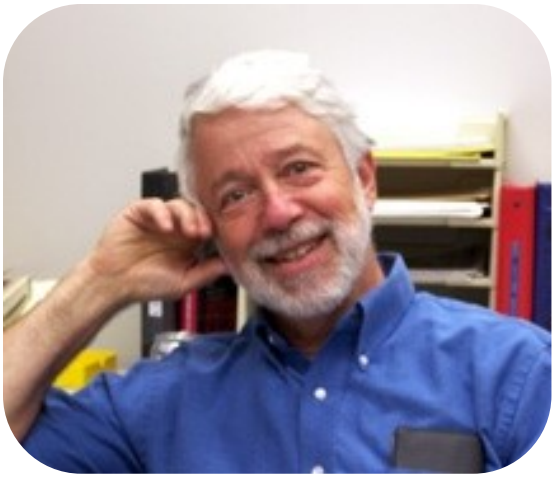
IOWA | Interdisciplinary Graduate Program in Genetics

2023 Student Retreat

"The DNA damageome and cancer, and drugging evolution of antibiotic resistance"

September 22–23
University of Iowa
Art Building West

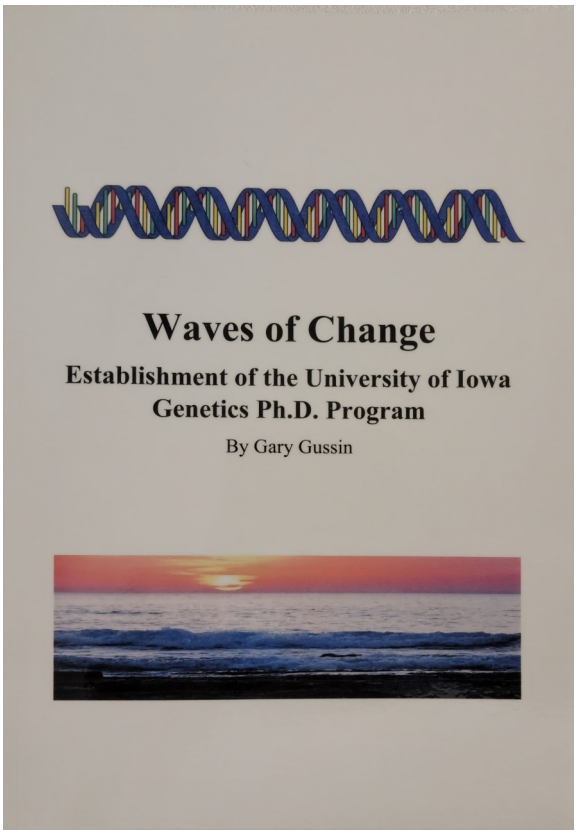


Dr. Gary Gussin
Professor Emeritus
Department of Biology

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.



IOWA | Interdisciplinary Graduate Program in Genetics

STUDENT RETREAT

Keynote Speaker

Susan Rosenberg Ph.D.

Professor of Molecular and Human Genetics

Ben F. Love Chair in Cancer Research

Baylor College of Medicine

Alumni Speakers

Tanner Koomar, Ph.D. (2021)

Data Scientist

Recursion Pharmaceuticals

Timothy Parnell, Ph.D. (2000)

Associate Director
Cancer Bioinformatics Shared Resource

Huntsman Cancer Institute
University of Utah

Friday, September 22

Public Keynote Talk—Susan Rosenberg, PhD
Biology Building East

3:30 Light refreshments available
4:00 *"Why cancers (and infections) beat us and how to turn the tables"*

Saturday, September 23

Genetics Program Events

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

8:30 **Opening Remarks**
Dr. Anna Malkova.—Retreat Committee Chair

8:40 **Student oral presentations—Session 1**
- Lucas Casten (Michaelson, Psychiatry)
- Bekah Peplinski (Dupuy, Anatomy & Cell Biology)
- Lucas Pietan (Casavant, Biomedical Engineering)

9:45 **Poster session 1**—odd numbered posters
(Refreshments available)

10:45 **Alumnus Speaker** (Remarks :Jake Michaelson, PhD)
Tanner Koomar Ph.D.
TechBio|BioTech: Trekking into a Rapidly Evolving Industry"

11:20 **Alumnus Speaker** (Remarks: Pamela Geyer, PhD)
Timothy Parnell
"From bench to bioinformatics"

12:00 **Director remarks**

12:05 **Lunch**

1:00 **Student oral presentations—Session 2**
- Anna Carver (Stevens, Psychiatry)
- Nathan Mohar (Wallrath, Biochemistry& Microbiology)
- Lindsey Snyder (He, Biology)

2:00 **Poster session 2**—even numbered posters
(Refreshments available)

3:15 **Keynote Speaker**
Susan Rosenberg Ph.D.
"The DNA damageome and cancer, and drugging evolution of antibiotic resistance "

4:30 **Closing Remarks & Award Ceremony**
Dr. Anna Malkova.—Retreat Committee Chair

IOWA | Interdisciplinary Graduate Program in Genetics

Table of Contents

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.
Professor, Department of Biochemistry
and Molecular Biology
Director, Genetics Ph.D. Program

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

Rob DuBay
Program Administrator
Genetics Ph.D. Program

Abby Dalton
Program Associate
Genetics Ph.D. Program

Genetics Retreat Chair, Anna Malkova, Ph.D.
Professor, Biology

Dr. Malkova is a Roy J. Carver Professor in the Department of Biology at the University of Iowa. Dr. Malkova graduated from St. Petersburg State University (Russia), where she obtained her Ph.D in Genetics. She received her postdoctoral training in Molecular Genetics at Brandeis University in the laboratory of Dr. James Haber. Before joining the University of Iowa in 2014, Dr. Malkova was an Associate Professor in the Department of Biology at the Indiana University Purdue University Indianapolis. Dr. Malkova's research is aimed to unravel mechanisms of DNA repair. In particular, she is interested in repair of double-strand DNA breaks (DSBs), which are dangerous because their imprecise or faulty repair often leads to mutations and chromosome aberrations that cause genetic diseases and cancer. Her research focuses on one pathway of DSB repair called Break-Induced Replication (BIR), which she discovered during her postdoctoral research at Brandeis University. Dr. Malkova has trained many Ph.D students and authored more than 50 manuscripts published in high-impact journals including *Nature, Cell, Molecular Cell and Nature Communications*.

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 Student Committee Members

Tim Nguyen
Van Otterloo Lab
Periodontics

Baylee Bruce
He Lab
Biology

Susan Rosenberg, Ph.D.

Ben F Love Chair in Cancer Research
Professor, Departments of Molecular & Human Genetics, Biochemistry, and Molecular Virology & Microbiology
Founder, Mechanisms in Cancer Evolution Program
Co-leader, Chromatin Biology Program, Dan L Duncan Comprehensive Cancer Center

"The DNA damageome and cancer, and drugging evolution of antibiotic resistance"

Dr. Rosenberg received a PhD in molecular biology at the University of Oregon working with Franklin Stahl, and did postdoctoral work with Miroslav Radman (Paris). Her lab studies molecular mechanisms that promote genome stability and instability, and their impacts on evolution, infectious disease, and cancer. Her lab originated technologies for studying DNA damage in living bacterial and human cells, and discovered mechanisms of mutation formation activated by stress responses, which potentially accelerate evolution when cells are poorly adapted to their environments, and are now evident from bacteria to cancer. Rosenberg founded the Gordon Research Conference on Molecular Mechanisms in Evolution, and founded and leads the Mechanisms in Cancer Evolution Program in the Dan L Duncan Comprehensive Cancer Center. She has received two NIH Director's Pioneer Awards, a WM Keck Foundation Award, the Biosphere and Humanity Medal (Russian Academy of Medicine), the Eli Lily / National Cancer Institute of Canada William Rawls Prize, the Young Scientist Award of the Genetics Society of Canada, and two Michael E DeBakey MD Awards for Excellence in Research. Dr. Rosenberg is a fellow (elected) of the American Association for the Advancement of Science, a member of the American Academy of Microbiology, served on the Senior Editorial Board of *SCIENCE*, and in various roles for the American Association for the Advancement of Science, currently Board of Directors (elected).

Alumnus Speaker: Timothy Parnell

Alumni 2000
Timothy Parnell

Associate Director, Cancer Bioinformatics Shared Resources
Huntsman Cancer Institute
University of Utah

"From bench to bioinformatics"

Tim was the third graduate student in Dr. Pamela Geyer's lab working on a thesis investigating Su(Hw) insulators and higher order chromatin structures. He defended in December 2000, but continued in the lab while his spouse, Emily, finished her PhD in the Geyer lab in 2002. They moved to the University of Utah in Salt Lake City in 2004, where Tim joined the lab of HHMI investigator Bradley Cairns at the Huntsman Cancer Institute as a post-doc studying chromatin remodeling in yeast, first through the application of microarrays and then next generation sequencing. This required the development of novel software development and bioinformatics skills. Tim joined the Bioinformatics Shared Resource core facility at HCI in 2013, and became the Associate Director in 2017. Tim resides in Salt Lake City with Emily and their two children, ages 16 and 13.

Alumni 2021
Tanner Koomar, Ph.D.

Data Scientist
Recursion Pharmaceuticals

"TechBio|BioTech:
Trekking into a Rapidly Evolving Industry"

Tanner Koomar received his PhD from the University of Iowa in 2021 in the lab of Dr. Jacob Michaelson. He specialized in applying machine learning to the genetic etiology of psychiatric and developmental conditions, particularly autism. His work focused on unifying rare and common genetic burden, and also applied systems biology approaches to understanding common traits, particularly language ability in adolescents. This research was built on a foundation of whole genome sequencing and detailed linguistic assessment collected over a decade. He also developed and piloted a computerized rapid language screener which could collect the large sample sizes needed to power common-variant studies.

Tanner is a data scientist at Recursion Pharmaceuticals, which utilizes deep-learning to map the relationships within and between the full human genome and millions of compounds via transcriptomic and phonemic read-outs. He initially performed analytics supporting Recursion's high throughput screening platform to track and optimize experimental execution and automation. In 2022, he joined a team building tools that enable bench scientists to rapidly plan, execute and analyze experiments to onboard new cell lines and genetic perturbation modalities. His work places an emphasis on large-scale data visualization. This is critical for the high throughput lab, which is capable of screening over a million compounds or genetic perturbations each week.

Student oral presentations—Session 1

Lucas Casten

The genetic architecture of modern human migration

Bekah Peplinski

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

Lucas Pietan

Prioritization of fluorescence in situ hybridization (fish) probes for differentiating primary sites of neuroendocrine tumors with machine learning

Student oral presentations—Session 2

Anna Carver

Overexpression of placental Igf-1 alters placental transport and leads to proliferation changes in the ventral forebrain that enlarges the striatum

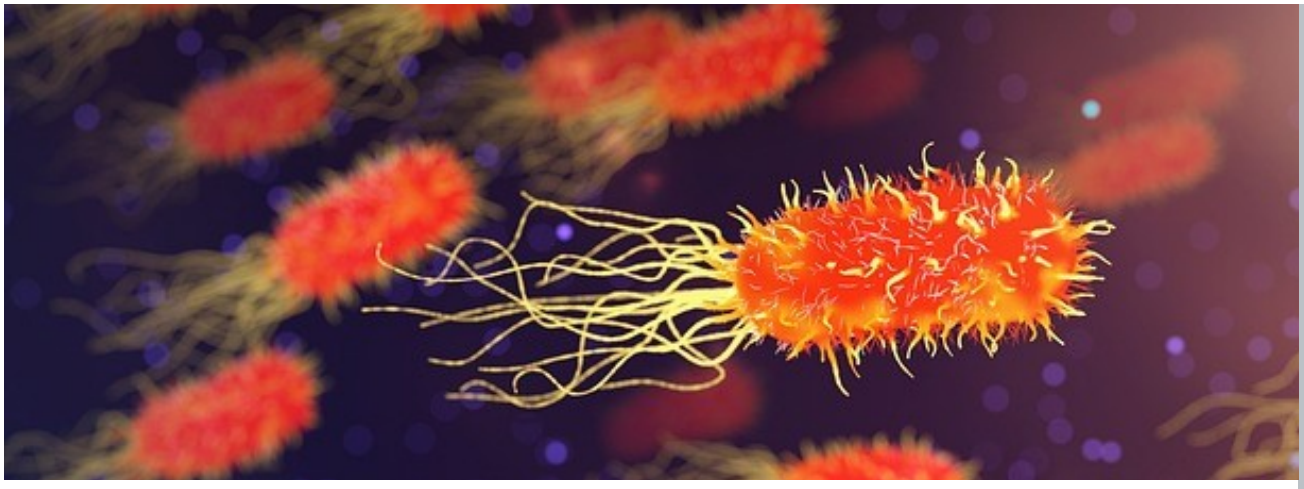
Nathan Mohar

DNA sequence variation in SMAD7 enhances LMNA-associated skeletal muscle disease severity and implicates TGFβ signaling as a therapeutic target

Lindsey Snyder

Why do transcription factors work collaboratively?

Abstract Number	Presenter	Lab	Department	Page Number
1	Lucas Casten	Michaelson	Psychiatry	19
2	Bekah Peplinski	Dupuy	Anatomy & Cell Biology)	20
3	Lucas Pietan	Casavant	Biomedical Engineering	21
4	Anna Carver	Stevens	Psychiatry	22
5	Nathan Mohar	Wallrath	Biochemistry	23
6	Lindsey Snyder	He	Biology	24



Abstract Number	Presenter	Lab	Department	Page Number
7	Emily Adelizzi	Martine Dunnwald	Anatomy & Cell Biology	20
8	Chloe Beck	Ben Darbro	Pediatrics	21
9	Hunter Brown	Tina Tootle	Biology	22
10	Baylee Bruce	Bin He	Biology	22
11	Muhammad Elsadany	Jake Michaelson	Psychiatry	23
12	Floyd Evans	Todd Scheetz	Ophthalmology	24
13	Emily Fontenoy	Andy Frank	Anatomy & Cell Biology	25
14	Anne Gleason	Jake Michaelson	Psychiatry	26
15	Matthew Koenig	Daniel Summers	Biology	26
16	Athena Kvamme	Chi-Lien Cheng	Biology	27
17	Lola Lozano	Budd Tucker & Robert Mullins	Ophthalmology	27
18	Swathi Mamillapalli	Ben Darbro	Pediatrics	28
19	Jordan Mayberry	Markus Kuehn	Ophthalmology	29
20	Sam Mellentine	Tina Tootle	Anatomy & Cell Biology	30
21	Marcelo Miranda Melo	Hatem El-Shanti	Pediatrics	31

Abstract Number	Presenter	Lab	Department	Page Number
22	Matt Miller	Chris Adams	Internal Medicine	31
23	Amanda Moy	Maurine Neiman & John Logsdon	Biology	32
24	Nate Mullin	Rob Mullins	Ophthalmology	32
25	Emma Navratil	Rob Mullins	Ophthalmology	33
26	Tim Nguyen	Eric Van Otterloo	Periodontics	34
27	Joseph Oberlitner	Sarit Smolikove	Biology	35
28	Omar Rabab'h	Ryan Boudreau	Internal Medicine	36
29	Tyler Rankin	Arlene Drack	Ophthalmology	36
30	Nikki Recka	Eric Van Otterloo	Periodontics	37
31	Maria Alejandra Rodriguez Herrera	Matt Potthoff	Neuroscience & Pharmacology	38
32	Sammy Santiago Valle	Alex Bassuk	Neurology	39
33	Lucretia Shumate	Scott Moye-Rowley	Molecular Physiology & Biophysics	40
34	Krislen Tison	Aislinn Williams	Psychiatry	41
35	Maria Fernanda Valdes Michel	Bryan Phillips	Biology	42
36	Yann Vanrobaeys	Ted Abel	Neuroscience & Pharmacology	43
37	Hayley Vaughn	Ben Darbro	Pediatrics	44
38	Blake Zimmerman	Cat Pinarro	Pediatrics	45

1. Genome-wide association study of migration in modern humans shows significant genetic overlap with education, risk-taking, health, and psychiatric disorders

Lucas G. Casten[1,2], Jacob J. Michaelson[2]

[1] Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, IA
[2] Department of Psychiatry, Carver College of Medicine, University of Iowa, Iowa City, IA

Human migration is a multifaceted phenomenon that impacts genetics, health, and society. Despite the profound impact of migration, little is known about how genetic variation may influence this behavior in contemporary humans. To characterize the effects of common genetic variants on modern migratory behavior, we conducted a Genome Wide Association Study (GWAS) on distance moved from birthplace in a large population sample of unrelated adults from the United Kingdom (UK Biobank, N = 282,178). Phenotypic analyses of this trait showed significant positive correlations with years of education and brain volume as well as a negative correlation with the number of medications being taken. Both twin based and molecular based methods provided significant heritability estimates (twin H^2 = 34.4%, unrelated sample SNP h^2 = 3.8%, suggesting the trait is amenable to genetic studies. The GWAS identified 27 independent significantly associated loci across the genome (p < 5e-08), with the strongest association coming from *rs40504*. Several genome-wide significant variants mapped to the *MEF2C* gene, which has been previously implicated in neurodevelopmental disorders.
Downstream analyses of the GWAS results showed significant genetic overlap with educational attainment, total brain size, religiosity, risk taking, bipolar disorder, autism, and health related traits. Finally, we validated these GWAS results in a held out sample of unrelated UK Biobank participants and found a polygenic propensity score using weights from our discovery GWAS was significantly predictive of actual migration distance (Pearson's R = 0.24, p < 2e-16, N = 1,156). Together, these results suggest that distance migrated from birthplace in modern humans is at least partially attributable to genetic factors. The genetic variants associated with further migration distance from birthplace have been linked with higher education levels and slightly increased risk for specific psychiatric disorders like bipolar disorder.

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

Rebekah M. Peplinski[1,2], Silvana Pedra Nobre[3], Jacob L. Schillo[1,2], Keely K. Ulmer[3], Yasmin A. Lyons[3], Jesse D. Riordan[2], Michael J. Goodheart[3], Adam J. Dupuy[1,2]

[1]Interdisiplinary Graduate Program in Genetics; University of Iowa, Iowa City, IA
[2]Department of Anatomy and Cellular Biology; University of Iowa, Iowa City, IA
[3]Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Iowa Hospitals and Clinics, Iowa City, IA

Low-grade serous ovarian cancer (LGSOC) has recently been characterized as a unique subtype of ovarian cancer with distinct molecular characteristics, clinical treatments, and therapeutic responses. Although LGSOC accounts for ~5% of ovarian cancers, the age of onset is younger (~45 years) than other subtypes and has a low response rate (~4%) to standard chemotherapy. One defining characteristic of LGSOC is frequent genetic alterations in the MAPK pathway, which has led to recent MAPK-targeted therapeutic clinical trials. MEK inhibition, particularly with trametinib, has been shown to be effective in patients with recurrent LGSOC. However, these patients eventually develop therapeutic resistance and progressive disease, with no remaining treatment options. Here we sought to characterize the biology of LGSOC and its response to trametinib in an effort to identify candidate mechanisms that may be targeted in combination with trametinib to improve therapeutic efficacy and outcomes for LGSOC patients.

After characterizing trametinib responses and identifying cytotoxic doses for a panel of LGSOC cell lines, we performed RNA sequencing on each cell line under basal conditions, and after 24-hour or 7-day exposure to an IC_{95} dose of trametinib. These experiments identified both acute and longer-term transcriptional changes that occur in response to trametinib. CRISPR screens targeting the kinome were also performed to identify synthetic lethal interactions between trametinib treatment and loss of function of specific protein kinases. Integrated analysis of these approaches identified several potential candidates (AKT, mTOR, CDKs, among others) that may be targeted in combination with trametinib to improve LGSOC patient outcomes. Experiments testing synergistic relationships between trametinib and other FDA approved inhibitors were performed. Using these combination therapeutic approaches could re-sensitize cells to trametinib treatment or prevent the emergence of resistance, thereby improving therapeutic efficacy. If successful, a similar approach could be used in a clinical trial to treat patients with LGSOC.

3. Prioritization of fluorescence in situ hybridization (fish) probes for differentiating primary sites of neuroendocrine tumors with machine learning

L Pietan[1,2], H Vaughn[1,3], J Howe[4], A Bellizzi[5], B Darbro[1,3], T Braun[1,2,6], T Casavant[1,2,6,7]

[1]Interdisciplinary Graduate Program in Genetics, University of Iowa
[2]Department of Biomedical Engineering, University of Iowa
[3]Stead Family Department of Pediatrics, University of Iowa
[4]Healthcare Department of Surgery, University of Iowa
[5]Department of Pathology, University of Iowa
[6]Center for Bioinformatics and Computational Biology, University of Iowa
[7]Department of Electrical and Computer Engineering, University of Iowa

Determining neuroendocrine tumor (NET) primary sites is pivotal for patient care. Among the approximate 170,000 NET cases in the United States, pancreatic NETs (pNETs) account for 17-20%, while small bowel NETs (sbNETs) constitute 50-55%, each demanding distinct treatment approaches. Many clinical tests can be performed to help identify the primary sites of NETs, including blood tests, imaging, and histological assessments with immunohistochemistry and fluorescence in situ hybridization (FISH) tests. The diagnostic power and prioritization of FISH assay biomarkers for establishing the primary site has not been thoroughly investigated using machine learning (ML) techniques. We trained several ML models on FISH assay metrics from 85 sbNET and 59 pNET samples for primary site prediction. Exploring multiple methods for imputing missing data, the impute-by-median dataset coupled with a support vector machine using a radial basis function (SVM-RBF) model achieved the highest classification accuracy of 93.1% on a held-out test set and 85.4% on the initial training set, with the top importance variables originating from the *ERBB2* FISH probe. Due to the greater interpretability of decision tree (DT) models, we fit DT models to ten dataset splits, achieving optimal performance with k-nearest neighbors (KNN) imputed data and a transformation to single categorical biomarker probe variables, with mean accuracy of 81.4% on the held-out test sets and 75.9% on the training sets. *ERBB2* and *MET* variables ranked as top performing features in 9 of 10 DT models and in the full dataset model. The full dataset DT model utilizes the *ERBB2, CDKN2A,* and *SMAD4* variables as splits with 3 of the 4 terminal nodes having predictive probabilities greater than 0.796. These findings offer probabilistic guidance for FISH testing, emphasizing the prioritization of the *ERBB2* FISH probe in diagnosing NET primary sites.

4. Overexpression of Placental *Igf-1* Alters Placental Transport and leads to Proliferation Changes in the Ventral Forebrain that Enlarges the Striatum

Annemarie Carver[1,2,3], Robert Taylor[2,3], Faith Fairbairn[2,3], Shanmukh Boggarapu[2,3,4], Sreelekha Kundu[2,3], and Hanna Stevens[1,2,3,5]

[1] Interdisciplinary Graduate Program in Genetics, University of Iowa, Iowa City, IA
[2] Department of Psychiatry, Carver College of Medicine, University of Iowa, Iowa City, IA
[3] Iowa Neuroscience Institute, Carver College of Medicine, University of Iowa, Iowa City, IA
[4]Department of Psychology, Saint Louis University, Saint Louis, MO
[5] Hawk-IDDRC, University of Iowa, Iowa City, IA

Changes to placental delivery of nutrients and hormones can alter fetal neurodevelopment. Our lab previously identified that prenatal stress, an established risk factor for neurodevelopmental disorders (NDDs), increases placental *Insulin-like growth factor 1* (*Igf-1*) expression. *Igf-1* is an essential hormone for neurodevelopment that is placentally produced prior to birth. The striatum, a brain region enlarged in some with NDDs, has high levels of the Igf-1 receptor. We hypothesized that placental *Igf-1* overexpression would lead to accelerated growth of the striatum, mimicking NDD phenotypes. To test our hypothesis, we induced placental overexpression of *Igf-1* via placental-targeted CRISPR manipulation on embryonic day 12 (E12). Males and females responded differently to this manipulation. At E14 and E18, female placentas displayed placental *Igf-1* overexpression, but males experienced lesser effects, potentially through a ceiling effect limiting overexpression. However, E14 overexpression male fetal bodies showed an increase in Igf-1 protein, suggesting an initial placental overexpression occurred that was later downregulated. Male overexpression placentas also showed downregulation of Igfbps and amino acid transporters. Conversely, female overexpression placentas upregulated amino acid transporters. Unsurprisingly, overexpression females showed increased body mass and placental efficiency at E14 while males did not. Interestingly, placental *Igf-1* overexpression did not increase proliferating cells in E14 ventral forebrain, a structure that gives rise to the striatum, but paradoxically decreased Ki67 while increasing S phase arrest in females only. Igf-1 is known to promote the transition from G1 to S phase, and its overexpression likely resulted in mistiming of carefully regulated embryonic brain development and altered cell cycle timing to prevent rapid maturation. At E18 we identified an increase in striatal volume in overexpression samples. In sum, placental *Igf-1* overexpression alters placental nutrient transport in a sex specific manner that may contribute to changes in fetal brain proliferation and morphogenesis that may be relevant to NDDs.

Funding: R01 MH122435, NIH T32GM008629, & NIH T32GM145441

5. Dna sequence variation in *smad7* enhances *lmna*-associated muscular dystrophy in drosophila models

NP Mohar[1,2], CJ Langland[2], BW Darbro[1,3], and LL Wallrath[1,2]
[1]Interdisciplinary Graduate Program in Genetics, University of Iowa
[2]Department of Biochemistry and Molecular Biology, Carver College of Medicine, University of Iowa
[3]Department of Pediatrics, Carver College of Medicine, University of Iowa

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 TGFβ/SMAD signaling pathway, that segregates with severe disease. To functionally test the *SMAD7* variant, we generated a Drosophila model containing both the *LMNA* mutation and the *SMAD7* variant. We demonstrate that the *SMAD7* variant has minimal effects on muscle function alone. However, the *SMAD7* variant enhances muscle defects caused by the *LMNA* mutation, consistent with a genetic modifier. In addition, the *SMAD7* variant increases TGFβ/SMAD signaling in muscle, suggesting a mechanism for the enhancement of muscle disease severity. To broaden our analysis, we sequenced the *SMAD7* gene in a cohort of 45 *LMNA*-MD and identified six additional variants. Taken together, our findings support *SMAD7* as a modifier gene for *LMNA*-MD and identifies the TGFβ/SMAD signaling pathway as a therapeutic target.

6. Why do transcription factors work collaboratively?

L Snyder[1], E O'Brien[2], J Zhao[2], T Cassier[2], Y Zhang[5], R Gordan[5], N Schnicker[3], X Xhou[4], B He[1,2]

[1] Interdisciplinary Program in Genetics, University of Iowa, Iowa City, IA
[2] Department of Biology, University of Iowa, Iowa City, IA
[3] Protein and Crystallography Core, University of Iowa, Iowa City, IA,
[4] Yale School of Medicine, New Haven, CT
[5] Duke University, Durham, NC

A prevalent feature in eukaryotic transcription is combinatorial regulation, or the requirement of two or more transcription factors (TFs) to co-activate a gene. This allows multiple upstream signals to influence downstream gene expression and provides a way to tune specificity by requiring a defined binding configuration for activation to occur. Why do certain TFs need other TFs (co-TFs) to function? How does combinatorial regulation evolve? We take advantage of the natural variation in co-TF dependence present in the Phosphate Starvation response TF in two yeast species to study the genetic and biophysical basis for coactivator dependence. In *S. cerevisiae*, the TF Pho4 (ScPho4) relies on Pho2, whereas in *C. glabrata*, Pho4 (CgPho4) exhibits lower dependence on Pho2 and activates 2-3 times more targets. Using chimeras of ScPho4 and CgPho4 coupled with a dual fluorescence reporter, we discovered that changes in the DNA binding domain (DBD), the putative Pho2-interaction domain, and a portion of the transactivation domain collectively contribute to Pho2 independence. Biolayer Interferometry and EMSA showed that CgPho4DBD binds ~4 times tighter than ScPho4DBD to the consensus motif *in vitro*, but the two TFs have no significant differences in specificity. CgPho4's transactivation domain has a higher activation potential than ScPho4 when assayed as a Gal4DBD fusion. Lastly, we show that CgPho4 is more capable of binding nucleosome-occluded DNA *in vivo* compared with ScPho4. Our findings indicate that decreased ability to bind both naked and nucleosome-bound DNA, along with reduced activation potential, contribute to coactivator dependence, all of which can be strengthened by amino acid substitutions and insertions, resulting in reduced coactivator dependence.

7. The periderm protein zfp750 is required for murine digit fusion but is dispensable for palatogenesis

Emily Adelizzi[1]*, Sunil Singh[2]*, Kaylia Duncan[3], Caleb Heffner[4], Stephen Murray[4], Robert A. Cornell[2], Martine Dunnwald[1]

*Contributed equally

1. Department of Anatomy and Cell Biology, University of Iowa
2. School of Dentistry, University of Washington
3. Children's Hospital of Philadelphia
4. Jackson Laboratories

Orofacial clefts (OFC) are common structural birth defects with a strong genetic under pinning but most of the heritable risk has not been assigned to any gene or locus. Candidate genes that harbor the missing heritability may operate in the same gene regulatory network (GRN) as known OFC risk genes. For instance, two OFC risk genes, *IRF6* and *GRHL3*, are involved in the GRN governing epithelial differentiation and periderm development. The periderm, which is a transient, single layer of flattened cells, covers developing epithelia of the oral cavity and presumptive epidermis of the early vertebrate embryo. Like IRF6 and GRHL3, ZNF750 governs epidermal differentiation but it is unknown if it regulates periderm differentiation or palatogenesis. Here we report that in zebrafish embryos, depletion of *znf750* or *grhl3* followed by RNA sequencing indicated that Znf750 acts downstream of Grhl3 to promote expression of a subset of periderm markers. We then moved to a murine model and generated homozygous *Zfp750* frame-shift mutants to determine if they exhibit periderm-associated defects. At E18.5, *Zfp750* mutant embryos have detached pinnae, open eyes, unfused digits, and increased incidence of oral adhesions, all consistent with a defect in the periderm. However, these mutants do not show defects palatogenesis, as all embryos showed proper palatal fusion at E15.5. RNA sequencing of E15.5 embryonic limbs reveals a modest reduction of some periderm markers in *Zfp750* mutants compared to wild types. Collectively, these data suggest that *Zfp750* promotes some periderm-dependent events but is dispensable for palatogenesis. However, the presence of oral adhesions in *Zfp750* mutants supports the possibility that *ZNF750* mutations may elevate risk for OFC in combination with other mutations or environmental insults.
Funded by NIH grants R01DE023575 (RAC), R01AR066739 (MD), U54OD030187 (SM), and T32GM145441 (EA).

8. Measuring Gene Burden to Investigate Phenotypic Heterogeneity in Chromosomal Disorders

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With approximately one out of every 150 babies born having a chromosomal disorder, determining the cause of phenotypic heterogeneity in these disorders is important in predicting the scope of comorbidities and overall disease severity. Even with the rise of genomic sequencing, the number of variants of unknown significance (VUS) showcases the gap in our knowledge, diagnostic power[1], and ability to interpret DNA changes. Genetic modifiers are a confounding genetic variation that when present for a specific disease can explain phenotypic heterogeneity; measuring genetic burden on relevant functional protein-protein interaction networks might lead to a better understanding of each separate phenotype in complex genetic diseases. Several genetic burden techniques have been created, however, many of these techniques fail to consider amino acid conservation, predicted pathogenicity scores, mutation type, and population minor allele frequency. In addition to genetic modifiers, DNA methylation also impacts phenotypic heterogeneity in disease, however, it is not accounted for in standard gene burden analyses. Headway has been made in the investigation of epigenetic markers as they relate to mendelian disease with several studies suggesting that specific epigenetic signatures, or EpiSigs, can be predictive of disease[3–5]. To date, no analysis or bioinformatic tool combines the impact of genetic burden with the impact of DNA methylation to explain phenotypic heterogeneity. Our objective is to create a model of genetic burden that includes pertinent genetic information alongside epigenetic data that is weighted according to its influence on gene function. This will help explain phenotypic heterogeneity in chromosomal disorders and will also be applicable to a wide range of genetic diseases and disorders making it an invaluable tool in holistic investigation of genetic diseases for researchers. For patients, it can provide explanation for their specific disease and possible avenues for therapies in the future.

1. Eisenberger, T. *et al.* Increasing the yield in targeted next-generation sequencing by implicating CNV analysis, non-coding exons and the overall variant load: The example of retinal dystrophies. *PLoS One* **8**, (2013).
2. Pinnaro, C. T. *et al.* Candidate modifier genes for immune function in 22q11.2 deletion syndrome. *Mol Genet Genomic Med* **8**, (2020).
3. Levy, M. A. *et al.* Functional correlation of genome-wide DNA methylation profiles in genetic neurodevelopmental disorders. *Hum Mutat* (2022) doi:10.1002/humu.24446.
4. Aref-Eshghi, E. *et al.* Evaluation of DNA Methylation Episignatures for Diagnosis and Phenotype Correlations in 42 Mendelian Neurodevelopmental Disorders. *Am J Hum Genet* **106**, 356–370 (2020).
5. Levy, M. A. *et al.* Novel diagnostic DNA methylation episignatures expand and refine the epigenetic landscapes of Mendelian disorders. *Human Genetics and Genomics Advances* **3**, 100075 (2022).

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

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Collective cell migration is a critical process in 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. Most studies exploring the roles of PGs in 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 experiments knocking down the $PGF_{2\alpha}$ synthase, Akr1B, in the border cells significantly delayed migration. Using available mutant and RNAi lines, we will delineate the $PGF_{2\alpha}$ signaling pathway, including the GPCR, 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 delay migration and result in an elongated cluster morphology. We expect this 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 in the capability to access nucleosome-occluded sites between ortholgous transcription factors

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Eukaryotic transcription factors (eTFs) frequently work in combinations, which allows for increased specificity and information integration. However, the general basis of why eTFs depend on one another for their activity is not well known. We know that this dependency is important for both cooperative binding and recruitment of the PolII complex. We hypothesize that in addition to these examples, TF co-dependence also stems from the inability of individual TFs to bind to nucleosome occluded DNA. In *S. cerevisiae,* the secreted phosphatase gene *PHO5's* promoter encodes a non-consensus, nucleosome-exposed binding site for the main TF, Pho4, as well as a consensus, nucleosome-occluded site. Past research showed that ScPho4 depends on a second TF, ScPho2, which co-bind to the exposed site, resulting in the remodeling of the -2 nucleosome and allowing ScPho4 to bind to the occluded consensus Pho4 site to activate gene expression. Remarkably, a Pho4 ortholog in the related yeast pathogen *C. glabrata* (CgPho4) is able to induce gene expression without Pho2 both in its native background and when expressed exogenously in *S. cerevisiae*. We hypothesize that unlike ScPho4, CgPho4 can bind directly to the nucleosome-occluded site. To test this, we introduced CgPho4 into a mutant *S. cerevisiae* strain containing a mutated *PHO5* promoter which lacks the exposed Pho4 site and is fused to a fluorescent reporter. Flow cytometry results showed that *PHO5* is activated to ~55% of the level when compared to the wild type *PHO5* promoter paired with CgPho4. Pho2 doesn't affect the mutant *PHO5* promoter activity with CgPho4. Preliminary ChIP results suggest that CgPho4 is able to bind to the occluded site when the exposed site is mutated. These findings suggest that CgPho4 may possess characteristic traits of a nucleosome displacing factor.

11. Mapping Brain Activity in Response to Drugs Using Deep Learning and Integrated Omics Data

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Drug development is a multifaceted endeavor necessitating a thorough comprehension of the intricate biological mechanisms governing drug effects. A pivotal aspect of drug development is pinpointing brain regions activated by drugs, either therapeutically or in terms of side effects. In this study, we present a novel approach to construct a brain activity map, showcasing the activation intensity of diverse brain regions in response to specific drugs.

The Allen Institute offers an extensive dataset of microarray gene expression profiles across various brain regions, accompanied by their spatial coordinates in the MNI-space. As this dataset covers only specific brain regions, we devised a deep learning model to predict gene expression in unmeasured regions (i.e., imputation). Subsequently, we established correlations between gene expression metrics and drug transcriptomic signatures from the LINCS project, quantifying each brain region's response to the drug.

To interpret the possible functional changes within the brain based on predicted drug maps, we integrated feature space data from the Neurosynth dataset. To validate our findings, we compared our tool's predictions with literature results for methylphenidate (MPH), demonstrating congruent patterns of regional activation. We observed that the patterns of regional activation predicted by our approach closely aligned with the functional connectivity changes linked to methylphenidate in the literature.

The implications of the resulting brain activity map are profound for drug development and personalized medicine. By developing a predictive tool for anticipating brain region activation in response to drugs, our research streamlines drug discovery, economizes time and resources, and enhances drug safety through side effect prediction. This methodology bridges the gap between genomics and neurology, providing a holistic understanding of how drugs modulate brain activity.

12. Revealing snps that modulate disease risk

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Genome-wide association studies (GWASs) have successfully identified single nucleotide polymorphisms (SNPs) associated with complex diseases, including macular degeneration, glaucoma, and diabetes. They allow researchers to locate genomic regions in which one (or more) genetic variation(s) alter the risk of developing the phenotype of interest. Our goal is to distinguish the risk-modulating SNPs in GWAS experiments from those that are merely in linkage disequilibrium with risk-modulating SNPs.

Risk-modulating SNPs are frequently assumed to alter amino acid sequences (cSNPs), such as the Y402H variation in Complement Factor H associated with increased risk of macular degeneration. However, many associated loci do not harbor cSNPs. In such cases, transcriptional regulation is the hypothesized mechanism through which a SNP modulates disease risk. Thus, each SNP represents a potential transcriptional regulator in which two alleles have different regulatory effects.

To efficiently evaluate thousands of candidate transcriptional regulators identified from a single GWAS, large-scale experiments such as Massively Parallel Reporter Assays (MPRAs) are required. To examine the contrasting effects of different alleles from numerous SNPs, we used Biallelic Targeted Self-Transcribing Regulatory Region Sequencing (BiT-STARR-Seq), an MPRA in which allele-specific oligos are integrated downstream from a reporter gene in an expression construct. Thus, sequencing of the resulting RNA transcripts measures the amount of transcription derived from each allele directly, allowing identification of SNP alleles with differential regulatory potential.

In an initial experiment, we applied BiT-STARR-Seq to a small set of SNPs near *LOXL1* associated with pseudo-exfoliation syndrome, all of which were in strong linkage disequilibrium with the peak association. This preliminary analysis identified seven SNPs that appear to modulate transcriptional regulation, for which confirmatory experiments are underway. Further BiT-STARR-Seq experiments are being designed to expand our investigation to n=993 SNPs in the *LOXL1* region associated with pseudo-exfoliation syndrome as well as SNPs associated with primary open-angle glaucoma.

13. Genetic links between sleep and a form of synaptic plasticity

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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.

The idea of homeostatic regulation has been extended to specific behaviors like sleep. One attractive hypothesis in the sleep field is known as the "synaptic homeostasis hypothesis" (SHY). This idea posits that animals use sleep as an off-line period to restore the brain's capacity for plasticity that will be needed during the daytime. Yet on a molecular level, SHY has not been tested rigorously and the data are mixed. Some data suggest that sleep increases synapse number and strength, while countervailing observations show global downscaling of synaptic strength during sleep. Regardless, if the general concept of SHY is correct, then perturbations that disrupt sleep should affect synaptic homeostasis. Perturbations that disrupt synaptic homeostasis, might also affect sleep.

The fruit fly *Drosophila melanogaster* offers an ideal system to test both predictions. Sleep behavior is well characterized in flies, as are forms of homeostatic synaptic plasticity. We recently conducted a genetic screen in Drosophila, examining canonical "sleep genes" for roles in maintaining normal synapse function. Of note, we found that disruptions of calcineurin signaling disrupt both sleep and synaptic homeostasis. We did a second pilot screen, this time testing if the loss of synaptic homeostasis factors causes sleep abnormalities. We found that *Drosophila* harboring loss-of-function conditions of *GluRIIA* (glutamate receptor subunit), *cacophony* (Ca_V2 subunit), mitochondrial complex I (MCI), or JAK-STAT signaling components all disrupted normal sleep, though in different ways for each. We will present our current data, as well as plans to expand our direct tests of SHY, using an extensive molecular and genetic toolkit available for *Drosophila melanogaster*.

14. Deep learning modeling to identify regulatory sequences in the brain

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Activity dependent gene expression in the brain allows for differential response to numerous stimuli. Changes in activity-dependent expression profiles have been associated with psychiatric diseases. Enhancers are an important component of regulating the rate of gene expression within a cell, but in contrast to other regulatory sequences such as promoters, enhancers sit at a much more variable distance from their target gene and can be found either up- or downstream. This makes it more difficult to correctly identify the target gene of individual enhancers.

We use publicly available sequences to fine tune a convolutional neural network to extract unique feature information. The model was originally trained to identify sequences from diverse tissues, which we intend to specialize to our classes of interest. We then use the output features to train a classifier model, which will predict the identity class of the provided sequences. Once the model has been trained, we intend to use in-silico mutagenesis to examine the effect of mutations on the enhancer potential of the different sequences.

15. Development of an *in-vitro* model for axonal proteostasis

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Maintenance of proteostasis is critical for preserving cell integrity and ensuring an appropriate response to a changing environment. Impairments in proteostasis can lead to a deleterious imbalance in protein concentration, localization, and degradation. Deficiencies in proteostasis have important consequences for neurons, which must persist and function for an entire human's lifespan. In the axonal compartment, isolation from the soma necessitates a unique approach to sustaining proteostasis within this compartment. Many studies on neuronal proteostasis focus on the soma rather than the axon such that specialized mechanisms residing in the axon compartment are still unknown. To fill this important gap, I've developed two in-vitro models to investigate local proteostasis networks in the axon. The first model utilizes cultured neurons from the dorsal root ganglion of embryonic mice and genetically encoded fluorescent reporters-fused with axon-targeting 5' and 3'UTRs to visualize newly synthesized proteins in axons as well as soma. The second model I have developed involves complete removal of a neuron's axons while maintaining cell integrity. New axon growth is then subsequently induced. I have employed both models in concert, along with live-cell imaging, to track the spatial and temporal fate of newly synthesized proteins and investigate how neurotoxic stressors and human disease models impact proteostasis networks. For example, I've introduced mutated forms of a tRNA synthetase linked to Charcot-Marie-Tooth disease in primary neurons and observed extensive axon degeneration and neuronal cell death. Further, I have observed a decrease in neuronal protein synthesis, a decrease that is not present during the initial phase of axon growth. These findings help elucidate Charcot-Marie-Tooth's unclear pathology. With this model system, genetic and pharmacological screens will identify critical nodes in axonal proteostasis networks, which have broad relevance to the growing list of proteinopathies and other neurological disorders characterized by loss of proteostasis.

16. Auxin's role in developing stem cells in the meristem of *ceratopteris richardii gametophytes*

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Plant stem cells are located in specialized structures called meristems that control the plant's growth throughout life with a complex network of signals. The haploid gametophyte phase of the model fern Ceratopteris (*Ceratopteris richardii)* consists of a single layer of cells with a multicellular marginal meristem that is easy to observe and manipulate. Previously, the Cheng Lab established a method for ablating individual "stem cells" within the marginal meristem to induce regeneration of a new meristem within a certain distance from the original. This system allows us to gain insight into the factors involved with meristem initiation.

The phytohormone auxin is known to be essential to many developmental processes in seed plants, including meristem development. However, its role in fern marginal stem cell identity is unclear. My preliminary results show that *CrTAA2*, a Ceratopteris gene predicted to encode an enzyme that catalyzes the first step of auxin biosynthesis, is dynamically expressed throughout meristem development and is expressed in the marginal meristem of the Ceratopteris gametophyte.

To understand auxin's involvement with regeneration of a new meristem, we visualized expression of *CrTAA2* and *CrWOXB*, a marker gene for meristematic activity in the gametophyte 3 days after ablation. We observed expression disappeared from the ablated meristem and appeared where the new meristem forms. To further examine auxin's role in this process, we treated gametophytes post-ablation with hormones including three different auxins, a competitive inhibitor of TAA, and an auxin transport inhibitor. We observed that treatment with high concentrations of two of the auxins or the transport inhibitor delayed meristem regeneration and affected the position of the new meristem.

17. Modeling VHL-associated hemangioblastomas *in vitro* using patient-derived stem cells

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Mutations *VHL* result in a cancer syndrome known as von Hippel-Lindau (VHL) disease, which predisposes individuals to retinal hemangioblastomas. Mutations are inherited in an autosomal dominant manner. Interestingly, clinical observations document significant phenotypic variability among patients within the same family carrying the same primary mutation. This suggests that genetic modifiers may influence disease severity. The purpose of this study was to combine patient-derived iPSCs and genome editing technologies to generate an in vitro model system suitable for investigating VHL disease mechanism and evaluating the role of novel genetic variants on phenotypic severity.

We obtained peripheral blood and dermal fibroblast cells from four family members diagnosed with VHL disease. DNA isolated from peripheral blood was Sanger sequenced. Dermal fibroblasts were characterized via a hypoxia gene expression array and subsequently reprogrammed into induced pluripotent stem cells (iPSCs). CRISPR-Cas9 ribonucleoproteins designed to cut upstream of the desired VHL locus were fabricated and delivered to patient iPSCs via the CellFE Zephyr microfluidics platform. T7E1 assay was used to evaluate cutting efficiency and select guides for homology dependent repair.

Sequencing of the *VHL* locus revealed a heterozygous pathogenic Tyr98His mutation in all four individuals. Two of the patients (ages 44 and 60) were determined to have clinically severe disease and two of the patients (ages 61 and 82) were determined to have clinically mild disease. Patient iPSCs from all four donors were demonstrated to be pluripotent via scorecard analysis and to have normal chromosomal structure and number via karyotyping. Following delivery of CRISPR-Cas9 ribonucleoproteins, a cutting efficiency of up to 30% at the targeted locus near the Tyr98His mutation was detected.

These results show that iPSC cell cultures can be effectively established from dermal punch biopsies of patients affected with VHL disease. Furthermore, CRISPR technology will enable the ability to model disease-associated hemangioblastomas *in vitro*.

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

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Neurodevelopmental disorders (NDD), including autism spectrum disorder and intellectual disability (ID), are highly prevalent. In the USA, ~67 million individuals have a diagnosis of NDD (3-17 yrs) and 10.5% (6.7 million) have ID causing significant socioeconomic burdens.

Pathogenic copy number variants (CNVs) have been found to have a significant impact on intellectual disability presentations. The increase in genomic testing, such as chromosomal microarrays (CMA) and whole genome sequencing (WGS), has led to the detection of an increasing number of "variants of unknown significance (VUS)". In this project, we hypothesize that disruption of genes within VUS CNVs that intersect with known microdeletion / microduplication syndrome (MMS) functional networks and protein-protein interaction (PPI) pathways will lead to similar phenotypes seen in established CNV disorders thus helping in ID VUS re-classification.

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

Our preliminary results using these clustering procedures showed that known microdeletion/duplication syndrome (MMS) CNVs shared a well-defined underlying functional network of interacting genes that can be genotypically quantified, phenotypically defined, and leveraged to interpret VUS CNVs. This methodology allows for candidate ID gene identification followed by functional testing using Gal4-UAS RNAi system and T-maze olfactory assay in the model system *Drosophila melanogaster* to identify genes involved in memory and cognition.

This project contributes to refining clinical interpretation and classification of ID and NDD VUS genetic lesions for more accurate diagnoses and potentially precision therapies. Furthermore, functional testing of VUS genes will describe novel gene-phenotype associations in ID patients.

19. Contribution of CD4 and CD8 cells to retinal ganglion cell loss in glaucoma

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Background: Loss of retinal ganglion cells (RGC) is the ultimate cause of vision loss in glaucoma. Although elevated intraocular pressure (IOP) has been identified as a main risk factor for the development of glaucoma, disease progression is likely due to multiple pathomechanisms. Previous studies in our laboratory have demonstrated that adoptive transfer of CD3+ cells from glaucomatous mice into healthy recipients results in progressive loss of RGC. Conversely, RAG mice lacking T or B cells, are profoundly protected from glaucomatous damage caused by elevated IOP. These findings indicate that adaptive immune responses are one of the factors leading to vision loss in glaucoma. The current study was carried out in order to determine the functional contribution of CD4+ and CD8+ T cells in this process.
Methods: Elevated IOP was induced in the eyes of C57Bl/6J controls, *Cd4* knockout (KO), and *Cd8* KO mice through intracameral injection of Ad5.Myoc^{Y437H} (n=25/group). IOP was measured weekly using rebound tonometry. Visual function was determined every month by measurements of the optokinetic response (OKR). RGC loss was assessed in whole-mounted retinas following immunohistochemical labeling of RGC with antibodies directed against RBPMS. After 4 months of elevated IOP, spleens were harvested, homogenized, and transferred into RAG recipient mice by intraperitoneal injection.
Results: After 4 months of elevated IOP, the OKR in controls had decreased by 0.097 cycle/degree (c/d), whereas visual acuity in *Cd4* KO decreased by 0.050 ($p=0.03$) and by 0.068 c/d in *Cd8* KO ($p=0.26$). RGC density decreased in all groups with elevated IOP ($p<0.0009$). Losses in neither *Cd4* nor *Cd8* KO were significant when compared to control mice. RAG mice receiving adoptive transfers of splenocyte preparations from these groups also developed RGC damage. 4 months after transplantation, OKR responses in RAG which had received control splenocytes decreased by 0.069 c/d ($p=0.032$), whereas those that received *Cd4* KO or CD8 ko splenocytes decreased by 0.048 c/d ($p=0.21$), or 0.083 ($p=0.009$), respectively. RGC density in recipient mice decreased from 3,404 cells/mm^2 in naïve RAG mice to 2,865 cells/mm^2 in control recipients, 2,938 cells/mm^2 in *Cd4* KO recipients, and 2,544 cells/mm^2 in *Cd8* KO recipients. No differences between *Cd4* or *Cd8* KO were found when compared to control recipients.

Conclusions: Neither absence of CD4+ or CD8+ cells resulted in robust RGC protection in donor or recipient mice. This suggests either compensatory mechanisms in *Cd4* and *Cd8* KO mice, or that other splenocyte cell populations contribute stronger to RGC loss than appreciated.

20. Specific prostaglandins are produced in the migratory cells and the surrounding substrate to promote *drosophila* border cell migration

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Collective cell migration – the coordinated movement of associated cells – is important for both normal development and tumor invasion. One poorly understood class of regulators of cell migration are prostaglandins (PGs), short-range lipid signals. To define the mechanisms whereby PGs regulate migration, we use the collective migration of the *Drosophila* border cells as a model. The cluster of border cells is specified from the follicular epithelial cells during Stage 9 of oogenesis, delaminates from the epithelium, and migrates collectively between the nurse cells (substrate). Prior work showed PG signaling is required for on-time migration and cluster cohesion. However, the particular PG or PGs controlling border cell migration remain unknown. To address this, we assessed the roles of three PGE_2 synthases (mPGES-1, mPGES-2, and cPGES) and the $PGF_{2\alpha}$ synthase Akr1B during border cell migration. Loss of cPGES or Akr1B, delays border cell migration. These findings support the model that cPGES and Akr1B are required for on-time border cell migration. Cell-specific RNAi knockdown experiments reveal cPGES acts in the substrate whereas Akr1B acts in the border cells to promote migration and Akr1B acts both in the border cells and the substrate to regulate cluster cohesion. We find that Akr1B and therefore, $PGF_{2\alpha}$ signaling is required for border cell integrin-based adhesions. Additionally, Akr1B limits myosin activity within the border cells, while cPGES limits myosin activity within both the substrate and the border cells. Both of these downstream effectors likely contribute to PG regulation of border cell migration. Together our data reveals two PGs, PGE_2 and $PGF_{2\alpha}$, are produced in different locations to promote border cell migration, revealing PGs act not only in the migrating cells but also in the microenvironment to drive migration. As PG signaling is highly conserved, these studies provide critical insight into the specific functions of individual PG signaling cascades in controlling collective cell migration and can be applied to understanding both developmental and pathological migrations.

21. MISSENSE VARIANT IN *KRT32* IS RESPONSIBLE FOR INEFFICIENT ANCHORING OF ANAGEN HAIR SHAFT TO ITS FOLLICLE

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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. We are currently examining a cohort of LAHS patients for variants in *KRT32* and performing downstream cell biology functional assays that provide functional evidence of the pathogenicity of the identified mutation. The understanding of the pathophysiology of LAHS would provide insight into the mechanism(s) that anchor the anagen hair shaft to its follicle.

22. Atf4-mediated activation of cdkn1a (p21) promotes muscle atrophy

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Skeletal muscle atrophy is an unmet medical need whose molecular mechanisms are not well understood. Previous work has established a causative role for the transcription regulatory protein ATF4, and its direct target gene Cdkn1a (p21) in skeletal muscle atrophy during a variety of stress conditions. To investigate the mechanism by which p21 promotes muscle fiber atrophy, we biochemically isolated skeletal muscle proteins that associate with p21 as it induces atrophy in vivo. We found that p21 interacts with, and directly inhibits, multiple Cyclin-CDK complexes in skeletal muscle fibers. Furthermore, we found that inhibition of Cyclin-dependent kinase 1 (CDK1) is sufficient to induce skeletal muscle fiber atrophy and is partially required for p21-mediated skeletal muscle fiber atrophy. Together, these results provide new insights into the molecular mechanisms of skeletal muscle atrophy by identifying a direct biochemical mechanism by which p21 expression in skeletal muscle fibers promotes muscle atrophy.

23. Analysis of chirality and de novo mutation in asexual snails

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Potamopyrgus antipodarum, also known as the New Zealand mud snail, is a freshwater species native to New Zealand. These snails exist either as an obligately sexual diploids or obligately asexual triploid or tetraploids. Both sexual and asexual snails are found coexisting throughout their native habitat.

In 2019, we discovered a *P. antipodarum* individual, nicknamed Sinistra, that has a shell that spirals to the left ("sinistral"). Sinistra is the first-ever *P. antipodarum* individual ever found to depart from the typical right-spiraling (dextral) chiral form. This individual was produced within an otherwise dextral asexual *P. antipodarum* lineage, suggesting that her unique phenotype arose within an asexual context. We are now using whole-genome sequencing data to compare Sinistra to her clonemates, with the goals of (1) uncovering the genetic basis of chirality, an important question in biology, as well as (2) estimating the rate of *de novo* mutation in an asexual line. *De novo* mutation is of broad interest both as the source of the raw material for evolution as well as the only mechanism by which asexual lineages can access new genetic variation.

24. LOSS OF NR2E3 DISRUPTS ROD PHOTORECEPTOR CELL MATURATION CAUSING A FATE SWITCH LATE IN HUMAN RETINAL DEVELOPMENT

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While dysfunction and death of light-detecting photoreceptor cells underlie most inherited retinal dystrophies, knowledge of the species-specific details of human rod and cone photoreceptor cell development remains limited. Here, we generate retinal organoids carrying retinal disease-causing variants in *NR2E3*, as well as isogenic and unrelated controls. Organoids were sampled using single-cell RNA sequencing across the developmental window encompassing photoreceptor specification, emergence, and maturation. Using single-cell RNA sequencing data, we reconstruct the rod photoreceptor developmental lineage and identify a branchpoint unique to the disease state. We show that the rod-specific transcription factor NR2E3 is required for the proper expression of genes involved in phototransduction, including rhodopsin, which is absent in divergent rods. NR2E3-null rods additionally misexpress several cone-specific phototransduction genes. Using joint multimodal (RNA and ATAC) single-cell sequencing, we further identify putative regulatory sites where rod-specific factors act to steer photoreceptor cell development. Importantly, these findings are strikingly different than those observed in rodent models of disease. Together, these data provide a roadmap of human photoreceptor development and leverage patient iPSCs to define the specific roles of rod transcription factors in photoreceptor cell emergence and maturation in health and disease.

25. Evaluation of senescence features in RPE and choriocapillaris in young, age-matched, and AMD human donor eyes.

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Cellular senescence is a hallmark of aging, contributing to both normal features of aging and pathologies of aging. Research on the role of senescence in the pathogenesis of age-related macular degeneration, particularly in the choroid, is limited. We conducted preliminary studies to test the hypothesis that telomere length in cells of the choriocapillaris and retinal pigment epithelium are shorter in AMD donor eyes as compared to both young and age-matched controls, and that senescence contributes to endothelial cell loss early in AMD.

Donor eyes were categorized as young (age < 40 years), age matched controls (age >71 years, without AMD) or AMD (age >71 years with early atrophic AMD). Average telomere lengths of genomic DNA were determined from 5 young, 6 aged, and 4 AMD bulk RPE/Choroid punches using a commercially available telomere length RT-qPCR assay. To address telomere lengths in specific layers of the choroid, we performed a pilot study utilizing laser capture microdissection (LCM) to collect choriocapillaris and RPE cells separately from fixed cryosections of young, aged, and AMD donor maculas. Finally, immortalized and primary choroidal endothelial cells were treated with H_2O_2 to induce senescence, followed by exposure to complement-intact or complement-depleted serum, and deposition of the C5b-9 membrane attack complex (MAC) was evaluated.

No statistically significant difference in telomere length was observed between bulk RPE-choroid or LCM-isolated RPE and choriocapillaris of young, aged or AMD donors. Overall, telomere lengths of RPE cells were longer than those of cells in choriocapillaris from the same donors, although this did not reach statistical significance. Senescence induction resulted in cells with larger surface area and an increased frequency of nuclei positive for senescence-associated heterochromatin foci markers (MacroH2A and HMGA2). Senescent cells and their subendothelial matrix showed increased MAC deposition, suggesting that senescence may contribute to vascular susceptibility to MAC in AMD.

26. Tfap2 transcription factors function in a frontonasal dysplasia-related neural crest gene regulatory network

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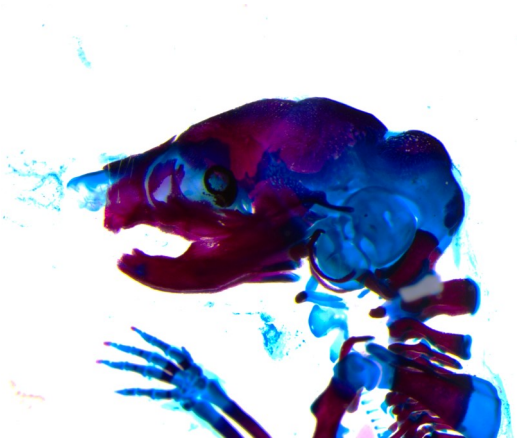
Neural crest cells (NCCs) form unique facial structures during embryonic development thanks to gene regulatory programs specifying each region. Yet, how these positional pathways are activated, particularly those for the medial and upper-face regions (i.e., midface), remains poorly understood. In this study, we found that combined loss of *Tfap2a* and *Tfap2b* genes at either pre- or late-migratory NCC stage resulted in a fully penetrant midface cleft compared to controls or loss of a single paralog in mice. Testing a post-migratory role for TFAP2 in midfacial NCCs, bulk and single-cell transcriptomics revealed that loss of *Tfap2* compromised the midface positional program. This included decreased transcript levels in *Msx1*, *Pax3/7*, and most notably members of the Frontonasal Dysplasia-related *Alx* family (*Alx1/3/4*). The latter is of interest because the *Tfap2*-associated midface cleft phenocopies those in *Alx* compound mutants, which have been attributed to impaired NCC differentiation and patterning. Combined ChIP- and ATAC-seq profiling suggested that *Alx* gene expression is directly dependent on TFAP2 function. In mouse, we observed that reducing *Alx3* gene dosage in single *Tfap2* mutant backgrounds resulted in midfacial anomalies synergistically increasing in both penetrance and phenotypic severity; similarly, zebrafish epistasis analyses suggest this midface pathway is conserved. Our cumulative findings illuminate TFAP2 as a key factor in reinforcing the midface gene regulatory programs. Ongoing studies aim to leverage this mouse model of Frontonasal Dysplasia to examine the epigenetic and cellular basis of midfacial clefting.



27. Outcomes of tagging DNA repair protein RAD-51 – The good and the bad

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Meiosis is an essential biological process such that defects in meiotic processes results in infertility. During meiosis, programmed double-strand breaks are generated by SPO-11 to make crossovers to ensure proper chromosome segregation and to promote genetic diversity. However, not all DSBs in meiosis are designated as crossovers (COs). In fact, most are designated as non-crossover (NCO) events. The sites of DSB formation are relegated to specific loci in the genome and thus are referred to as meiotic hotspots. Most organisms have these hotspots (e.g., humans, mice, yeast). Hotspots can be identified by immunoprecipitating SPO-11 or the proteins that process DSBs, such as RAD-51.

C. elegans meiotic DSBs were previously mapped using RAD-51. However, this method cannot currently be used to identify DSB sites (*e.g.,* in various mutants) since the RAD-51 antibody used is no longer commercially available. We developed several epitope tagged versions of RAD-51 in an attempt to allow for more universal use to map/mark meiotic DSBs. Several tagged versions we developed are fully functional and behave similar to wild type RAD-51 in all the assays used so far. In the process of generating this strain, however we stumbled upon interesting consequences of tagging RAD-51. Here, we characterize six epitope tagged versions (five developed by our lab) of the *rad-51* gene. Four versions are homozygously viable. Defective tagged RAD-51 strains display a reduction in embryonic viability due to unresolved DSBs. There is an accumulation of RAD-51 foci in defective epitope tagged versions of RAD-51 while homozygous viable versions display a minor reduction in repair kinetics but normal resolution. In those with accumulation of RAD-51 foci, the CO pathway appears to resolve without issue while NCO resolution is impaired, which is unlike *rad-54* or *rad-51* nulls. Taken together, these results suggest the possibility for parsing out the decision-making mechanism between COs vs NCOs to repair SPO-11-dependent DSBs in meiosis in these defective versions of RAD-51. Additionally, homozygous viable versions will aid in eventual ChIP-Seq for mapping loci prone to specific types of damage.

28. Regulation and dynamics of intercalated disc transcriptomes

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Intercalated discs (ICDs) are dynamic and complex structures that couple cardiomyocytes (CMs) electrically and mechanically to synchronize their contraction which is vital for heart pump function. *Dysregulation of ICD structure and protein composition has been implicated in many heart diseases*. In addition to their known structural roles, ICDs likely also serve as a hub for local protein synthesis in CMs. Studies have found enhanced local translation at ICDs with enrichment of specific mRNAs. *Despite evidence for both ICD localized RNAs and local translation in CMs, no transcriptome-wide unbiased approaches have been used to profile RNAs at ICDs, and few efforts have been made to identify RNA-binding proteins (RBPs) that mediate RNA localization to ICDs*. To begin addressing this, we developed a nearly impossible "ICD-seq", We identified hundreds of unique mRNAs that are significantly enriched at the ICD, and these mRNAs were found to harbor sequence motifs that are bound by several RBPs that have been implicated in heart disease. Follow-up studies are needed to compare human and mouse ICD profiles and further explore how ICD transcriptomes are altered in disease and begin to delineate the underlying mechanisms. Our *overarching hypothesis* is that select subsets of functionally related mRNAs are trafficked to and translated at the ICD, by conserved means depending upon microtubules and an array of molecular motors and RBPs, and that these processes are perturbed during cardiac stress and contribute to downstream detriments in heart function. To begin addressing this, we propose the following aims:

AIM 1: Compare human and mouse ICD RNA profiles and assess how they "re-wire" in cardiac stress and disease.

AIM 2: Characterize functional RNA motifs and RBPs that direct mRNA localization to ICDs.

This work will deliver key resources to better understand an important biological realm that remains largely underexplored in the heart.

29. Subretinal Gene Therapy in the Bardet Biedl Syndrome Type 10 mouse

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Bardet-Biedl Syndrome type 10 (BBS10) is an autosomal recessive disease-causing retinal degeneration leading to blindness. Mutations in *BBS10* affect the chaperonin protein complex which assembles the BBSome. Without a proper BBSome, photoreceptors die. Currently, there are no treatments to help *BBS10* patients. Subretinal gene augmentation therapy has shown promise in slowing vision loss in inherited retinal degenerations, but the effects may not be durable. The purpose of this study is to analyze treatment efficacy of subretinal gene augmentation therapy in the *Bbs10*$^{-/-}$ mouse and to prolong the treatment effect.

A *Bbs10* knockout mouse model (*Bbs10*$^{-/-}$) was maintained both on a SV129 and a mixed genetic background. The human BBS10 gene was cloned into shuttle plasmids driven by the rhodopsin kinase (RK) promoter. Plasmids were packaged into an AAV2/8 viral capsid. *Bbs10*$^{-/-}$ mice received a single subretinal injection of 2 µL of AAV8-RK-*hBBS10* at low dose (4 x 10^8 vg/µL), medium dose (4 x 10^9 vg/µL), or high dose (3.74 - 4 x 10^{10} vg/µL). Electroretinography (ERG) was performed at 1-, 2-, and 3-, months post-injection (PI). At 2-month PI, *Bbs10*$^{-/-}$ eyes treated with AAV8-RK-h*BBS10* had higher ERG amplitudes in light-adapted but not in dark-adapted ERGs. The highest amplitudes were found in the high dose group. At 3- months PI, amplitudes were still higher in the treated eyes, however amplitudes had declined compared to 2 months.

Two methods are proposed to make efficacy more durable: dual subretinal injections to increase the area of retina rescued, and N-acetyl cysteine (NAC) supplementation to reduce oxidative stress.

In summary, mice treated with AAV8-RK-*hBBS10* exhibit better cone activity in a dose-dependent manner, but the effect is not durable. Future research will be directed at using the highest dose tested combined with dual injections or NAC supplementation.

30. Epidermal loss of prmt5 leads to the emergence of an atypical keratinocyte-like cell population and defective stratification

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During development, the initially single-layered epithelium of the presumptive skin requires precise coordination of cell proliferation and differentiation to execute epidermal stratification. Defects in these events during development lead to congenital anomalies whereas accumulation of harmful mutations in adulthood can lead to carcinoma. Protein Arginine Methyl Transferase 5 (PRMT5) -an enzyme that catalyzes methylation of arginine residues in critical proteins, including histones and transcription factors- is upregulated in carcinoma and correlates with poorer prognosis. While inhibition of PRMT5 has been shown to have anti-cancer properties, the mechanisms behind this effect are unknown. Interestingly, PRMT5 has been identified as necessary to maintain a progenitor, stem-cell fate in both germ-cell and limb development as well as a variety of cancers. Therefore, we hypothesize that PRMT5's methylation of histones and transcription factors drives a gene expression program that impedes differentiation allowing the maintenance of a stem-cell phenotype. To test this hypothesis, we used conditional mouse genetics to delete *Prmt5* from the early (E7.5) ectoderm. Consistent with a critical role for PRMT5 during this process, epithelial loss of *Prmt5* resulted in gross skin defects, reduced skin barrier function, and reduced postnatal viability. Histological analyses of control and mutant skin revealed severe defects in epidermal stratification, including major reduction of the proliferative basal layer. Molecularly, sc-RNA/ATAC-seq suggests that loss of PRMT5 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. Collectively, our findings have identified a critical role for PRMT5 in epidermal development and provide a novel model in which to dissect its molecular function in this process, providing insight into its role during tumor progression.

31. 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 have postulated 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 non-alcoholic steatohepatitis (NASH) could be repurposed for the treatment of AD.

Most therapeutics for AD have focused on targeting Aβ with little success. To date, very little is known about the therapeutic 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.

32. The Neuroprotective Effect of Naltrexone in a MOR Knock-Out Temporal Lobe Epilepsy Mouse Model

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[4] Principal Investigator

Through previous studies done in our lab using high throughput transcriptome profiling, we identified Naltrexone (NTX) as a potential disease-modifying candidate capable of preventing or slowing the progression of epileptogenesis. NTX is a mu opioid receptor (MOR) antagonist used to treat alcohol and opioid abuse. MOR is a G-protein coupled receptor that when overstimulated it causes neuroinflammation that leads to epileptogenesis. In contrast, MOR antagonism reduces proinflammatory mediators and attenuates neuroinflammation. To understand the mechanism by which NTX may promote neuroprotection, we evaluated the effect of NTX in a MOR knock out mouse model where seizures were induced using chemo-convulsants such as pilocarpine (Pilo) and pentylenetetrazol (PTZ). Results are promising as NTX demonstrated protective effect against acute seizures. Surprisingly, NTX showed a protective effect in MOR KO mice as well, indicating that another receptor is in play.

33. Characterization of functionality of candidate interactors in the pdr1 and upc2a azole resistance pathways 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. Our lab has recently discovered a connection between the ergosterol biosynthesis pathway and this Pdr1/CDR1 drug efflux system. The transcription factor Upc2A is well known to control expression of ergosterol biosynthetic genes like ERG11. Our recent data demonstrate that Upc2A also controls the expression of both PDR1 and CDR1, establishing a direct link between two pathways that regulate azole resistance. Using biochemical techniques, we have found a number of proteins which interact with Pdr1 and Upc2A. 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 made an auxin-inducible form of Spt5. This strain allows us to acutely deplete Spt5 from the cell upon addition of the plant hormone auxin. This mutant shows no growth defect in the absence of auxin, while showing a significant growth defect in the presence of auxin. We will use this degradable form of Spt5 to examine the effect of its loss on the transcription of PDR1, CDR1, and ERG11.

34. Single-cell transcriptomic analysis on the cerebellum of mice with the 16p11.2 microduplication mutation

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Schizophrenia (SCZ) is a complex genetic disorder and mutations such as the copy number variant 16p11.2 microduplication (16p11.2dup) can cause SCZ. 16p11.2dup is associated with microcephaly, suggesting that the duplicated genes are involved in neurodevelopment. Computational analysis of publicly available bulk RNA-seq data from mice with 16p11.2dup shows significant transcriptional dysregulation in the cerebellum, but the specific cell types affected are unknown, and there are no published analyses of cerebellum-dependent behaviors in this mouse line. Therefore, we performed a behavioral assessment with delay eyeblink conditioning, which is a cerebellum-dependent form of learning. We observed that 16p11.2dup mice show impaired learning ($p<0.01$) and delayed response to the conditioned stimulus ($p<0.05$) compared to WT littermates. To better understand the neuropathological basis of microcephaly in the 16p11.2 mutation, we compared key cerebellar parameters between 16p11.2dup and WT mice, specifically investigating Purkinje cell density and molecular and granular layer thickness. Our findings reveal no significant difference between mutant and control mice. We would like to investigate the cell types that are primarily affected by the 16p11.2 duplication and how they can contribute to microcephaly. We will perform single nucleus RNA-seq using the Parse Biosciences nuclei fixation kit followed by their Evercode WT Mini v2 kit on the whole cerebellum of male and female 16p11.2dup mice and WT littermates. Using the R package toolkit Seurat, we will cluster nuclei with similar gene expression profiles which is crucial for unraveling the cellular heterogeneity present in the cerebellum. Dimensionality reduction techniques, such as PCA and t-SNE, will be used to look at the dataset in a lower-dimensional space. By condensing the complex gene expression data, these techniques will allow for the identification of patterns and relationships among the different cerebellar cell populations. Overall, our data show that 16p11.2 dup is associated with a deficit in cerebellum dependent behavior. The single nucleus RNA-seq data will help specify which cerebellar cell types are most affected by 16p11.2dup and may accelerate the discovery and development of new diagnostic and therapeutic strategies for schizophrenia.

35. SYS-1/beta-catenin inheritance and regulation by Wnt-signaling during asymmetric cell division.

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Asymmetric cell division (ACD) allows daughter cells of a polarized mother to acquire different developmental fates. In *C. elegans* , the Wnt/β-catenin Asymmetry (WβA) pathway oversees many embryonic and larval ACDs; here, a Wnt gradient induces an asymmetric distribution of Wnt signaling components within the dividing mother cell. One terminal nuclear effector of the WβA pathway is the transcriptional activator SYS-1/β-catenin. SYS-1 is sequentially negatively regulated during ACD; first by centrosomal regulation and subsequent proteasomal degradation and second by asymmetric activity of the β-catenin "destruction complex" in one of the two daughter cells, which decreases SYS-1 levels in the absence of WβA signaling. However, the extent to which mother cell SYS-1 influences cell fate decisions of the daughters is unknown. Here, we quantify inherited SYS-1 in the differentiating daughter cells and the role of SYS-1 inheritance in Wnt-directed ACD. Photobleaching experiments demonstrate the GFP::SYS-1 present in daughter cell nuclei is comprised of inherited and *de novo* translated SYS-1 pools. We used a photoconvertible DENDRA2::SYS-1, to directly observe the dynamics of inherited SYS-1. Photoconversion during mitosis reveals that SYS-1 clearance at the centrosome preferentially degrades older SYS-1, and this accumulation is regulated via dynein trafficking. Photoconversion of the EMS cell during Wnt-driven ACD shows daughter cell inheritance of mother cell SYS-1. Additionally, loss of centrosomal SYS-1 increased inherited SYS-1 and, surprisingly, loss of centrosomal SYS-1 also resulted in increased levels of *de novo* SYS-1 in both EMS daughter cells. Lastly, we show that daughter cell negative regulation of SYS-1 via the destruction complex member APR-1/APC is key to limit both the *de novo* and the inherited SYS-1 pools in both the E and the MS cells. We conclude that regulation of both inherited and newly translated SYS-1 via centrosomal processing in the mother cell and daughter cell regulation via Wnt signaling are critical to maintain sister SYS-1 asymmetry during ACD.

36. Spatial transcriptomics reveals unique gene expression changes in different brain regions after sleep deprivation.

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Sleep deprivation has extensive effects on both the brain and behavior, impacting memory, attention, and metabolism. Previous studies have primarily investigated changes in gene expression within individual brain regions. However, the uniformity or heterogeneity of sleep loss's effects on the brain remains unclear. In this study, we employ spatial transcriptomics to assess the impact of short-term sleep deprivation on the entire brain of male mice.
Our findings reveal substantial differences in gene expression across the brain because of sleep deprivation, with the most significant alterations observed in the hippocampus, neocortex, hypothalamus, and thalamus. Using a rank-sum test like Kruskal-Wallis and stringent thresholds (FDR < 0.001 ; fold-change > |1.2|), differentially expressed genes and their regulatory direction exhibited significant variation among the different regions. Notably, the hippocampal region exhibited the highest sensitivity, characterized by a substantial decrease in gene expression, especially in RNA processing-related molecular functions. Interestingly, the neocortex demonstrated the second highest sensitivity, displaying significant and robust upregulation of gene expression, primarily associated with transcription factor binding, ubiquitin ligase activity, and protein kinase activity. Additionally, we conducted deconvolution analysis using reference scRNA-seq datasets to investigate the gene expression profiles in individual cortical layers (L2-3, L4, L5, and L6) and specific hippocampal subregions (CA1, CA2, CA3, Dentate Gyrus, stratum radiatum, and stratum oriens).

Importantly, we have developed bioinformatic tools enabling the registration of tissue sections and gene expression data into a unified anatomical space, facilitating a comprehensive comparison of gene expression patterns across the entire brain.
Our results suggest that distinct molecular mechanisms acting in discrete brain regions underlie the biological effects of sleep deprivation.

37. Functional copy number alterations as diagnostic and prognostic biomarkers in neuroendocrine tumors

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Neuroendocrine tumors (NETs) can arise in multiple organs throughout the body. Sixty percent of NET patients have advanced metastatic liver disease before they are diagnosed, often leading to exploratory procedures to determine primary tumor site. Functional copy number alterations (fCNAs) are DNA copy number changes with concordant differential gene expression. Given that fCNAs are less likely to be bystander genetic lesions, we hypothesized that they could be used as diagnostic (site of origin) and prognostic (event free survival) NET biomarkers.

To identify candidate fCNAs, we integrated chromosomal microarray (CMA) and RNA-seq differential gene expression data from 31 pancreatic (pNET) and 33 small bowel neuroendocrine tumors (sbNET) as well as 47 early disease progression (<24 months) and 17 late disease progression (>24 months) patients. Candidate fCNAs that could accurately differentiate these groups were then replicated using fluorescence in situ hybridization (FISH) on formalin-fixed, paraffin-embedded (FFPE) tissues in a larger cohort of 60 pNETs and 82 sbNETs. Logistic regression analysis was performed to determine the predictive ability of these biomarkers as well as the assay performance metrics of sensitivity, specificity, and area under the curve.

Our results indicate that fCNAs at chromosomal loci 9p21.3, 17q12, 18q21.2, and 19q12 may be used as diagnostic NET biomarkers and can be utilized in clinical testing to aid in determination of primary tumor site for patients with metastatic liver disease of pNET or sbNET origin.

38. Investigating the impact of a NMUR1 variant on calcium flux in mammalian cells

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NMUR1 is a G-protein coupled receptor found on the cell membrane. It binds with neuropeptide ligands with the native ligand being Neuromedin U (NMU). When bound it changes conformation leading to the activation of the coupled g-protein which will then, in turn, begin a signal cascade via the release of GTP. One element of this cascade is the stimulation of a phospholipase C which ends up releasing IP3 molecules into the cytosol. These IP3 molecules bind to IP3 receptors on the ER which releases calcium ions into the cytosol. Our interest in NMUR1 is due to a variant identified in one of our families which leads to individuals having a growth hormone deficiency ending up with stunted growth. This variant is located in the region of the NMUR1 that interacts with the G-protein. Our functional investigation into this family is focused on identifying the impact of this variant and identifying if there is an impact on the calcium response in the cell as a method of determining if the interaction with the G-protein is still functional in the variant. We will do this with HEK293 cells transfected with both the wild type and mutant NMUR1. By stimulating these cells with NMU25 (a synthetic version of the natural ligand NMU), the calcium response can be compared between both versions of the protein. Both fluorescent microscopy and flow cytometry will be used to test this response. Investigating this variant will provide insight into how the NMUR1 receptor impacts overall growth and energy intake.


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