IOWA

Interdisciplinary Graduate Program in Genetics

2024 Student Retreat

September 27th – 28th

University Capital Center

STUDENT RETREAT

Keynote Speaker

Danielle M. Dick, Ph.D.

Director, Rutgers Addiction Research Center
Greg Brown Endowed Chair in Neuroscience

Robert Wood Johnson Medical School
Rutgers University

Public Talk: *"Using Genetics to Stop Addiction Before it Starts: Science Fiction or Science Fact?"*

Keynote Talk: "The Genetics of Substance Use Disorders: Novel Approaches to Identify Genes and Develop Tailored Prevention/ Intervention"

Alumni Speakers

Wesley Goar, Ph.D.
(2019 Sheffield/Scheetz labs)

Senior Bioinformatics Scientist
The Steve and Cindy Rasmussen Institute for Genomic Medicine
Nationwide Children's Hospital

"Oh, the Places You'll Go: The Many Pathways of Bioinformatics"

Kellie Schaefer-Swale, Ph.D.
(2020 Mahajan/Bassuk labs)

Senior Scientist
Translational Biomarkers
Arrowhead Pharmaceuticals

"My Experiences Working in Industry"

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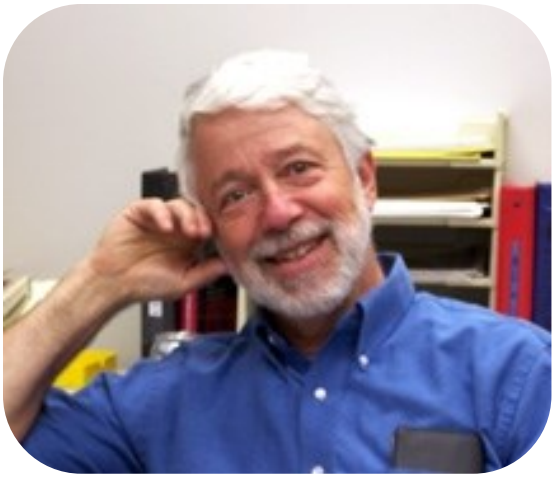
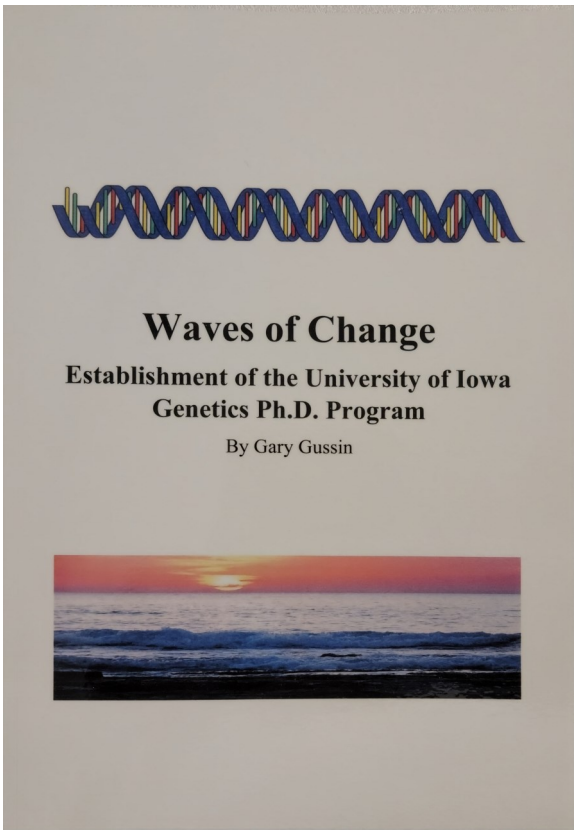


Dr. Gary Gussin
Professor Emeritus
Department of Biology

University of Iowa

Dr. Gary Gussin is a Professor Emeritus in the Department of Biology. Dr. Gussin was instrumental in the establishment of the Interdisciplinary Graduate Program in Genetics during the 1960's and was elected as the first Chairperson of the Program in 1975. Dr. Gussin published a book in 2021 titled *Waves of Change: Establishment of the University of Iowa Genetics Ph.D. Program*. The book recounts the early years of the program and successes, struggles, and contributions by the many faculty and administrators that helped develop the Program.

Wave of Change can be obtained by providing a minimum $20 donation to the Interdisciplinary Graduate Program in Genetics.



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Retreat Schedule

Friday, September 27
Biology Building East, 101 Kollros Auditorium

Public Keynote Talk—Danielle Dick, PhD

3:20 Light refreshments available
3:30 *"Using Genetics to Stop Addiction Before it Starts: Science Fiction or Science Fact?"*

Saturday, September 28
University Capital Center, Conference Center on 2nd Floor

8:00am **Check-in & continental breakfast**

8:30 **Opening Remarks**
Lori Wallrath, Ph.D.—Director
Jake Michaelson, Ph.D.—Retreat Committee Chair

8:40 **Student oral presentations—Session 1**
- Chloe Beck (Darbro lab), abstract #1
- Joseph Oberlitner (Smolikove lab), abstract #2
- Julianna Koenig (Summers lab), abstract #3

9:45 **Poster session 1**—odd numbered posters

10:45 **Alumnus Speaker** (Remarks: Alex Bassuk, PhD)
Kellie Schaefer-Swale, Ph.D.
"My Experiences Working in Industry"

11:20 **Alumnus Speaker** (Remarks : Val Sheffield, Ph.D.)
Wesley Goar, Ph.D.
"Oh, the Places You'll Go: The Many Pathways of Bioinformatics"

12:00 **Lunch**

1:00 **Student oral presentations—Session 2**
- Marcelo Miranda Melo (El-Shanti lab), abstract #4
- Krislen Tison (Williams lab), abstract #5
- Nikki Recka (Van Otterloo lab), abstract #6

2:00 **Poster session 2**—even numbered posters

3:15 **Keynote Speaker**
Danielle Dick, Ph.D.
"The Genetics of Substance Use Disorders: Novel Approaches to Identify Genes and Develop Tailored Prevention/Intervention"

4:30 **Closing Remarks & Award Ceremony**
Jake Michaelson, Ph.D.—Retreat Committee Chair

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Program History

The Interdisciplinary Graduate Program in Genetics at the University of Iowa is a broad-based training program that incorporates an expansive range of techniques, model organisms, and approaches to actively address critical issues in biology, medicine, evolution, and genomics, with genetics as a common intellectual thread. Its purpose is to optimally prepare trainees to be at the forefront of genetics research and become future leaders in any area of modern genetics, from bioinformatics and -omics to molecular genetics, gene discovery and mapping, cancer and medical genetics, and personalized medicine. Our mission for society is to train a diverse cohort of graduate students in the broad discipline of genetics who are highly prepared and well-equipped to educate all members of society, who will increasingly need to understand genetic aspects of their own family's health care and individualized genomes.

The Genetics Program curriculum is designed to provide a solid and broad foundation in genetics and foster strong independent critical thinking skills and multidisciplinary training to equip students to meet modern challenges. Throughout the span of their educational training, there is emphasis on rigor and reproducibility, responsible conduct and ethical, safe practices and inclusivity in the sciences. The Genetics Program also provides flexibility tailored to individual needs to ensure student success. In addition, the program offers a Computational Genetics subtrack for students who want to develop strengths in the biological aspects of genetics and computational approaches to analyzing large and diverse sets of genomic and genetic data. Students thus equipped have been extremely successful in filling a growing niche in the contemporary science workforce. Research opportunities within the program span the spectrum of genetics, from bacterial to model organism to human genetics, and from developmental genetics to evolution, from epigenetics to genomics to disease mechanisms.

After several formative years of interdisciplinary research activity in Genetics on the University of Iowa campus, the Interdisciplinary Graduate Program in Genetics was approved as a degree-granting PhD program in 1975. This program that began with 7 primary faculty has grown to currently having 76 faculty and 45 students in four colleges and 16 academic departments across our campus, with almost 200 graduates. While the history of the program makes the University and the state of Iowa proud, it is the continued pursuit to change and incorporate novel ideas, modern methods, and societal needs that keeps successfully preparing our students for their scientific careers. We have an consistent track record of program completion, on-time graduation rates, publications, and awards, as well as career advancement to postdoctoral fellowships, faculty positions at research-intensive universities and primarily undergraduate institutions, and biotechnology companies. Since 1997, the Genetics Program has been supported by a T32 Predoctoral Training Grant from the National Institutes of Health, last awarded in July 2022.

Lori Wallrath, Ph.D.
Director
Professor, Department of Biochemistry and Molecular Biology

Bryan Phillips, Ph.D.
Associate Director
Professor, Department of Biology

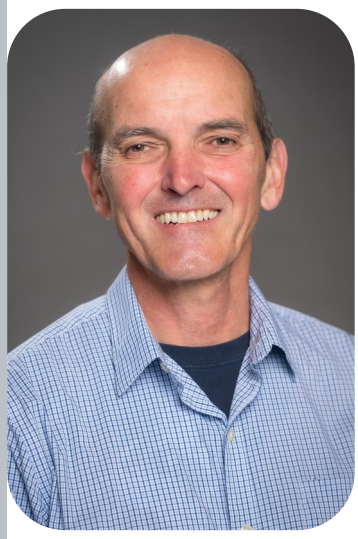
Rob DuBay
Program Administrator

Sydney Schmeltz
Program Associate

Genetics Retreat Co-chair, Jake Michaelson, Ph.D.

Professor, Psychiatry & Neuroscience

Dr. Jake Michaelson is a Roy J. Carver professor in Psychiatry and Neuroscience and the Division Director of Computational and Molecular Psychiatry at the University of Iowa. His lab studies the effect of genetic variations on the development of the brain, with specific applications in autism and language impairment. He earned his B.S. and M.S. in biological engineering at Utah State University before earning his PhD in computational biology at the Technische Universität Dresden in Germany in 2010. After his time in Germany, he joined the lab of psychiatric geneticist Jonathan Sebat at UC San Diego, where he completed his postdoctoral training and published several of the earliest papers dealing with whole genome sequencing in autism. In 2013 he joined the faculty at the University of Iowa, and his current research is supported by NIH, the Simons Foundation, and the Carver Trust.



Genetics Retreat Co-chair, Andrew Russo, Ph.D.

Professor, Molecular Physiology and Biophysics

Dr. Andy Russo is a Professor of Molecular Physiology and Biophysics and Professor of Neurology at the University of Iowa. He received his PhD in Biochemistry from UC Berkeley and did postdoctoral training at UCSD on the neuropeptide CGRP. The focus of Dr. Russo's research is the molecular basis of migraine. He is currently using mouse genetic models to study how CGRP contributes to the pain and altered sensory processing of migraine. The overall goal of his studies is to develop effective diagnostic and therapeutic strategies for migraine and post-traumatic headache.

Genetics Student Committee Members

Baylee Bruce
He Lab

Biology

Lucas Casten
Michaelson Lab

Psychiatry

Ellen Koufer
M. Schultz Lab

Pediatrics

Danielle Dick, Ph.D.

Director, Rutgers Addiction Research Center
Greg Brown Endowed Chair in Neuroscience

"The Genetics of Substance Use Disorders: Novel Approaches to Identify Genes and Develop Tailored Prevention/Intervention"

Danielle M. Dick, Ph.D. is a tenured Professor of Psychiatry at Rutgers Robert Wood Johnson Medical School, where she serves as the inaugural director of the Rutgers Addiction Research Center at the Brain Health Institute and holds the Greg Brown Endowed Chair in Neuroscience. She received her Ph.D. in Psychology in 2001 from Indiana University and subsequently completed a postdoctoral fellowship in the Department of Medical and Molecular Genetics. She was on the faculty at Washington University, St. Louis from 2003 – 2007, and Virginia Commonwealth University from 2007 – 2022, before joining Rutgers University. Her research involves studying how genetic predispositions interact with environmental factors to contribute to patterns of substance use/dependence and related behavioral disorders across development. She has served as the Principal Investigator (PI) or site PI on 16 National Institutes of Health (NIH) grants, and Co-Investigator on another 9 NIH grants, with grant funding totaling >55 million dollars. She has >425 peer-reviewed publications, and has won numerous national and international awards. She has been named as one of the top 1.5% most highly cited researchers in the world across all fields of science and is an internationally recognized and award-winning expert on genetic and environmental influences on substance use and mental health in youth. She is passionate about bringing research to the public in ways that are engaging and accessible; her first book "The Child Code: Understanding your child's unique nature for happier, more effective parenting" is out now from Penguin Random House.

Alumnus Speaker: Wesley Goar, Ph.D.

Alumni 2019
Wesley Goar, Ph.D.

Senior Bioinformatics Scientist
The Steve and Cindy Rasmussen Institute
for Genomic Medicine
Nationwide Children's Hospital

"Oh, the Places You'll Go: The Many Pathways of Bioinformatics"

Dr. Goar received his PhD in Genetics (Computational) at the University of Iowa under the direction of Drs. Val Sheffield and Todd Scheetz. During his doctoral training he studied a variety of rare inherited diseases, with a focus on developing bioinformatics pipelines and performing variant/data analysis. Following his PhD, he worked as a Senior Bioinformatics Scientist and the Manager of Bioinformatics at Immortagen LLC. Dr. Goar managed and trained the bioinformatics team, developing NGS pipelines for exome sequencing and targeted cancer panels, as well as designing analysis protocols for variant calling and classification. Additionally, he created clinical algorithms, refined personalized cancer diagnostic reports, supervised database management, and oversaw commercial relationships and inventory. In March of 22' he left Immortagen and joined the Steve and Cindy Rasmussen Institute for Genomic Medicine (IGM) at Nationwide Children's Hospital as a Senior Bioinformatics Scientist (Wagner Lab). Dr. Goar manages the development of the Variation Categorizer interface, a tool designed to automate and streamline somatic variant analysis using community guidelines. He is also heavily involved in the global community serving as:

- Cancer Genomics Consortium (CGC) - Board Member
- CGC - Genomic Resources Development Committee
- Variant Interpretation for Cancer Consortium (VICC) - Program Manager
- VICC Gene Fusion Oncogenicity Workgroup - Project Coordinator
- Assigned Expert for the Global Alliance for Genomics & Health
- ClinGen Somatic - Established Significance Somatic Cancer Variant Curation Expert Panel

Dr. Goar is very passionate about data standards to ensure precise and accurate representation of data while enabling knowledge sharing to support improved patient outcomes.

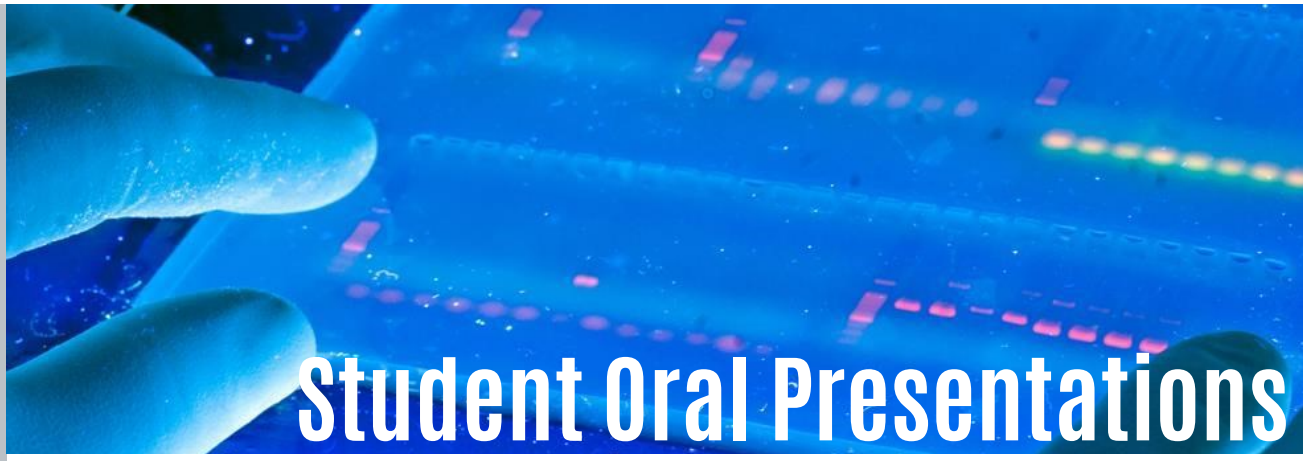
Alumni 2020
Kellie Schaefer-Swale, Ph.D.

Senior Scientist

Translational Biomarkers Group
Arrowhead Pharmaceuticals

"My Experiences Working in Industry"

Dr. Kellie (Schaefer) Swale joined the Graduate Program in Genetics at the University of Iowa in 2014. She performed her thesis research in Dr. Vinit Mahajan's lab, which centered around understanding the underlying mechanisms of a rare, complex eye disease, Neovascular Inflammatory Vitreoretinopathy (NIV). In 2017 the Mahajan Lab moved to Stanford University, and Dr. Swale continued her thesis work there. She successfully defended her thesis, "Insights into the mechanisms underlying calpain-related pathology," in December 2020. From there, she began her career in industry at Adverum Biotechnologies in Redwood City, CA. At Adverum, Dr. Swale worked as a Scientist in Research and Discovery on gene therapy approaches for ocular pathologies. In late 2022 she moved to Madison, WI and began her current position of Senior Scientist in the Translational Biomarkers group at Arrowhead Pharmaceuticals. In her current position, Dr. Swale develops assays to test biomarkers in clinical trial samples and oversees the testing of those samples.



Student Oral Presentations

Session 1 - 8:40am—9:40am

Chloe Beck

"Using DNA Methylation to Investigate Phenotypic Heterogeneity"

Joseph Oberlitner

"Characterization of defective tagged RAD-51 – stalling repair – potential tool use"

Julianna Koenig

"Development of an in-vitro model for Charcot-Marie-Tooth disease"

Session 2 - 1:00pm—2:00pm

Marcelo Miranda Melo

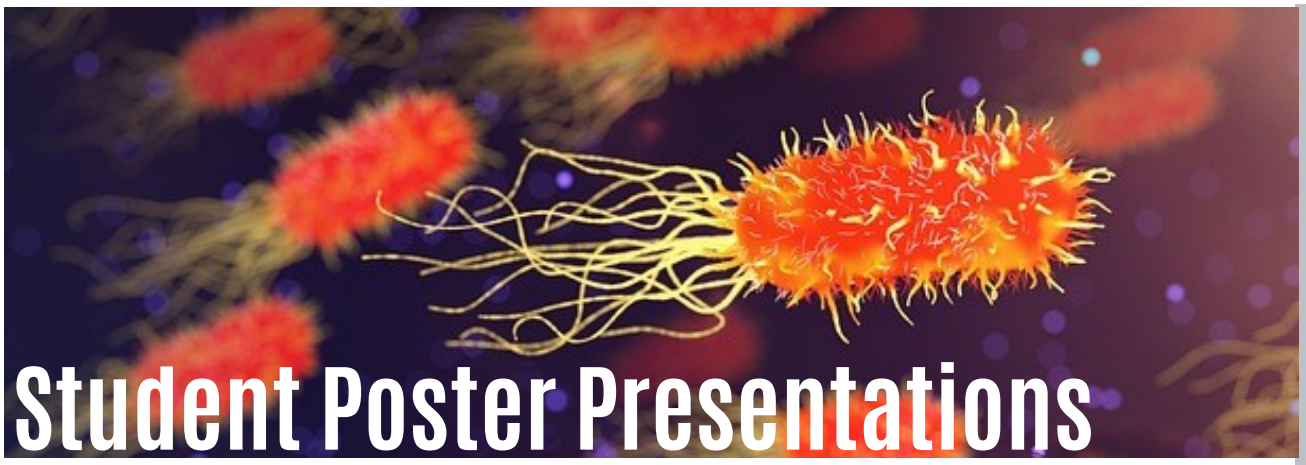
"Missense Variant in KRT32 is Responsible for Inefficient Anchoring of Anagen Hair Shaft to its Follicle"

Krislen Tison

"Transcriptomic analysis on the cerebellum of mice with the 16p11.2 microduplication mutation"

Nikki Recka

"Epidermal loss of PRMT5 leads to the emergence of an atypical keratinocyte-like cell population and defective stratification"



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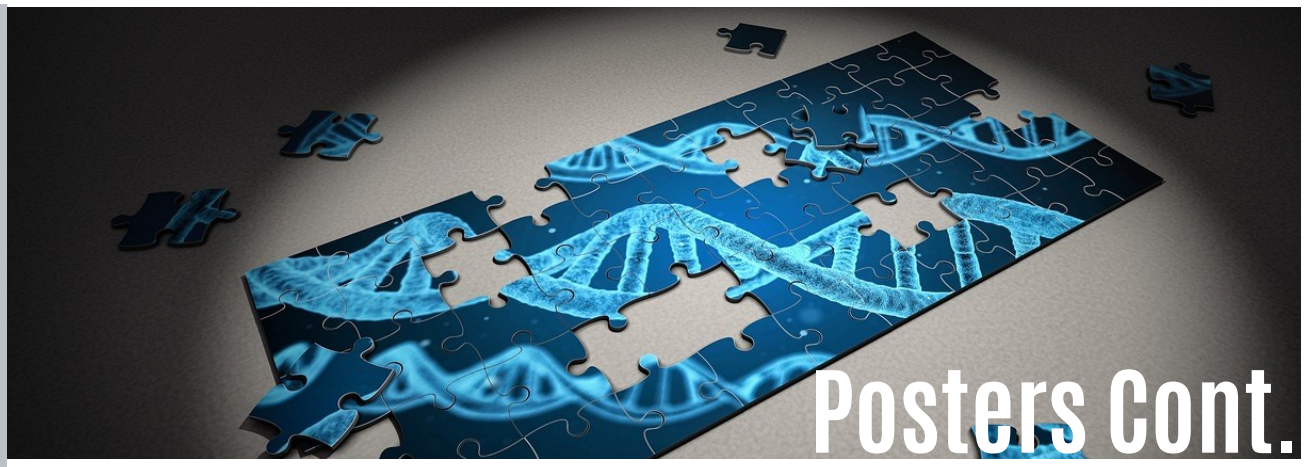

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1. Using DNA Methylation to Investigate Phenotypic Heterogeneity

CB Beck[1 2 3], EHC Kovács[4], ME Gaine[5 6], JA Wemmie[6], VA Magnotta[6 7], CT Pinnaro[1 2], BW Darbro[1 2 3]

[1] Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, IA

[2] Stead Family Department of Pediatrics, Iowa City, IA

[3] Shivanand R. Patil Cytogenetics and Molecular Laboratory, Iowa City, IA

[4] Department of Neuroscience and Pharmacology, University of Iowa, Iowa City, IA

[5] Department of Pharmaceutical Sciences and Experimental Therapeutics (PSET), College of Pharmacy, University of Iowa, Iowa City, IA

[6] Department of Psychiatry, University of Iowa, Iowa City, IA

[7] Department of Radiology, University of Iowa, Iowa City, IA

Background: DNA methylation, a known regulator of disease, has great influence on gene expression but is not accounted for in standard gene burden analyses (GBA). GBA is used to measure phenotypic heterogeneity (PH) in genetic diseases. We propose the first GBA that includes genetic information alongside DNA methylation. As phase one for this project, our goal is to create a novel weighting scheme (burden metric) that can be used with DNA methylation array data.

Methods: To identify the most relevant CpG sites for GBA, we first filtered CpG sites according to tissue-based methylation variability of the locus, variation attributed to change in nucleotide (and not methylation), and presence or absence of encompassing repetitive sequence. We further intersected this list of CpG sites with those present on the Illumina 450k, EPICv1, and EPICv2 arrays. We then scored each CpG site with our novel Burden Estimate from Weighted Integration of Site-specific Epigenetic changes (BeWISE) where each CpG is weighted according to its presence within a functional region of the genome.

Results: Our filtered list included ~65,000 sites attributed to 12,007 genes and contained CpG sites enriched for promoter, enhancer, and insulator regions, highlighting the connection between gene regulation and DNA methylation. To test the ability of the BeWISE score to differentiate affected from unaffected individuals, we utilized a cohort with bipolar disorder (BD) (n = 122) and controls (n = 69) as previously published results implicate genetics and epigenetics in the pathogenesis of BD. Using our BeWISE score aggregated by gene, we trained a random forest classifier to distinguish BD and controls with over 80% accuracy.

Conclusions: The BeWISE score can delineate between cases and controls with high accuracy and return meaningful results. Using this BeWISE score combined with genetic information may help explain PH of a wide range of genetic diseases.

2.Characterization of defective tagged RAD-51 – stalling repair – potential tool use

Joseph Oberlitner[1,2], Maggie Tinman[1]*, Aasthika Das[1]*, Emily Koury[1], Nicola Silva[3], Sarit Smolikove[1,2]

1 Department of Biology, University of Iowa, Iowa City, Iowa, 52242
2 Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, Iowa, 52242
3 Department of Biology, Masaryk University, Brno, Czech Republic
*Denotes undergraduate author

DNA double-strand breaks (DSBs) are the most harmful form of DNA damage, contributing to the formation of mutations and serving as a driver for cancer development. However, DSBs are formed on purpose in the development of gametes (sperm and eggs). DSBs are required for crossover formation, which are essential for successful segregation of chromosomes during meiosis, thereby generating viable gametes; and for promoting genetic diversity. In meiosis, DSBs are generated by SPO-11. Broken ends of the chromosome are resected to generate single-stranded DNA overhangs. These single-stranded pieces of DNA are coated by single-stranded binding protein, RPA, then subsequently replaced by RAD-51. RAD-51 aids in the initiation of repair of DSBs by invading a region of homology on an unbroken chromosome. RAD-51 is removed by RAD-54.L after invasion to allow for repair to occur. In the germline, DSBs are repaired by two main mechanisms either via the non-crossover (NCO) pathway or the crossover (CO) pathway. Although the CO pathway is responsible for accurate separation of chromosomes during meiosis, most DSBs are repaired using the NCO pathway.

Tagging different repair proteins allows us to interrogate how the germline decides between repair outcomes (CO vs NCO repair). Our lab has generated a separation of function allele of *rad-51*, *rad-51::FLAG*. This defective allele displays slowed repair kinetics, resulting in an accumulation of RAD-51 foci in meiotic prophase I. *rad-51::FLAG* worms also display checkpoint activation via an extended region of phosphorylated serine 8 of SUN-1 as a result of delayed repair, however, this level of checkpoint activation is less than that of a *rad-51* null. In addition to this, we investigated if *rad-51::FLAG* shows aberrant RAD-51-RAD-54.L colocalization by conducting a proximity ligation assay. The rad-54.L::HA *rad-51::FLAG* worms display an accumulation of RAD-51/HA PLA foci in late pachytene whereas the peak of RAD-51/HA PLA foci in *rad-54.L::HA* worms is in mid-pachytene. This suggests that RAD-51::FLAG filaments have issues being removed, thus stalling at an intermediate step in repair.

The defects in RAD-51 disassembly in *rad-51::FLAG* mutants lead to formation of chromosomal fragments. These defects are similar in their magnitude to ones observed in *rad-51* or *rad-54* null mutants. In these mutants, it was shown that these effects are due to perturbation in both CO and NCO repair pathways. To test whether *rad-51::FLAG* shares a similar phenotype, we use GFP::COSA-1, a marker for COs. In *C. elegans*, each chromosome pair acquires one CO leading to wild-type number of six GFP::COSA-1 foci. A *rad-51* null displays an average of 2.3 GFP::COSA-1 foci while a *rad-54.L* null displays an average of 3.4 GFP::COSA-1 foci. However, unlike a *rad-51* null or a *rad-54.L* null, *rad-51::FLAG*, displays 6 GFP::COSA-1 foci. Given that rad-51::FLAG worms display checkpoint activation and chromosomal fragments, these results suggest that CO repair is slow appears to proceed normally, while the NCO pathway appears to be perturbed, given our accumulation of RAD-51 foci. These results suggests that NCO vs CO has distinct recombination intermediates, consistent with our earlier published work that DSB timing is linked to repair pathway choice.

This project was funded by NSF grant #2027955.

3. Development of an in-vitro model for Charcot-Marie-Tooth disease

J.A. Koenig[1,3] & D.W. Summers[1,2,3]

[1]Department of Biology, University of Iowa, Iowa City, IA

[2]Iowa Neuroscience Institute, Carver College of Medicine, University of Iowa, Iowa City, IA

[3]Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, IA

Maintenance of proteostasis is critical for preserving cell integrity and appropriate responses to a changing environment. Impairments in proteostasis can lead to a deleterious imbalance in protein synthesis, localization, and degradation. Such impairments have important consequences for neurons, which must function for a human's entire lifespan. Charcot-Marie-Tooth disease (CMT) describes a group of inherited peripheral neuropathies. A common feature in certain instances of these neuropathies is mutations in aminoacyl-tRNA synthetases (ARS). This suggests that a defect in the protein synthesis aspect of proteostasis may underly the unclear etiology of CMT. However, the mechanism(s) through which ARS mutations may cause the sensory and muscular phenotypes observed in CMT patients remains unknown. To address this gap in knowledge, I developed an in-vitro model for the effects of mutant ARSs in sensory neurons. This model utilizes neurons cultured from the dorsal root ganglion (DRG) of embryonic day 14 mice and genetically encoded fluorescent reporters to visualize newly synthesized proteins in live neurons. Once grown, DRGs undergo chemical axotomy to remove axons while maintaining cell integrity. The axons are subsequently regenerated. This approach allows me to assess axon regeneration dynamics in a mutant ARS background. In this DRG culture model, lentiviral expression of a CMT associated mutant tyrosyl-tRNA synthetase (mYARS) induces dose-dependent axon degeneration and cell death when compared to wildtype and empty vector controls. Further, I observed a significant decline in neuronal protein synthesis with mYARS. However, axon regeneration is unaffected in the mYARS condition. These findings demonstrate that expression of mYARS in DRG cultures induces axon degeneration, mirroring a prominent event in many CMT neuropathies. Furthermore, these findings help elucidate CMT's etiology. The unaffected regrowth dynamics raises the possibility of the existence of factors present in neurons during the initial axon growth stages. These factors confer resistance to the synthesis dampening effect.

4.Missense Variant in *KRT32* is Responsible for Inefficient Anchoring of Anagen Hair Shaft to its Follicle

Marcelo Melo[1], Elizabeth Phillippi[1], Hatem El-Shanti[1]

[1] Department of Pediatrics, University of Iowa, Iowa City, IA;

Loose Anagen Hair Syndrome (LAHS) is characterized by excessive shedding or easily pluckable terminal hair during its active growth (anagen) phase. Although the prevalence of LAHS is estimated to be 2 cases per million, we believe that this is an underestimate due to under-reporting. This disorder is thought to be due to abnormal anchoring of the hair shaft to the hair follicle. Several genes have been implicated in LAHS, including pathogenic variants of *KRT75* (keratin 75) as well as pathogenic variants in *PPP1CB* and *SHOC2* in Noonan syndrome with loose anagen hair (NSLH). Our group identified a likely pathogenic variant in *KRT32* (keratin 32) that is responsible for autosomal dominant LAHS and segregating with the disorder in a large kindred. The identified *KRT32* missense variant (NM_002278.3; c.296C>T; NP_002269.3; p. Thr99Ile) replaces a highly conserved threonine at position 99 with an isoleucine residue. This amino acid is evolutionarily conserved, specifically in mammals, suggesting that variation has a high likelihood of being pathogenic. Keratin 32 is known to heterodimerize with keratin 82 to form the intermediate filament network within the cuticle of the hair shaft. We are currently performing functional assays in the form of protein-protein interactions and microscopy that provide functional evidence of the pathogenicity of the identified mutation. We hypothesize that the variant T99I in *KRT32* prevents the heterodimerization between the two keratins which is a mandatory interaction for proper anchorage of the hair shaft to the follicle. The understanding of the pathophysiology of LAHS would provide insight into the mechanism(s) that anchor the anagen hair shaft to its follicle, which could have implications in other disorders associated with anagen hair loss.

5. Transcriptomic analysis on the cerebellum of mice with the 16p11.2 microduplication mutation

Krislen Tison[1,2], Kamilla Jacobo[1,2], Cessily Hayes[1,2], Hunter Halverson [1,2,], Krystal Parker[1,2], Marie Gaine[1,2,3], Aislinn Williams[1,2]

[1]Department of Psychiatry, University of Iowa, Iowa City, Iowa; [2]Iowa Neuroscience Institute, University of Iowa, Iowa City, Iowa; [3]Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, Iowa

Schizophrenia is a severe and chronic illness where patients suffer with hallucinations, delusions, and cognitive dysfunction. Medications can reduce hallucinations and delusions, but currently available treatments do not improve cognitive symptoms. Recent work suggests that the cerebellum is involved in cognitive dysfunction in schizophrenia, but the mechanisms underlying this are unknown. To address this, we employ copy number variant (CNV) 16p11.2 duplication mice (16p11.2dup), a genetic mouse model of schizophrenia, to study gene expression changes within various cerebellar cell types. We hypothesized that the cerebellum is an important site of pathology in 16p11.2dup. We first performed a deferential gene expression computational analysis of publicly available RNAseq data from 16p11.2dup mice and saw significant transcriptional dysregulation in the cerebellum between genotype and sex. This led us to generate our own transcriptomic profiles for 16 mice across both sexes and genotypes from the cerebellum using paired end Illumina sequencing. Differential gene expression analysis identified several genes with high expression levels, including *Myo16*, *Gabra5*, *Gadl1*, *Meis2*, and *Egr3*. Gene Ontology (GO) analysis further highlighted biological processes enriched in the dataset, with a significant association found for the term "associative learning". The cerebellum, traditionally viewed as a motor structure, has also been implicated in associative learning. Given the involvement of *Gabra5* and *Meis2* in learning-related processes, it is plausible that these genes influence cerebellar circuits involved in associative learning. Overall, our findings shed light on the transcriptional landmark changes occurring in the cerebellum in a valid mouse model and can potentially accelerate the discovery and development of new diagnostic and therapeutic strategies for schizophrenia.

6. Epidermal loss of PRMT5 leads to the emergence of an atypical keratinocyte-like cell population and defective stratification

N Recka[1,2,3], A Simmons-Burnett[4], R Cornell[5], E Van Otterloo[2,3]

[1] Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, IA

[2] Department of Anatomy and Cell Biology, University of Iowa, Iowa City, IA

[3] College of Dentistry and Dental Clinics, University of Iowa, Iowa City, IA

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During development, the single-layered epithelium of the presumptive skin requires precise coordination of cell proliferation and differentiation for proper epidermal stratification. Defects in these events lead to congenital anomalies whereas accumulation of harmful mutations in adulthood can cause carcinoma. Protein Arginine Methyl Transferase 5 (PRMT5) -an enzyme that catalyzes methylation of arginine residues in histones and transcription factors- is upregulated in various carcinomas, correlating with poorer prognosis. While inhibition of PRMT5 exhibits anti-cancer properties, the underlying mechanisms are unknown. PRMT5 has been identified as necessary for maintenance of stem-cell fate in limb development and cancer. Therefore, we hypothesize that PRMT5's methylation of histones and transcription factors drives a gene expression program hindering differentiation, maintaining a stem-cell phenotype. Conditional mouse genetics deleting Prmt5 from early (E7.5) ectoderm resulted in gross skin defects, reduced skin barrier function, and reduced postnatal viability. Histological analyses revealed severe defects in epidermal stratification, including basal layer reduction. Molecularly, sc-RNA/ATAC-seq suggests PRMT5 loss leads to the emergence of an atypical keratinocyte-like cell population not found in control samples. Ongoing work aims to clarify this atypical cluster and how its presence may disrupt epidermal development. Our findings reveal PRMT5's crucial role in epidermal development, offering a model to dissects its molecular function and gain insights into its role in tumor progression.

7. Arhgap29 regulates epithelial morphology, contractility, and stiffness

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ARHGAP29, Rho GTPase Activating Protein (GAP) 29, functions in the cyclical regulation of RhoA, a major modulator of the actomyosin cytoskeleton. Arhgap29 is expressed in many cell types, including epithelial cells, where it promotes the inactivation of RhoA. Arhgap29 has also been implicated in a range of tissue morphogenesis events, including palatogenesis, however what role Arhgap29 plays in different cell types during this process remains unknown. Using an ectoderm-cell specific mouse knockout of Arhgap29, we found a significant, yet incompletely penetrant cleft palate in mutants compared to wild-type controls. Further phenotypic characterization revealed a thickened oral epithelium at the tip of the palatal shelves, a disorganized lingual epithelium, and an increase in epithelial cells positive for phospho-Myosin regulatory light chain (pMRLC) and a-smooth muscle actin, two markers of actomyosin contractility. To further investigate the mechanism by which Arhgap29 contributes to cleft palate, we knocked down Arhgap29 in epithelial cells *in vitro* and observed increased cell spreading and reduced proliferation. Immunofluorescent staining for the actomyosin cytoskeleton showed an increase in filamentous actin structures and an increase in pMRLC, suggesting an increase in contractility. To determine what effect these cytoskeletal changes may have on cell stiffness, we performed atomic force microscopy (AFM). AFM revealed that Arhgap29 knockdown cells were significantly stiffer compared to control cell lines. Together, these data point to a model in which Arhgap29 promotes the remodeling of the actomyosin cytoskeleton of epithelial cells required during palatogenesis.

8. Profiling the Evolution of Acquired Stress Response Genes in the Pathogenic Yeast *Candida glabrata* through Hermes Transposon mutagenesis.

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Candida glabrata is an opportunistic pathogen closely related to the pathogenic *C. bracarensis* and *C. nivariensis* as well as the non-pathogenic species within *Nakaseomyces* clade, including, *N. delphensis* and *N.bacillisporus*. These species, along with *Saccharomyces cerevisiae*, the baker's yeast, exhibit significant evolutionary diversity compared to *C.albicans* and several other pathogenic yeast in its clade. This evolutionary diversity has raised critical questions in the field regarding the genetic factors underlying the independent evolution of pathogenicity, virulence, host colonization and drug resistance among budding yeast.

To address these questions, we focus on stress responses, particularly acquired stress resistance (ASR), which involves preparing an organism for the ensuing environmental changes after encountering an initial stressor. We utilize transposing mutagenesis followed by cell sorting and sequencing (Tn-seq), coupled with computational and functional studies to identify upstream regulators of ASR. Preliminary results suggest that *C.glabrata* strains with transposon insertions in essential genes may be involved in oxidative stress (H_2O_2) and nutrient (Phosphate) starvation responses. By elucidating the molecular players in ASR, this study aims to uncover how signaling networks can be rewired to allow organisms to respond to novel environmental stimuli using existing networks. This understanding may idenify actionable candidate genes relevant to the evolution of pathogenicity, virulence and drug resistance in budding yeast.

9. Defining the prostaglandin $F_{2\alpha}$ signaling pathway promoting collective cell migration

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Collective cell migration is critical for development, wound healing, and cancer metastasis. In species from Drosophila to mammals, one mechanism promoting migration is prostaglandin (PG) signaling. PGs are small lipid signaling molecules that promote cell migration by activating G-protein coupled receptors (GPCRs) and initiating signaling cascades in autocrine or paracrine signaling. Most studies investigating PG signaling during cell migration have knocked out or inhibited PG signaling globally. Thus, it remains largely unknown what cells, the migratory cells or the cells in the microenvironment, produce which PGs to drive migration. Further, the downstream mechanisms by which individual PG signaling cascades contribute to migration remain poorly understood. This project will use the established model of invasive, collective cell migration of the Drosophila border cells to uncover the roles of the understudied PG, $PGF_{2\alpha}$, in promoting collective cell migration. Previous findings lead to the model that the migratory cells produce $PGF_{2\alpha}$ via its synthase, Akr1B, and the substrate produces PGE_2 via its synthase, cPGES, to promote on-time migration. Knocking down the $PGF_{2\alpha}$ synthase, Akr1B, in the border cells significantly delayed migration, reduced integrin enrichment on the border cell membranes, and increased activated myosin in the border cells. Using available mutant and RNAi lines, we will uncover the $PGF_{2\alpha}$ signaling pathway downstream of the $PGF_{2\alpha}$ receptor, including the Gα protein, and the downstream effectors, required for on-time border cell migration. Based on previous experiments where $PGF_{2\alpha}$ synthase was knocked down within the border cells, impairing downstream pathway components is expected to phenocopy loss of Akr1B. We expect this signaling pathway to be conserved across organisms and contribute to other invasive, collective cell migration processes. Understanding the roles of specific PG species and their signaling events in collective cell migration will advance the field's understanding of development, and guide advancements in regenerative and cancer therapies.

10.Evolution of Pioneer Factor Ability Between Orthologous Transcription Factors

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Pioneer Factors (PF) are a special class of Transcription Factors (TFs) that are defined by their ability to access closed chromatin and open it up to allow for transcription. In eukaryotes, PFs are recognized for their crucial role, especially in specifying cell fate during development. PF's role in other biological processes outside development is still poorly understood. Another limitation in our understanding of PF activity is whether it changes during evolution. Existing studies implicitly treat PF activity as a fixed property of a TF. However, genome-wide screens have shown that TFs vary quantitatively in their PF activities, suggesting the possibility that PF activities are evolvable and could diverge between orthologous TFs. If true, this would have profound implications for the evolution of gene regulation and the consequent phenotypes. In preliminary studies, our lab has found strong evidence for such variation in PF activity between two orthologous TFs in related yeasts. In the baker's yeast *S. cerevisiae*, the basic Helix-Loop-Helix TF, Pho4 (ScPho4), fails to induce the reporter gene when the promoter contains only a nucleosome-occluded motif. By contrast, the orthologous Pho4 in the related pathogenic yeast, *C. glabrata* (CgPho4), is capable of inducing the same mutant promoter. My central hypothesis is that the difference in the PF ability of divergent Pho4 orthologs is due to CgPho4's ability to bind nucleosome occluded sites and recruit chromatin remodelers and these mechanisms stem from differences in protein sequences. This research aims to determine CgPho4's ability to bind nucleosome-occluded motifs genome-wide and investigate the consequent changes in nucleosome occupancy and gene expression. This will contribute to our knowledge of how PF ability evolves.

11. 16p11.2 Microdeletion Induces Sex-Specific Defects in Placental Development in Mice

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Neurodevelopmental disorders (NDDs) are extremely common. The prenatal environment significantly influences NDD risk which may include how NDD risk genes affect placental function. However, placental disruptions in mouse models of NDD risk genes remain unexplored. Our objective was to investigate placental function in the 16p11.2 microdeletion model (16p del). 16p del in humans is associated with NDDs. Mice modeling this deletion display sex-specific behavioral phenotypes relevant to NDDs, mirroring the male bias observed in human NDDs. We hypothesized that 16p del would alter placental growth and function with more severe phenotypes observed in male placentas. To test this, wildtype female mice were bred with hemizygous 16p del males, and placental weight, fetal weight, placental morphology, and placental gene expression were assessed.16p del males showed 50% expression level in all three 16p del genes at three embryonic timepoints. Unexpectedly, embryonic day 16 (E16) 16p del female placentas exhibited comparable expression to wildtype *Mapk3*, a 16p del gene that is a major regulator of placental development. Male 16p del placental mass was increased at E16 and E18 compared to litter-matched wildtypes; females showed no group differences. This increased 16p del male placental weight may have been related to increased E16 decidual proportion we found in 16p del males, a phenotype typically associated with poor placental perfusion. Further evidence of poor placental perfusion was seen at E18 as 16p del males had a significant decrease in total placental sinusoidal area. There were no group differences in female placental morphology. Furthermore, 16p del females showed mid-gestation promotion of angiogenic and growth pathways expression in the placenta, conversely 16p del males showed downregulation of these pathways in late-gestation. The sex-specific placental abnormalities in 16p del mice likely impact postnatal development and may contribute to early developmental origins of male bias in NDDs.

12. Genome sequencing uncovers rare and common genetic mechanisms in the development of human language ability

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Language is the foundation of human social interaction, education, commerce, and mental health. The heritability underlying language is well-established, but our understanding of its genetic basis remains unclear. To illuminate the language-specific contributions of rare and common variation, we performed high-coverage whole genome sequencing in N=350 unrelated individuals who received longitudinal deep language phenotyping throughout childhood. We conducted polygenic score (PGS), gene, and gene set-based analyses of these phenotypes. Additionally, we describe a novel Evolutionary Stratified Polygenic Score (ES-PGS) analysis to identify evolutionary events important to phenotypic variation.

We found that language phenotypes were pervasively associated with behavior and mental health. As expected, PGS for cognitive performance had the strongest associations with language. PGS for psychiatric conditions are associated with childhood language abilities, including; ADHD PGS and core language ability ($r = -0.13$, $p = 0.02$), as well as schizophrenia PGS and receptive language ($r = -0.16$, $p < 0.01$). Interestingly, our ES-PGS analysis found the fastest-evolved regions of the human genome (HAQERs) have a disproportionately large impact on core language ability, but not on nonverbal IQ. This finding was replicated in a large independent sample of > 30,000 individuals, establishing HAQERs as crucial elements in human language development. Rare variant analyses found that burden in genes related to cognitive performance, human-chimp divergence, autism, and schizophrenia were all associated with impaired core language ability.

These results offer genetic evidence supporting the link between neuropsychiatric risk and childhood language ability. Both common and rare variant analysis showed evidence that human-specific selection patterns and known cognitive performance loci contribute to core language skills.

13. Enhanced Brain Connectivity Supports Processing Speed: Evidence from 7T Structural, Diffusion, and Resting-State MRI

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Processing speed (PS) is one of the fundamental nonverbal cognitive domains influenced by structural and functional brain connectivity. While previous research has focused on this relationship, studies have often been limited by the sample size and the lack of integration of different MRI modalities. To address these limitations and investigate the basis of PS, we employed a comprehensive multimodal approach, integrating high-resolution (7T) structural MRI, diffusion imaging, and resting-state fMRI in a neurodiverse cohort (n=45).

Several metrics assessing white matter integrity and tracts geometrics including Fractional Anisotropy (FA), Axial Diffusivity (AD) and Quantitative Anisotropy (QA) were extracted from diffusion imaging, with a particular focus on the corpus callosum. Resting-state fMRI was used to measure the fractional Amplitude of Low-Frequency Fluctuations (fALFF) to capture intrinsic brain activity across multiple regions. We evaluated PS through Wechsler intelligence tests (WISC-V and WAIS-IV), the NIH Toolbox, and other developed language tasks to be correlated with extracted neuroimaging metrics.

We found significant associations between the corpus callosum FA (ρ: 0.44, p-value: 0.005), QA (ρ: 0.47, p-value: 0.002), and total radius of tract end regions (ρ: 0.51, p-value: 0.0007) with PS, indicating that enhanced axonal density and integrity support faster cognitive processing. We also observed a strong link between calculated fALFF values in key brain networks and measured PS, particularly in the SalVentAttnB network.

Future work will include predicting transcriptomic profile of different brain regions per individual using eQTL data and comparing it to neuroimaging metrics. Our approach of integrating neuroimaging and genetics in a neurodiverse sample offers a unique and holistic view of the PS cognitive domain.

14. Identifying Polymorphisms that Modulate Disease Risk through Changing Gene Expression

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For more than two decades, genome-wide association studies (GWASs) have successfully identified polymorphisms in human genomic loci associated with complex diseases, including cancers, neurodegenerative diseases, and glaucoma. Despite these successes, GWASs rarely identify the causal, risk-modulating variations in these loci. This is the next major step required for understanding the factors affecting disease risk. Some associated polymorphisms are potentially causal, yielding an amino acid sequence change. However, most polymorphisms associated with complex diseases are not predicted to alter the amino acid sequence. Thus, the molecular mechanism underlying the risk-modulation is unknown.

We and others hypothesize that many GWAS-identified loci alter disease risk by altering gene expression, i.e., we propose that variants in associated loci alter disease risk by altering the transcription rate of nearby genes. We term these elements expression modulating variants (emVars). We have identified several putative emVars using a massively parallel reporter assay called Bi-allelic Targeted Self-Transcribing Active Regulatory Region Sequencing (BiT-STARR-Seq). BiT-STARR-Seq uses a plasmid reporter system, in which custom-designed oligos are cloned downstream from a promoter element such that the sequence potentially regulates its own transcription. For a given variant, oligos representing reference and alternate alleles are designed and cloned into constructs. We determine the relative effect of each allele by the relative prevalence of each allele in the RNA from next-generation sequencing. Using BiT-STARR-Seq, we have identified putative emVars near *LOXL1*, which is associated with pseudo-exfoliation syndrome (XFS), in HEK-293T and HLE-B3 cells. We are currently expanding our investigation to include a comprehensive evaluation of polymorphisms surrounding *LOXL1* for XFS and associations for primary open-angle glaucoma and neovascular macular degeneration. Future steps include validating emVars using RT-PCR and computational methods. The goal of this research is to identify molecular mechanisms that reveal therapeutic targets to prevent or delay the blindness caused by XFS-related glaucoma.

15. Factors that control synaptic homeostasis maintenance also support sleep

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The brain and all the tissues that it excites govern animal behavior. To keep behaviors controlled, nervous system output needs to stay within physiological bounds. At the levels of neurons, synapses, or circuits, this means that homeostatic forms of regulation are needed. One model synapse where this form of regulation is studied is the *Drosophila* neuromuscular junction (NMJ). Our lab and others have found that at the NMJ, a small group of factors has been identified as critical for the long-term maintenance of synaptic homeostasis. These factors can respond to acute challenges to the synapse but cannot maintain synaptic homeostasis with long-term challenges.

The idea of homeostatic regulation has been extended to specific behaviors like sleep. The synaptic homeostasis hypothesis (SHY) in the sleep field posits that sleep is used as an off-line period to restore the brain's capacity for plasticity that is needed during wakefulness. This idea has not been rigorously tested at a molecular level. If the general concept of SHY is correct, perturbations that disrupt sleep would affect synaptic homeostasis, and likewise, disruptions to synaptic homeostasis would also affect sleep. Previous research has shown that disruptions in a subset of canonical sleep genes can also cause disruptions to synaptic homeostasis. Here we find that loss-of-function mutations in some of the synaptic homeostasis maintenance factors also show sleep phenotypes. When compared to wild type disruptions to the genes encoding GluRIIA (glutamate receptor subunit), RyR (the Ryanodine Receptor), ITPR/IP3R (inositol trisphosphate receptor), and Src64B (Src family kinase), all show disruptions to the patterns seen in *Drosophila* sleep. They each have truncated or extended sleep, specific to daytime or nighttime periods. We will continue to test the synaptic homeostasis maintenance factors to determine which factors are important for sleep and how their synaptic functions might inform their roles in sleep.

16. Cardiomyocyte-Specific Deletion of Med13 & Med13L Results in Dysregulated Gene Expression and Lethal Systolic Heart Failure

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In 2022, 1 in every 5 deaths was due to cardiovascular disease which is one of the leading causes of death in the United States. Previous studies have linked mutations in the mediator complex, specifically Mediator 13 (med13) and Mediator 13 Like(med13L), with both congenital heart defects and cardiovascular diseases. The core mediator complex is comprised of approximately 26 subunits and interacts with RNA polymerase II and DNA-bound transcription factors to create the pre-initiation complex. Within the mediator complex, there is a reversibly disassociating kinase submodule consisting of Cyclin Dependent Kinase 8 (cdk8), Cyclin C, Mediator 12 (Med12), and Med13. There is also a mutually exclusive paralog of Med13, Mediator 13 Like. Previously Med13 and Med13L have been shown to be partially redundant in preimplantation embryo development, but this has yet to be investigated in the adult heart. Therefore, these studies investigate the critical nature of Med13 and Med13L in adult cardiomyocytes for normal cardiac function. We created a Med13 and Med13L tamoxifen induced - cardiac specific knockout mouse and treated with Tamoxifen at 8 weeks of age. We found a severe decrease in ejection fraction with a median survival time of 6 weeks post tamoxifen. The knockout mice also have an increase in heart weight: body weight ratios. To elucidate the role Med13 and Med13L have on cardiac gene expression, we performed bulk RNAseq on ventricles at 4 weeks post-tamoxifen and saw an increase in extracellular organization and fibrosis pathways, as well as a decrease in calcium and potassium channel RNA. Taken together, these studies demonstrate that Med13 and Med13L function partially redundantly in the heart and they play a crucial role in cardiac physiology and ensuring normal mediator-regulated RNA -pol II dependent transcription.

17. Identifying proteostatic regulators of mutant Niemann-Pick type C1 protein using Sleeping Beauty transposition

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Niemann-Pick type C1 is a rare, pediatric lysosomal storage disorder with autosomal recessive inheritance. The disease is caused by monogenic mutations in the *NPC1* gene which encodes the lysosomal cholesterol transporter NPC1. Symptoms of Niemann-Pick type C1 are diverse, but affected children commonly experience severe neurodegeneration, seizures, hepatosplenomegaly, liver cirrhosis, and early death. The most common disease-causing mutation is an isoleucine to threonine mutation ($NPC1^{I1061T}$) which is rapidly degraded in the ER. Importantly, $NPC1^{I1061T}$ is functional if trafficked to the lysosome. This observation spurred interest in understanding NPC1 degradation for the development of proteostasis modulators. However, little is known about the regulators of $NPC1^{I1061T}$ degradation. Here we are taking advantage of Sleeping Beauty transposition mutagenesis to screen for the regulators of NPC1 folding, trafficking, and degradation. We've tagged the HeLa $NPC1^{I1061T}$ protein with mScarlet to serve as a cellular functional readout for alterations in trafficking caused by the Sleeping Beauty insertions. When trafficked correctly, mScarlet shows high levels of fluorescence. When sequestered in the ER due to misfolding, the fluorescence level is very low. By FACS sorting for $NPC1^{I1061T}$ cells with high mScarlet signal, we can identify mutations which alter the behavior of this reporter and thus alter the $NPC1^{I1061T}$ phenotype. Through next generation sequencing and identification of recurrent insertion sites, we hope to develop a more complete picture of the regulators of $NPC1^{I1061T}$ protein homeostasis.

18. Blinded with Science: Investigating how a class of anti-cancer drugs causes retinal dysfunction

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Neurofibromatosis Type 1 (NF1) is a rare cancer predisposition syndrome which can cause tumors in neural crest cell-derived tissues like skin and nerves. Patients with NF1 are frequently treated with inhibitors of the Ras-MEK-ERK/MAPK pathway which effectively decreases tumor cell survival and proliferation. A subset of patients treated with these inhibitors develop a drug-associated retinopathy whereby the retina detaches from the underlying monolayer of retinal pigment epithelial (RPE) cells, leading to decreased visual acuity. While the mechanism of this retinopathy is unknown, it is hypothesized that these inhibitors disrupt normal RPE functioning, leading to fluid accumulation under the retina.

To test this hypothesis, we obtained skin biopsies from two female patients with NF1 (one of which developed drug-induced retinopathy) and one female patient without NF1. We reprogrammed isolated fibroblasts from these biopsies into induced pluripotent stem cells (iPSCs) which we then differentiated into RPE cells. During this time, we determined the optimal dose for cell culture of the clinically used MEK-inhibitor, selumetinib, in an immortalized line of RPE cells. We identified 1 uM as the optimal dose as it did not decrease cell viability as measured with an MTS assay, and it inhibited downstream protein activation at comparable levels to higher doses. We repeated the cell viability assay on a control line of our iPSC-derived RPE cells. We also treated these cells with 1 uM of selumetinib for 24 hours and evaluated inhibition of downstream protein activation and normal RPE cell functioning by measuring the ability of the cells to phagocytose rod photoreceptor outer segments, maintain transepithelial electric resistance, and secrete vascular endothelial growth factor.

Upon completion of these experiments, we will have a better understanding of the role RPE cells might play in this anticancer-drug-induced retinopathy.

19. Network Based Stratification of Known Microdeletion and Duplication Syndromes to Improve Interpretation of Variants of Unknown Significance

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In USA, ~67 million individuals are diagnosed with neurodevelopmental disorders (NDD) and ~10.5% among them have intellectual disabilities (ID) leading to major socioeconomic issues. Globally, ~1-3% of world population have ID diagnoses and the incidence is increasing. However, comprehensive genetic diagnostic methods are not yet fully explored in NDD and ID populations.

Phenotypic heterogeneity is common in patients with ID and is influenced by different pathogenic copy number variants (CNVs). The increase in NDD and ID genomic testing via chromosomal microarrays (CMA) and whole genome sequencing (WGS) has led to the identification of an increasing number of "variants of unknown significance (VUS)" both at the sequence level as well as CNVs. In this project, we hypothesize that specific protein-protein interaction networks are common between established microdeletion / microduplication syndrome (MMS) syndromes and VUS CNVs in patients with similar phenotypes. This enables ID VUS re-classification to establish NDD and ID genetic diagnoses.

To study this hypothesis, we will use the CMA and WGS data from ~7000 pediatric patients seen at the University of Iowa. We will analyze the detected VUS CNVs from our database in the context of known MMS PPI disease networks and phenotypes. We will adopt the Human Phenotype Ontology (HPO), pathway analyses, network-based stratification (NBS), and community detection algorithms to first identify the underlying significant ID PPI networks, genotype based patient clusters and subsequently reclassify the VUS CNVs based on their stratification with known CNVs.

Our preliminary experiments using these clustering procedures were successful in genotypically quantifying and phenotypically defining the functional interacting genetic variants in the context of known MMS CNVs for interpreting and re-classifying the detected VUS CNVs. This methodology allows for candidate ID gene identification which when followed by Gal4-UAS RNAi functional testing accompanied by T-maze olfactory assay in the Drosophila melanogaster model system to identify novel genetic variants in cognition and memory thus helping in VUS CNV interpretation.

This project contributes to more accurate diagnoses and potentially precision therapies in NDD and ID populations by refining clinical interpretation and classification of ID and NDD VUS genetic lesions. Furthermore, novel gene-phenotype associations in ID patients can be described by functional testing of the detected VUS genes.

20. Elevated IOP facilitates T cell mediated RGC loss

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Glaucoma is a neurodegenerative disease that causes loss of retinal ganglion cells (RGC), and subsequently, loss of vision. We have previously shown that adoptive transfer of CD3+ cells from glaucomatous animals into healthy recipients results in progressive loss of RGC, suggesting a functional role of adaptive immunity in the pathology of the disease. A crucial first step in this process is the extravasation of T-cells into the immune-privileged retina. This study sought to determine if elevated IOP facilitates extravasation using donor mice with a fixed T-cell receptor against GFP (*Just EGFP Death Inducing*, JEDI) and Thy1-GFP+ recipient mice that express GFP in a subset of RGC.

Mild IOP elevation was induced in Thy1-GFP+ mice through intracameral injection of Ad5.Myoc^{Y437H} (n=12). Thy1-GFP+/- control animals received intracameral injections of Ad5.empty (n=11-12/per group). Following injection, IOP was monitored by rebound tonometry and GFP+ cells were imaged by fundoscopy for 7 weeks. 4 weeks following injection, spleens from donor JEDI mice were harvested and transferred into recipient Thy1-GFP mice. At week 4.5 following intracameral injection, one group received an intraperitoneal injection of lipopolysaccharide (LPS). At the conclusion of the experiment, retinas were collected and visualized using confocal and widefield fluorescence microscopy.

Following injection and adoptive transfer, all mice experienced RGC loss on fundoscopy. Losses are similar in mice that did not receive splenocytes (-4.46%), those that received splenocytes but without elevated IOP (-2.83%), and those receiving splenocytes and LPS (-4.5%). However, mice receiving splenocytes and experienced elevated IOP lost significantly more RGC (-18.43%, p=0.0097). In these animals RGC loss was particularly noticeable in the central retina.

Slight elevation in IOP facilitates T cell-mediated degradation of RGC. LPS-mediated weakening of the blood-retina barrier by itself is insufficient to achieve RGC loss, suggesting that additional IOP-mediated mechanisms are required to overcome retinal immune privilege.

21. *SMAD7* is a modifier gene of *LMNA*-associated muscular dystrophy and a therapeutic target

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Mutations in *LMNA* cause a collection of diseases known as laminopathies, which include multiple types of muscular dystrophy (*LMNA*-MD). *LMNA*-MD is sensitive to genetic background, as individuals with the same *LMNA* mutation (including siblings) can have clinically distinct diagnoses and/or variable disease severity. Here, we describe a family in which four siblings with the same *LMNA* mutation exhibit highly variable muscle disease severity. Using whole genome sequencing, we identified a variant in the *SMAD7* gene, encoding a negative regulator of the SMAD signaling pathway, that segregates with severe disease. Functional tests in Drosophila support this variant as an enhancer of muscle disease severity. The *LMNA* mutation activates the SMAD pathway in muscle, and this activation is enhanced by the *SMAD7* variant, providing a mechanism for disease enhancement. Furthermore, overexpression of wild type Drosophila *SMAD7* can reduce SMAD signaling and rescue muscle defects caused by mutant lamins, implicating the SMAD pathway as a therapeutic target.

Translation of this genetic rescue to treatment with FDA-approved TGFβ/SMAD inhibitors is currently underway. Additionally, we have identified six additional *SMAD7* variants in the broader *LMNA*-MD population. We are currently testing whether these additional *SMAD7* variants are able to enhance muscle defects caused by multiple *LMNA* mutations, supporting the broad efficacy of *SMAD7* as an *LMNA*-MD modifier gene. Collectively, our data represents the first report of an *LMNA*-MD modifier gene that has been functionally tested in a model organism and implicates the SMAD pathway as a therapeutic target in the disease.

22.Metabolomic Profiling of Aging Human Choroid

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Age-related macular degeneration (AMD) is a leading cause of vision loss in older adults and has both environmental and genetic components. While previous metabolomics studies used plasma or serum to identify potential biomarkers of AMD, it is unknown how these findings correlate to the metabolomic state of the eye. In this study, we performed metabolomic analysis of snap-frozen choroid tissue from human donor eyes.

First, peripheral choroid and macular choroid were compared to determine the suitability of peripheral choroid as a proxy for macular choroid. Liquid chromatography mass-spectrometry analysis for a panel of 230 metabolites was conducted by the University of Iowa Metabolomics Core. Peripheral and macular choroid showed a difference in 13 out of 230 metabolites with $p<0.05$; no metabolites were significant with Bonferroni correction. We then analyzed tissue from the posterior supranasal quadrant of 87 donors (88 eyes) covering young (n=8, 21-42y), aged control (n=38, 70-97y), early/intermediate AMD (n=21, 71-96y), geographic atrophy (n=7, 76-97y), and macular neovascularization (n=14, 70-97y). This cohort was also genotyped for a common CFH polymorphism (Y402H) using SNP microarrays. Comparisons were made between male and female, young and control, AMD and control, and between CFH genotypes. Eyes from aged controls showed significantly more quinic acid, uric acid, and trimethylamine N-oxide than young controls (corrected $p<0.05$). When comparing AMD to control eyes, and in all other comparisons, no significant differences were found.

This study provides insight into the metabolome of the choroid in young and aged donors, as well as in various states of AMD progression. Further directions would include investigation into the role of specific metabolites that were found to be different between groups of interest.

23. Mechanisms reinforcing the midfacial neural crest positional identity

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Cranial neural crest cells display epigenetic plasticity, allowing them to respond to local signals and form an organized facial skeleton during embryonic development. Yet, what remains unclear are factors reinforcing their positional identities. Here, we show that TFAP2 transcription factors are a key candidate in this process in midfacial neural crest cells shaping the forehead, nose, and cheeks. Concerted inactivation of *Tfap2a* and *Tfap2b* in neural crest cells at either early or late stages results in overlapping severe midfacial cleft and skeletal abnormalities typifying frontonasal dysplasia. Single-cell transcriptomics revealed compromised expression of numerous components of the midface positional program, most notably ALX transcription factor genes *Alx1*, *Alx3*, and *Alx4*. CUT&RUN and ATAC-seq analyses suggest that TFAP2A and TFAP2B directly occupy regulatory elements associated with ALX gene loci to influence their activity and promote gene expression. Reducing *Alx3* dosage in TFAP2 mutant backgrounds exacerbated midfacial phenotypes without altering those in the jaw, demonstrating positional specificity of this regulatory node. Our findings identify TFAP2 transcription factors as key regulators reinforcing the midfacial neural crest identity and provide a framework for dissecting mechanisms controlling cranial neural crest positional identities.

24. Identifying Principles of Tissue Partitioning During Mouse Mandibular Epithelial Patterning

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A principle of embryonic development is the sharp partitioning of a homogenous tissue into defined domains. Domain precision is often achieved through the intersection, competition, and resolution of opposing gene regulatory networks, conferring differential cell-fate decisions. However, a mechanistic understanding of how this is achieved is lacking. We recently identified a functional opposing network establishing the oral-aboral axis of the mandibular epithelium. While initially diffuse and overlapping, we identified that the transcription factors (TFs) SOX2 and PITX (PITX1/2) form a complementary expression domain with the TF TFAP2 (TFAP2A and TFAP2B) along the oral-aboral axis, respectively. Highlighting the functional significance of these opposing networks, loss of oral TFs led to the expansion of aboral programs orally. Conversely, loss of aboral TFs led to the expansion of oral programs aborally—culminating in the production of oral structures (teeth) in the otherwise skin-fated epithelium. Ongoing work includes using mouse genetics to identify how these opposing networks are initially established, CUT&RUN assays to assess how they influence each other's genome-wide binding, and protein-protein interaction studies to determine whether competition is mediated through direct binding. Our model provides a tractable genetic system to mechanistically dissect principles of tissue portioning.

25. Identifying mechanisms to target in combination with trametinib to improve therapeutic efficacy in low-grade serous ovarian cancer

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Background

Low-grade serous ovarian cancer (LGSOC) is a unique subtype with distinct molecular and clinical characteristics. Although standard chemotherapy is ineffective (~4% response rate), the presence of frequent genetic alterations in the MAPK pathway have led to targeted therapeutic clinical trials, including with the MEK inhibitor trametinib. Unfortunately, after initial response, patients develop progressive disease, with no remaining treatment options. Here, we sought to characterize biological responses of LGSOC to trametinib and molecular mechanisms underlying resistance that may be co-targeted to improve therapeutic efficacy and outcomes for LGSOC patients.

Results

LGSOC cell lines initially showed high sensitivity to trametinib, followed by gradual development of spontaneous resistance. Genetic screening and molecular profiling identified several candidate genes and pathways (including FAK, MAPK, and PI3K-AKT) as being involved in therapeutic resistance. Western blotting confirmed upregulation of PI3K-AKT signaling after trametinib treatment. Subsequent experiments demonstrated a synergistic effect between trametinib and capivasertib, an FDA-approved AKT inhibitor.

Methods

Responses to trametinib were assessed in a panel of LGSOC cell lines using cell viability assays and crystal violet staining. A kinome-targeted CRISPR screen was conducted to identify specific protein kinases whose loss induced synthetic lethality in the context of trametinib treatment. Western blot analyses and multi-drug assays were performed to validate pathways implicated in trametinib response and identify synergistic interactions with other FDA-approved inhibitors.

Conclusions

Targeting AKT in combination with MEK enhanced therapeutic efficacy in a panel of LGSOC cell lines. A similar approach could be used clinically to improve outcomes for patients with LGSOC.

26. Regulation and dynamics of intercalated disc transcriptomes

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Intercalated discs (ICDs) are complex and essential structures in the heart. ICDs dysregulation is implicated in heart diseases. ICDs also likely serve as hubs for local protein synthesis in CMs. Studies have found enhanced local translation at ICDs, but only a few ICD-enriched mRNAs are known (e.g., Dsp).

Despite evidence for ICD-localized RNAs and local translation in CMs, no transcriptome-wide unbiased approaches have profiled RNAs at ICDs. To address this, we developed "ICD-seq," to enable high-throughput laser capture microdissection of immunostained ICDs from fresh-frozen cardiac tissue sections. We captured >20,000 ICDs from mouse heart tissues, isolated RNAs, and performed RNA-seq. We identified hundreds of unique mRNAs significantly enriched at the ICD, with enriched mRNAs harboring sequence motifs known to bind specific RBPs implicated in heart disease. Furthermore, **the ICD-localized transcripts are enriched for mRNAs trafficked to cell leading edges in an APC/Kif1c-dependent fashion**. Pilot studies applying ICD-seq to nonfailing human heart tissues revealed strong correlation to mouse data, supporting conserved processes and functional importance. **Our overarching hypothesis** is that select subsets of functionally related mRNAs are trafficked to, anchored, and translated at the ICD via conserved mechanisms dependent on microtubules, molecular motors, and RBPs, and that these processes are perturbed during cardiac stress, contributing to downstream detriments in heart function. To address this, we propose the following aims:

Aim 1: Assess how ICD RNA profiles "re-wire" in cardiac stress and disease. We will define ICD transcriptomes in humans and mice across different conditions of cardiac stress and disease to understand the translational significance of ICD RNA regulation. Bioinformatics will identify new candidate RNA motifs and RBPs regulating ICD RNAs during cardiac stress.

Aim 2: Assess how inducible CM-specific loss of Apc or Kif1c in mice alters mRNA localization to ICDs and cardiac structure and function. We will induce CM-specific knockout (KO) of Apc and Kif1c, perform ICD-seq on heart samples, and verify if "top hit" candidate mRNAs show de-enrichment from ICDs after KO. We will also determine the effects of inducible CM-specific Apc or Kif1c KO in adult mice.

Impact: This work will yield key resources and insights into local RNA regulation in the heart.

27. N-Acetylcysteine ameliorates loss of the electroretinogram b-wave in a Bardet-Biedl Syndrome Type 10 mouse model

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In this study, a mouse model of *Bbs10*$^{-/-}$ was orally treated with N-Acetylcysteine (NAC). Mice were provided with water bottles containing water only or NAC dissolved in water at a concentration of 7 mg/mL and pH of 4 for 4 months. Outer nuclear layer (ONL) thickness was measured monthly and compared to *Bbs10*$^{+/-}$ controls. Electroretinogram (ERG) were conducted monthly in dark and light adapted conditions. Finally, immunohistochemistry was performed to observe synaptic localization within the retina. Results obtained from 9 *Bbs10*$^{-/-}$ mice on NAC, 9 *Bbs10*$^{-/-}$ on plain water, 9 *Bbs10*$^{+/-}$ mice on NAC, 6 *Bbs10*$^{+/-}$mice on plain water.

NAC supplementation significantly (p = 0.0002; bonferoni one-way ANOVA) ameliorates the progressive degeneration of the retinal ONL on OCT in Bbs10-/- mice. There is no statistical difference between water and NAC a-waves. The b-wave amplitudes are statistically different between the NAC and water treated mice, with NAC treated mice displaying higher b-wave amplitudes compared to water (Welch's t-test, p = 0.0029). The a- and b-wave are proportionally correlated to each other, meaning that if the a-wave decreases a proportional decrease in the b-wave is expected. However, we show that *Bbs10*$^{-/-}$ a- and b-waves are not proportionally correlated at four months old. But, when mice are treated with NAC, we show there is a significant recovery of proportionality using a simple regression model (p = 0.0036). Finally, it was noted that in immunohistochemistry in the *Bbs10*$^{-/-}$ mice, there was a retraction of synapses into the ONL, indicating mislocalized synaptic terminals. Quantification revealed a statistically greater number of synapses in the ONL of the nob phenotype compared to the b-wave (p = 0.0492; ordinary one-way ANOVA).

These findings suggest NAC as a promising therapeutic intervention for managing BBS10-related retinal degeneration by mitigating oxidative stress and supporting retinal synaptic function.

28. THE THERAPEUTIC POTENTIAL OF FGF21 IN ALZHEIMER'S DISEASE

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Alzheimer's Disease (AD) is a progressive neurodegenerative disorder characterized by neuronal death and impairments in memory, language, and spatial navigation. While the pathophysiology and progression of AD has been linked to brain accumulation of amyloid beta (Aβ) and neurofibrillary tangles (NFTs), recent studies postulate that Alzheimer's disease might be a manifestation of metabolic disorders. Hyperglycemia and obesity have been associated with dementia and cognitive decline, and many AD patients show impairments in glucose tolerance or present diabetes.

Fibroblast growth factor 21 (FGF21) is an endocrine hormone primarily liver derived hormone that signals to tissues expressing the traditional FGF receptor (FGFR1) and a co-receptor known as β-klotho (KLB). FGF21 has an effect in several physiological processes, including the enhancement of insulin sensitivity and regulation of glucose homeostasis, all mediated through actions on the central nervous system (CNS). Likewise, FGF21 analogs that are currently used for the treatment of obesity, diabetes, and Metabolic dysfunction-associated steatohepatitis (MASH) could be repurposed for the treatment of AD.

Most therapeutics for AD have focused on targeting Aβ with variable success rates between patients. A consistent symptom across AD patients is disrupted brain energetic metabolism, suggesting a prospective area of study for therapeutic targets to treat this disease. To date, very little is known about the potential of FGF21 in treating Alzheimer's disease. Some studies have suggested that FGF21 may prevent neurodegeneration and pathological deficits in animal models of Alzheimer's disease through mechanisms influencing cell death, Aβ neurotoxicity and oxidative stress. Considering this, we hypothesize that FGF21 signals through the CNS, preventing neurodegeneration and rescuing neuronal plasticity to enhance memory and cognitive functions through affecting the hippocampus. Consequently, we aim to explore the utility of FGF21 to treat cognitive decline associated with AD and related dementias.

29. CHARACTERIZATION OF FUNCTIONALITY OF CANDIDATE INTERACTORS IN THE *PDR1* AZOLE RESISTANCE PATHWAY OF *CANDIDA GLABRATA*.

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Candida glabrata (Cg) is an opportunistic yeast which can cause infections in immunocompromised patients. These infections are often treated with azole drugs. Since azole drugs target the ergosterol biosynthesis pathway, specifically inhibiting an enzyme encoded by the *ERG11* gene, an inevitable consequence of the widespread use of azole drugs has been the development of resistant forms of Cg. These resistant organisms are often found to have a hyperactive form of a transcription factor called Pdr1. Pdr1 stimulates the expression of a membrane transporter protein called Cdr1 that is thought to act as a drug pump, exporting azole drugs out of the cell. Using biochemical techniques, we have identified a number of proteins which interact with Pdr1. We are determining if these co-purifying proteins act as regulators of these key transcription factors. A Pdr1-interacting protein called Spt5 has been the focus of recent studies. *SPT5* is known to be an essential gene in other organisms, responsible for RNA polymerase II elongation in transcription. To analyze the functional role of Spt5 in azole resistance and Pdr1-dependent gene transcription, we have developed an auxin-inducible form of Spt5. This strain allows for acute depletion of Spt5 from the cell upon addition of the plant hormone auxin, leading to a significant growth defect in the presence of auxin. We will use this degradable form of Spt5 to examine its effect on the transcription of *PDR1, CDR1*, and *ERG11*. When Spt5 is depleted, cells grown in the presence of fluconazole demonstrate reduced induction of *PDR1* and *CDR1*. Additionally, Spt5 recruitment to *PDR1* and *CDR1* is increased in the presence of fluconazole. This research has demonstrated that Spt5 is involved in the response to azole stress. To confirm that Spt5 is specific to the Pdr1/*CDR1* drug efflux system, we will determine how depletion of Spt5 impacts strains containing loss-of-function and hyperactive *PDR1* alleles.

30.The Influence of X Chromosome Parent-of-Origin on Glycemia in Individuals with Turner syndrome

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Turner Syndrome (TS) consists of a spectrum of karyotypic disorders where one of the sex chromosome is partially or completely absent. These TS patients exhibit higher rates of Diabetes Mellitus (DM) than the general population, the cause for which is poorly understood. Several TS phenotypes have been linked to parent-of-origin effects based on whether the maternal or paternal X chromosome remains intact. Thus, the increased rate of DM may be related to Xchr parent-of-origin.

The aim of this study was to determine the impact of Xchr parent-of-origin on glycemia in TS. A total of 81 individuals with 45,X karyotype from the TS: Genotype Phenotype study had Xchr parent-of-origin assessment and completed a 3-hour oral glucose tolerance test. Parallel-slopes multiple linear regression modeling was used to test whether Xchr parent-of-origin, age, and/or body mass index (BMI) significantly predicted incremental area under the glucose curve (iAUC). A second analysis included 62 additional individuals with 45,X mosaicism.

All three factors predicted iAUC in the 45,X cohort with statistical significance. Maternal Xchr monosomy was found to be associated with higher blood glucose during the test as compared to paternal Xchr monosomy. When including the additional group of individuals with 45,X mosaicism, Xchr parent-of-origin was no longer found to be significant while the overall model remained significant.

This data shows the presence of a parent-of-origin effect on blood glucose in TS. These findings suggest the possibility of imprinted genes on the X chromosome that contribute to the DM phenotype and could be relevant in both future TS studies as well as broader DM research.


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