and memory subsets (CD62L, S1PR1 and CD103). In addition, targeted-ablation of different Smad proteins alter the expression levels of different transcription factors that participate in fate determination, including Eomes and KLF2. We identified that while Smad proteins regulate the gene expression in canonical TGFb pathway, they also have TGFb independent functions in determining the fate of activated CTLs. Particularly, we show that Smad4 is required for the expression of KLRG1 and acts as a suppressor of CD103, independent of TGFb. This study reveals a novel role for Smad signaling cascade in guiding the fate decisions of activated CTLs.
and tissue localization via multiple intersecting signaling pathways. This work is supported by NIH grants AI056172 and AI123864.

33. Ju Chen – UConn Health

IL-17 inhibits CXCL9/10-mediated recruitment of CD8+ cytotoxic T cells and regulatory T cells to colorectal tumors

Ju Chen1, Xiaoyang Ye1,2, Elise Pitmon1, Mengqian Lu1,3, Jun Wan2,4, Evan R. Jellison1, Adam J. Adler1, Anthony T. Vella1 and Kepeng Wang1

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The IL-17 family of cytokines are potent drivers of colorectal cancer (CRC) development. We and others have shown that IL-17 mainly signals to tumor cells to promote CRC, but the underlying mechanism remains unclear. IL-17 also dampens Th1-armed anti-tumor immunity, in part by attracting myeloid cells to tumor. Whether IL-17 controls the activity of adaptive immune cells in a more direct manner, however, is unknown. Here, we show that IL-17 inhibits the recruitment of CD8+ cytotoxic T lymphocytes (CTLs) and regulatory T cells (Tregs) during early stage CRC development, and that the loss of IL-17 signaling resulted in increased CTL infiltration to colorectal tumors, as well as elevated production of the anti-inflammatory cytokines IL-10 and TGF-β. IL-17 signaling also inhibits the production of T cell attracting chemokines CXCL9 and CXCL10 by tumor cells. Conversely, the inability of hematopoietic cells to respond to CXCL9/10 resulted in decreased tumor infiltration by CTLs and Tregs, decreased levels of IL-10 and TGF-β, and increased numbers of tumor lesions. Together, these data indicate that IL-17 promotes early stage CRC development by inhibiting CD8+ CTL and Treg recruitment by downregulating CXCL9/10 production.

34. Chuan Li – UConn Health

Macspectrum yields unprecedented resolution of full-spectrum macrophage activation states in atherosclerosis

Chuan Li1, Antoine Menoret1,4, Cullen Farragher2, Zhengqing Ouyang3,4, Anthony T. Vella1, Beiyan Zhou1,4

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Macrophages are key to innate immunity, tissue homeostasis, and critically contribute to the pathogenesis of atherosclerosis and other cardiovascular diseases. In response to various stimuli, macrophages display highly diversified activation states that differentially orchestrate atherosclerosis development, and targeting the heterogeneous atherosclerotic macrophage subsets has been suggested as a potential strategy for therapy. However, despite the concept of multifaceted action of macrophages is well-accepted and described by several models, no current model can precisely depict and annotate their complex and dynamically regulated in vivo features. To address this major knowledge gap, we created a two-index platform, MacSpectrum (macspectrum.uconn.edu). This was accomplished using our original algorithms which were integrated with macrophage single-cell RNA sequencing data. The capability of MacSpectrum to segregate macrophage heterogeneity was well-supported by its performance on samples from human and murine species, under in vitro and in vivo conditions, and in various formats formats. Our platform consistently
predicted the previously reported features of macrophages in atherosclerosis and a more monocytic / less inflammatory phenotype associated with regression phase; further, MacSpectrum revealed novel characteristics of atherosclerotic macrophages, including a fine-mapped dynamic shift of their activation states, unprecedented regulatory factors, and signalling pathways potentially orchestrating pathogenesis, and unique signature genes of atherosclerotic macrophage subpopulations. In summary, MacSpectrum will provide a novel and comprehensive platform to annotate macrophage activations under sophisticated conditions in atherosclerosis and other CVDs. Application of this model to circulating monocytes and macrophage transcriptome profiles revealed novel, atherosclerosis-specific gene signatures and potential targetable pathways to facilitate precision medicine development.

35. Tao Lin – UConn Health

UBXN3B regulates adaptive immunity to viral infection

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UBXN3B belongs to the ubiquitin regulatory X domain-containing proteins (UBXNs) family, which are likely involved in diverse biological processes. However, the physiological functions of UBXN3B remain largely unknown. We first examined the changes in disease pathogenesis and antiviral immune responses of gene deficient animals and cells. Our previous study showed physiological evidence that UBXN3B positively regulates stimulator-of-interferon genes (STING) signaling. Here we present the physiological evidence that UBXN3B regulates adaptive immune response to both DNA and RNA viral infection. We generated the tamoxifen inducible UBXN3B systemic conditional knockout mice as invivo model. The Ubxn3b−/− mice showed dramatically decreased virus specific IgG antibody production after either herpes simplex virus 1 (HSV-1) or O'nyong nyong virus (ONNV) infection. However, the virus specific IgM antibody are not susceptible compare to the wild type mice. We also employed the Sting−/− mice and there are no deficient immune responses compared to wild type mice. Taken together, our results indicate that UBXN3B regulates adaptive immunity to viral infection likely in a STING-independent manner. However, much less is known about the molecular mechanism of this phenomena. We are currently continuing to address this knowledge gap of exciting understanding of the role of UBXN3B in antiviral immune responses.

36. Wojciech Rosikiewicz – The Jackson Laboratory

TET2 deficiency reprograms the germinal center B-cell epigenome and silences genes linked to lymphomagenesis

Wojciech Rosikiewicz¹*, Xiaowen Chen¹*, Pilar M. Dominguez², Hussein Ghamlouch³, Said Aoufouchi⁴, Olivier A. Bernard², Ari Melnick², Sheng Li¹,5,6

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*These authors contributed equally

The TET2 DNA hydroxy-methyltransferase is frequently disrupted by somatic mutations in diffuse large B-cell lymphomas (DLBCLs), a tumor that originates from germinal center (GC) B-cells. Herein we show that TET2 deficiency leads to DNA hypermethylation of regulatory elements in GC B-cells, associated with silencing of
the respective genes. This hypermethylation affects the binding sites and target genes of transcription factors including those involved in exit from the GC reaction and involves pathways such as B-cell receptor, antigen presentation, CD40, and others. Normal GC B-cells manifest a typical hypomethylation signature, which is caused by AICDA, the enzyme that mediates somatic hypermutation. However, AICDA induced demethylation is markedly impaired in TET2 deficient GC B-cells, suggesting that AICDA epigenetic effects are partially dependent on TET2. Finally, we find that TET2 mutant DLBCLs also manifest the aberrant TET2-deficient GC DNA methylation signature suggesting that this epigenetic pattern is maintained during and contributes to lymphomagenesis.

37. Jenny Suarez-Ramirez – UConn Health

The local lymph node becomes a repository for PD1+ Tissue-resident memory (Trm) CD8 T cells during Influenza virus infection

Jenny E. Suarez-Ramirez and Linda S. Cauley

Department of Immunology, UConn Health

Heterogenous populations of antiviral memory CD8 T cells participate in the prospective cytotoxic T lymphocytes (CTL) response to Influenza A virus (IAV) infection, including local populations of Tissue resident memory CD8 T cells (Trm) to virus specific CTLs in the mediastinal lymph node (MLN) until at least 60dpi. During this time the MLN contains large numbers of endogenous Trm cells which are uniformly PD1+ and express αEβ7 integrin (CD103) in the presence of transforming growth factor β (TGFβ). Naïve CD8 T cells that were transferred to IAV infected mice 30dpi, entered the reactive lymph node and initiated an abortive proliferative response upon antigen stimulation. Furthermore, these partially activated CTLs upregulated CD103 expression upon exposure to TGFβ, which indicated the potential to become canonical Trm cells. In contrast, central memory CD8 T (Tcm) cells were insensitive to TGFβ stimulation and did not respond to the persisting viral peptides. And early proliferative response indicates that PD1+ Trm cells in the MLN amplify the response in the lung Trm cells upon reinfection.

38. Asa Thibodeau – The Jackson Laboratory

Functional characterization of human immune cell epigenomes with deep convolutional neural network models

Asa Thibodeau, Cheng-Han Chung, Radu Marches, Robert J. Rossi, Jacques Banchereau, Duygu Ucar

The Jackson Laboratory for Genomic Medicine

Over the last decade, the primary focus of disease biology has shifted from coding to non-coding sequences in the human genome. Genome-wide association studies (GWAS) revealed that over 90% of disease-associated genetic variants are found in non-coding sequences and lead to dysregulated gene expression programs through disrupted functions of cis-regulatory elements (cis-REs). Cis-REs regulate gene expression in a cell-specific manner and play a critical role in individual and condition specific gene expression programs. Assay for Transposase Accessible Chromatin using sequencing (ATAC-seq) broadly captures open chromatin regions (OCRs), including cis-REs. However, determining the functional annotations (e.g., enhancers, insulators) of these OCRs from ATAC-seq data alone remains a challenge. For this purpose, we developed deep convolutional neural network (CNN) models using novel encoders for ATAC-seq data that can infer promoters, enhancers, and insulators from ATAC-seq open chromatin maps. Models were trained on ATAC-seq data from 4 cell types (monocyte, GM12878, HSMM, and K562) using ChromHMM annotations in these cell types as ground truth. Overall performance of these models achieved 0.86 precision on average when applied to held out test data. Next, we applied these models on ATAC-seq data from 133 individuals.
(437 samples from sorted immune cells, 118 samples from peripheral blood mononuclear cells (PBMCs)). Predictions in primary immune cells uncovered cis-REs that are cell-specific and harbor disease-related loci, in addition to cis-REs that are specific to cellular responses as well as clinical information (e.g., age and sex of individuals).
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