National Human Genome Research Institute

30 YEARS 1989 - 2019

November 25 - 26

2019 NHGRI Symposium

National Institutes of Health | Clinical Center | Building 10

2019 NHGRI Symposium

Program and Abstracts

NIH Clinical Center | Building 10

National Institutes of Health
Bethesda, Maryland

November 25 - 26

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Office of the Scientific Director
National Human Genome Research Institute
National Institutes of Health
Building 50, Room 5222
Bethesda, Maryland 20892-8002
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November 2019

Welcome to the 7th Annual NHGRI Symposium!

For the past six years, the annual NHGRI Symposia have provided an exciting forum for the exchange of scientific and research ideas, forging of new collaborations, and networking across the Institute. A number of space and scheduling issues led us to use a different format and location for the 2019 Symposium.

The first day of the Symposium will mix in-house scientific presentations, two keynote presentations, and our annual GREAT Awards Ceremony. Following oral and poster presentations from NHGRI staff in the morning, we will welcome Dr. Siddhartha Mukherjee for our first keynote presentation. Dr. Mukherjee is a noted biologist, oncologist, and author. After lunch, we will hold the annual GREAT Awards Ceremony, during which we will honor the accomplishments of NHGRI staff over the past year. We will then welcome Dr. Shirley Tilghman, President Emerita and Professor of Molecular Biology and Public Affairs at Princeton University, for our second keynote presentation.

The second day of the Symposium will feature another set of oral and poster presentations from NHGRI staff, along with a clinically-focused session highlighting work ongoing in the NHGRI Intramural Clinical Research Program.

We hope that you enjoy these two days by celebrating the Institute's accomplishments with each other!

Warm regards,

Eric Green, M.D., Ph.D.
Director, NHGRI

Daniel Kastner, M.D., Ph.D.
Scientific Director, NHGRI

2019 NHGRI Symposium

Agenda

Monday, November 25

8:45 a.m. - 9:00 a.m.	Welcome Remarks Eric Green, M.D., Ph.D. Dan Kastner, M.D., Ph.D.	Masur Auditorium
9:00 a.m. - 10:00 a.m.	Oral Presentations A	Masur Auditorium
10:00 a.m. - 11:30 a.m.	Poster Session A Information Tables	FAES Terrace & FAES Classrooms 1-4
11:30 a.m. - 12:30 p.m.	Keynote Presentation Siddhartha Mukherjee, M.D., D.Phil. *Assistant Professor of Medicine – Hematology/Oncology* Columbia University Medical Center	Masur Auditorium
12:30 p.m. - 1:30 p.m.	Lunch on Your Own	Building 10 Cafeterias
1:30 p.m. - 3:30 p.m.	NHGRI GREAT Awards Ceremony	Masur Auditorium
	Keynote Presentation Shirley M. Tilghman, Ph.D. *President Emerita,* *Professor of Molecular Biology and Public Affairs* Princeton University	
4:30 p.m. - 6:30 p.m.	Networking Event	Rock Bottom Restaurant, Downtown Bethesda

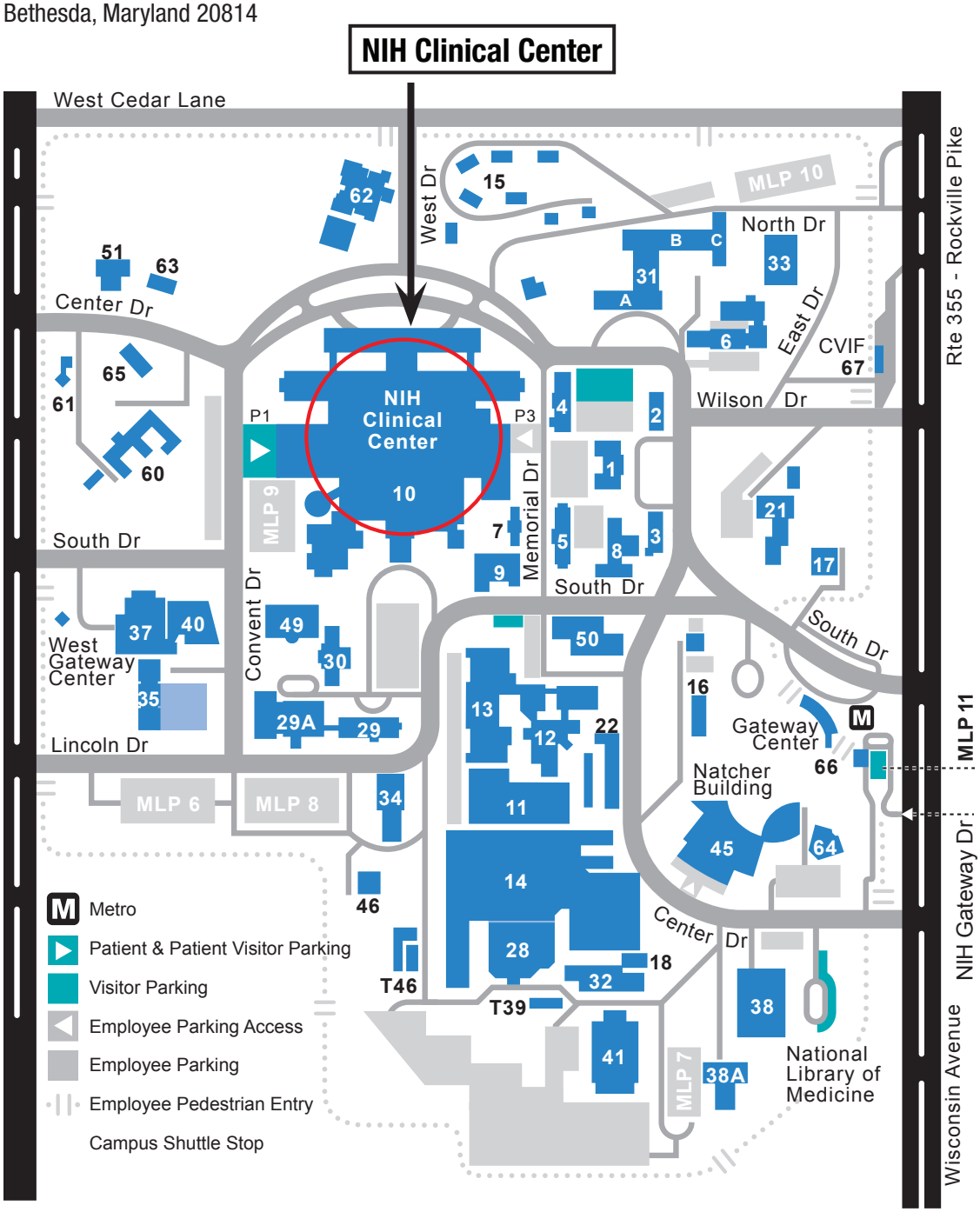
Tuesday, November 26

9:00 a.m. - 10:00 a.m.	Oral Presentations B	Masur Auditorium
10:00 a.m. - 11:30 a.m.	Poster Session B	FAES Terrace
11:30 a.m. - 12:30 p.m.	Doctor-Patient Conversation *A Lifetime of Uncertainty:* *Living with RUNX1 Deficiency* Paul Liu, M.D., Ph.D.	Masur Auditorium
12:30 p.m. - 12:45 p.m.	2019 NHGRI Symposium Poster Awards 2019 Intramural Research Training Awards 2019 Outstanding Faculty Mentorship Award	Masur Auditorium
12:45 p.m. - 12:50 p.m.	Closing Remarks Masur Auditorium Eric Green, M.D., Ph.D. Dan Kastner, M.D., Ph.D.	

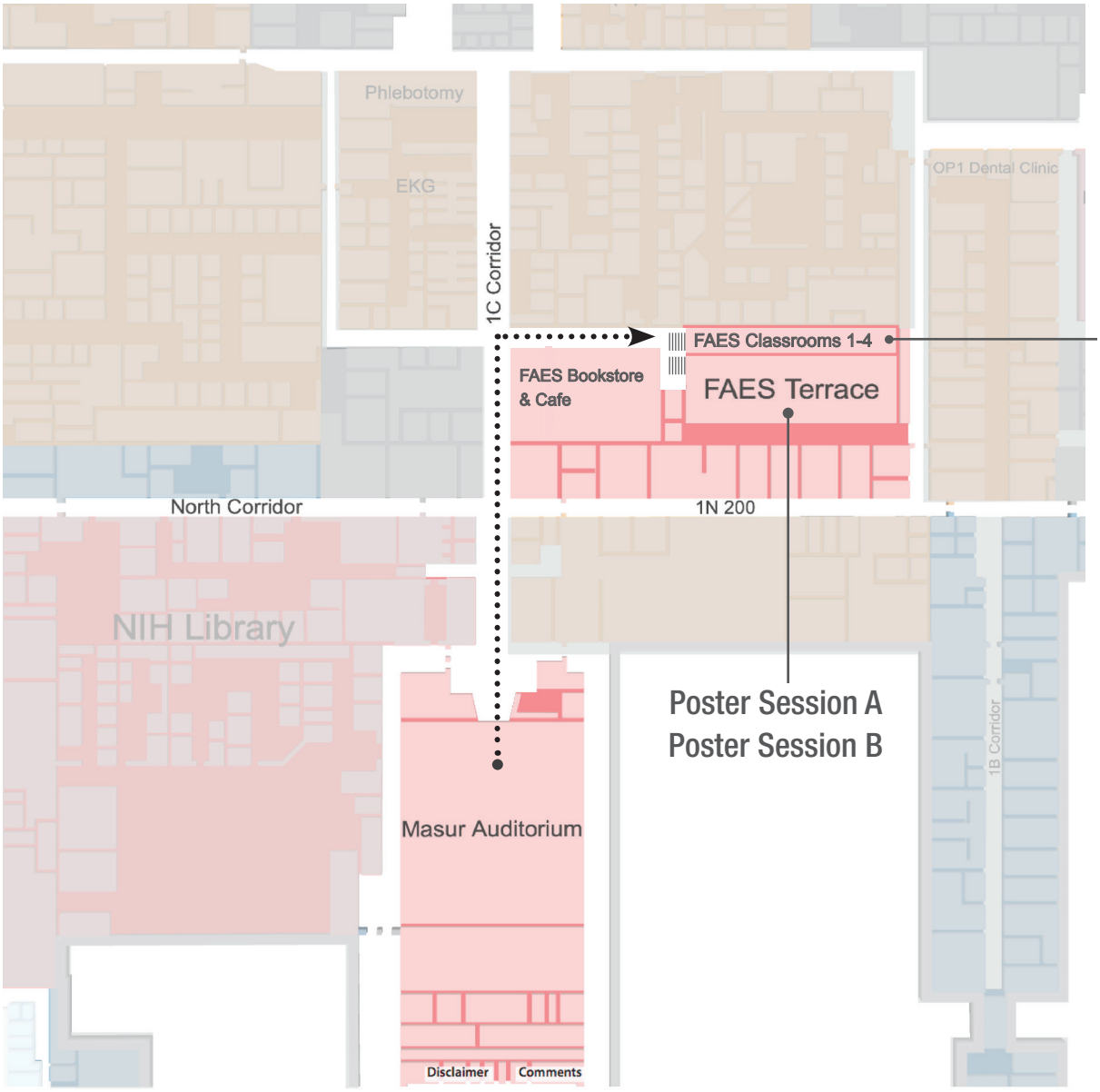
NIH Campus Map

NIH Clinical Center | Building 10

10 Center Drive
Bethesda, Maryland 20814



Poster Sessions | FAES Terrace, Classrooms 1-4



Information Tables

Poster Session A
Poster Session B

Please Note:

FAES Classrooms are located just downstairs from the FAES Terrace.

NIH Clinical Center | Building 10

Information Tables | FAES Classrooms 1-4

The following NHGRI offices will be hosting information tables during the poster session on Monday, November 25th.

- NHGRI Bioethics Core (BC)
- NHGRI Communcations and Public Liaison Branch (CPLB)
- NHGRI Education and Community Involvement Branch (ECIB)
- NHGRI Ethics Branch (EB)
- NHGRI Inclusion Task Force (ITF)
- NHGRI Information Technology Branch (ITB)
- NHGRI Management Analysis and Workforce Development Branch (MAWDB)
- NHGRI Protocol Service Center (PSC)

Guidelines for Poster and Oral Presentations

Authors of Poster Presentations

Each author of a poster presentation will find the number of his/her abstract on the assigned poster board. Tacks, tape, and other supplies will be available. The area provided for your visual materials will be a 48 x 48 inch tack board. Authors of all sessions should be at their displays and available to answer questions during the entire session.

Poster presenters should mount their displays at the following times:

Monday, November 25

Poster Session A	Last names A - Kat	10:00 a.m. – 11:30 a.m.

Posters in Session A should be mounted no later than 10:00 a.m. and removed by 11:30 a.m.

Tuesday, November 26

Poster Session B	Last names Kaz - W	10:00 a.m. – 11:30 a.m.

Posters in Session B should be mounted no later than 10:00 a.m. and removed by 11:30 a.m.

Authors of Oral Presentations

Authors of oral presentations will have a maximum of 10 minutes allocated for their presentations and 5 minutes for discussion. Strict adherence to this schedule will be the responsibility of the speakers and the chairperson. Each speaker in a session should arrive in the lecture hall at least thirty (30) minutes prior to the start of the session.

Monday, November 25

Oral Presentations A	O1 through O4	9:00 a.m. – 10:00 a.m.

Tuesday, November 26

Oral Presentations B	O5 through O8	9:00 a.m. – 10:00 a.m.

Oral Presentations

Monday, November 25

Oral Presentations A
9:00 a.m. – 10:00 a.m.

Jamie Diemer, Ph.D., Oncogenesis and Development Section

Xin Huang, Ph.D., Microbial Genomics Section

Senta Kapnick, Ph.D., Metabolism, Infection and Immunity Section

Prashant Sharma, Ph.D., Undiagnosed Diseases Program

Tuesday, November 26

Oral Presentations B
9:00 a.m. – 10:00 a.m.

Jorge Rodriguez-Gil, D.Phil., Genomics, Development and Disease Section

Erin Jimenez, Ph.D., Developmental Genomics Section

Christopher Marcum, Ph.D., Social Network Methods Section

Arang Rhie, Ph.D., Genome Informatics Section

NHGRI Symposium Keynote Presentation

Monday, November 25, 11:30 a.m. - 12:30 p.m.

Siddhartha Mukherjee, M.D., D.Phil.
Assistant Professor of Medicine – Hematology/Oncology
Columbia University Medical Center

Siddhartha Mukherjee is a pioneering physician, oncologist, and author who has redefined our public discourse on human health, medicine and science. A profoundly influential voice in the scientific community, he is best known for his books, *The Emperor of All Maladies: A Biography of Cancer*, which earned him the 2011 Pulitzer Prize, and *The Gene: An Intimate History* which won international awards and was recognized by *The Washington Post* and *The New York Times* as one of the most influential books of 2016. His published works exhibit an outstanding literary skill that has left an indelible mark on our culture, as *The Emperor of All Maladies* has been adapted into a documentary by filmmaker Ken Burns, and was included among *Time* magazine's 100 best nonfiction books of the past century.

Dr. Mukherjee's achievements as a writer and educator build upon his career as a renowned medical scholar. His groundbreaking studies into the composition and behavior of cancer cells have pushed the boundaries of modern medicine. His innovative research signals a paradigm shift in cancer pathology, and has enabled the development of treatments that reach beyond current pharmaceutical models toward new biological and cellular therapies. Serving as a professor of medicine at Columbia University and as a staff cancer physician at the university's medical center, Dr. Mukherjee generates hope for countless patients and families around the world, while revolutionizing our blueprint for healing. He writes for the *New Yorker, The New York Times Magazine* and many other publications, has received numerous awards for his scientific work, has published his original research and opinions in journals such *Nature, Cell and the New England Journal of Medicine*, and lives in New York City with his wife and daughters.

NHGRI Symposium Keynote Presentation

Tuesday, November 26, 2:30 p.m. - 3:30 p.m.

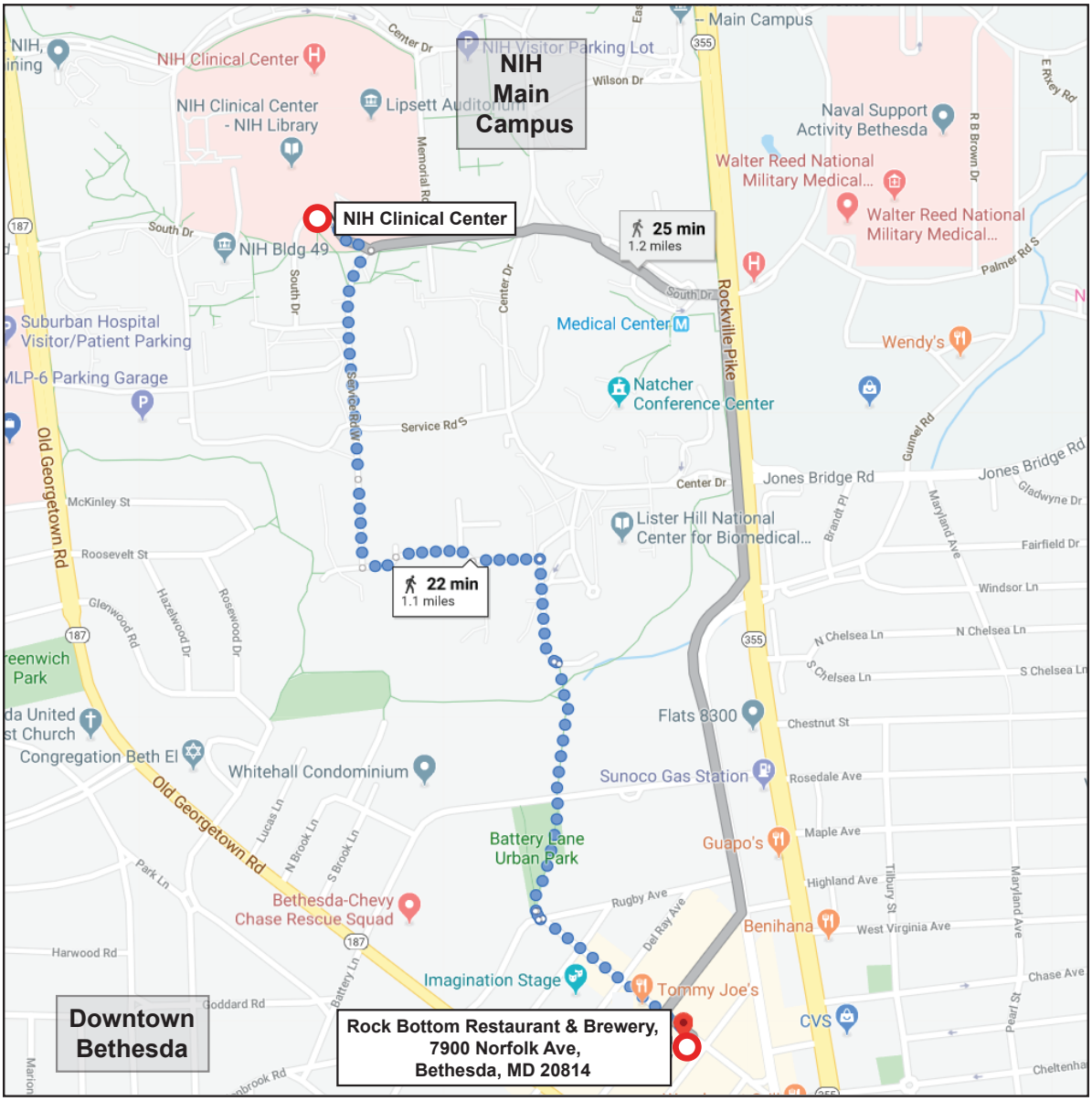
Shirley M. Tilghman, Ph.D.
President Emerita and Professor of Molecular Biology and Public Affairs,
Princeton University

Shirley M. Tilghman was elected Princeton University's 19th president on May 5, 2001 after serving on the Princeton faculty for 15 years. Upon the completion of her term in June of 2013, she returned to the faculty. During her scientific career as a mammalian developmental geneticist, she studied the way in which genes are organized in the genome and regulated during early development, and was one of the founding members of the National Advisory Council of the Human Genome Project for the National Institutes of Health.

Dr. Tilghman is an Officer of the Order of Canada, the recipient of a Lifetime Achievement Award from the Society for Developmental Biology, the Genetics Society of America Medal, and the L'Oreal-UNESCO Award for Women in Science. She is a member of the American Philosophical Society, the National Academy of Sciences, the National Academy of Medicine and The Royal Society of London. She serves as a trustee of Amherst College, the Institute for Advanced Study, and the Simons Foundation. She serves on the Science Advisory Board of the Chan Zuckerberg Initiative, is a director of The Broad Institute, and a Fellow of the Corporation of Harvard College.

Map to Networking Event

Monday, November 25, 4:30 p.m. - 6:30 p.m.



Walk 1.1 miles, 22 min

NIH Clinical Center, Building 10, NIH Main Campus
From NIH Clinical Center, walk south past Buidling 38, National Library of Medicine.
Exit the NIH main campus through the south pedestrian exit.
Continue along the Bethesda Trolley Trail path until it turns into Norfolk Ave.
Continue on Norfolk Ave for 4 blocks.
Rock Bottom Restaurant & Brewery, 7900 Norfolk Ave, Bethesda, MD 20814

2019 NHGRI Symposium

Oral Presentation Abstracts

Single Cell RNA-Sequencing to Uncover Cell Type-Specific Gene Expression Changes During Inv(16) Leukemia Initiation

O1
Oral

Jamie Diemer, Ph.D.

A subset of acute myeloid leukemias (AMLs) are marked by chromosomal abnormalities such as inversions and translocations affecting *RUNX1* or *CBFB.* For example, an inversion of chromosome 16 is seen in patients with the M4Eo subtype of AML, which leads to the expression of a fusion protein, CBFβ-SMMHC. This fusion protein acts as a driver of leukemogenesis but there are many unanswered mechanistic questions about the leukemogenic process. In this study, we have utilized single cell RNA-sequencing in a conditional knock-in mouse model (Kuo et. al, 2006), to profile the early changes of hematopoietic cell populations based on gene expression analysis in individual cells.

In total, cDNAs from more than 14,000 c-Kit+ bone marrow cells were sequenced from six mice in total—two each of a) wild-type, b) CBFβ-SMMHC expressing mice that were pre-leukemic, and c) leukemic CBFβ-SMMHC expressing mice. We find that the earliest hematopoietic compartment negatively affected by the expression of the fusion protein are those that express markers of late erythroid differentiation such as Rhag and Klf1. We also identify a population of cells that share many markers of the erythroid lineage but also co-express many abnormal genes, including known CBFB-SMMHC target genes such as *Il1rl1* and *Csf2rb*. The genes expressed in this population expand in the Kit(+) population of two leukemic mice, demonstrating the clonal expansion of this cell type. The early identification of these abnormal cells that expand to take over the progenitor population of the bone marrow enable an unbiased examination of markers which may be useful in the monitoring of leukemogenesis or the identification of novel therapeutic targets.

Tackling *Candida auris*, an emerging multi-drug resistant human fungal pathogen

O2
Oral

Xin Huang, Ph.D.

The emerging human pathogen, *Candida auris*, was first identified in 2009 from a clinical culture of a patient in Japan. Over the past decade, *C. auris* has emerged on multiple continents, including North America, as independent clades on different continents. It is highly resistant to azoles, with some strains resistant to all three major classes of antifungals. *C. auris* colonizes a patient's skin and persists on environmental surfaces, allowing it to spread rapidly through healthcare settings. Asymptomatic colonization on the skin by *C. auris* can lead to bloodstream infections through intravenous lines and its high antimicrobial resistance renders these infections recalcitrant to treatment. These factors prompted both the US Centers for Disease Control and Prevention (CDC) and the Public Health England to issue multiple clinical alerts in 2016 and 2017. Outbreaks have occurred in UK and US hospitals that have proven difficult to control, with an associated mortality rate of up to 60%.

In a collaboration between CDC and NHGRI, we aimed to develop sequencing protocols for *C. auris* outbreak samples from US healthcare facilities. We also developed mouse models to directly investigate risk factors for *C. auris* colonization.

We demonstrated that amplicon sequencing results corroborated with culturing results and helped in the development of more rapid molecular testing. Amplicon sequencing provides insight into the skin fungal and bacterial community profiles that are fundamental for microbially directed therapies. Using murine models, we first established that *C. auris* is primarily a skin colonizer. Surprisingly, we discovered long-term residence of *C. auris* within the deeper skin of wild-type mice as long as 4 months after it was cleared from the skin surface, which would complicate infection control measures in humans. We also discovered long-term skin colonization by *C. auris* for up to 4 months in an immune deficient mouse model, consistent with epidemiological survey data that immune deficiency is a risk factor for *C. auris* colonization. This mouse model is currently being used to test prevention and decolonization methods suitable for human populations.

Transmitochondrial cytoplasmic hybrids permit the study of mtDNA variation and immune cell function

O3
Oral

Senta Kapnick, Ph.D.

MtDNA encodes thirteen protein subunits of the respiratory chain responsible for generating cellular energy through oxidative phosphorylation (OXPHOS). Perturbations in OXPHOS can lead to mitochondrial disease (MD), clinically heterogenous disorders caused by mutations in nuclear or mitochondrial-encoded genes. While MDs manifest most severely in tissues with high energy requirements, patient reports reveal the emerging phenotype of immune dysfunction. Immune cells undergo metabolic changes that determine their ability to function. Mitochondria play a central role in regulating these metabolic pathways, thus immune cell function and mitochondrial-mediated metabolism are intimately linked. However, the metabolic consequences of OXPHOS defects and how they produce immunodeficiencies in MD patients remains unclear. To address this, we generated transmitochondrial cytoplasmic hybrids ("cybrids") as a tool for studying mtDNA variation. Cybrids incorporate mtDNA from different donors on a common nuclear background. We produced mtDNA-depleted (Rho0) cells from human Jurkat T-cell and monocyte THP-1 lines using a viral nuclease that triggers mtDNA depletion. To generate cybrids, we performed PEG-mediated fusion of Rho0 cells with platelets from healthy donors. Reconstitution was confirmed through PCR, microscopy, and Seahorse studies, showing loss/consequent restoration of cybrid respiration. These data establish a novel method for producing Rho0 lines and provide evidence that we can generate cybrids to study mtDNA variation, excluding the influence of nDNA. Work is underway producing/characterizing cybrids using donor material from patients with MD disease. This approach will shed light on how disease-associated mtDNA variants impact cellular immunity, and aid in treatment of immune dysfunction in patients with mtDNA-associated MD.

Analysis of a *de novo* mutation in *CUL3* reveals a novel mechanism of familial hyperkalemic hypertension

O4
Oral

Prashant Sharma, Ph.D.

Hypertension is a principal risk factor for cardiovascular diseases. Familial hyperkalemic hypertension, also known as pseudohypoaldosteronism type II (PHAII) or Gordon syndrome is a rare Mendelian form of hypertension caused by the imbalance between renal salt reabsorption and K^+ and H^+ excretion resulting in hyperkalemia and metabolic acidosis. Mutations in *CUL3* have been associated with PHAII (PHA2E), however, a consensus mechanism by which they cause disease has not emerged. CUL3 (Cullin 3), is a critical subunit of the Cullin 3-RING ubiquitin ligase (CRL3) complex. It acts as a scaffold protein for the other CRL3 subunits in order to facilitate the ubiquitination of the substrates including With-no-lysine (K) kinases (WNKs), which control potassium homeostasis and blood pressure by regulating the activity of Na^+Cl^- cotransporter (NCC) along the distal convoluted tubule of the kidney.

Here we define a mechanistic role of CUL3 in hyperkalemic hypertension by investigating the effect of a novel *de novo* heterozygous mutation (NM_001257198.1: c.1420_1431del12; p.Phe474_Met477del) found in a NIH Undiagnosed Diseases Program patient, who presented with elevated serum potassium, hypertension, global developmental delay, short stature, and frequent pulmonary and gastrointestinal infections. Analysis of patient-derived dermal fibroblasts demonstrated increased conjugation of mutant CUL3 with Nedd8 (neddylation) and reduced stability of total CUL3. Co-expression of mutant CUL3 with deneddylase Jun activation domain-binding protein-1 (JAB1) in cultured kidney cells revealed co-interaction but the loss of functional activity of JAB1 to recycle mutant CUL3. Consequently, the ability of JAB1 to form a homodimer and regulate the levels of WNK1 is significantly reduced. Analysis of urinary exosomes from the proband and unaffected sibling showed increased activation of NCC in the proband, likely contributing to electrolyte abnormalities and higher blood pressure. These results suggest a fundamental role of CUL3 in electrolyte abnormalities and hypertension. Additionally, we found that mutant CUL3 causes a dysregulation of JAB1 function. This interaction has identified a new regulatory mechanism for JAB1, which lies at the crossroads of numerous signaling pathways that may lead to a better understanding of many disease pathologies.

Genetic Background Modifies Phenotypic Severity and Longevity in a Mouse Model of Niemann-Pick Disease Type C1

O5
Oral

Jorge Rodriguez-Gil, D.Phil.

Niemann-Pick disease type C1 (NPC1) is a rare, fatal neurodegenerative disorder characterized by lysosomal accumulation of unesterified cholesterol and glycosphingolipids. These subcellular pathologies lead to phenotypes of hepatosplenomegaly, neurological degeneration, and premature death. NPC1 is extremely heterogeneous in the timing of clinical presentation and is associated with a wide spectrum of causative NPC1 mutations. To study the genetic architecture of NPC1, including the clinical and genetic heterogeneity seen in this patient population, we have generated a new NPC1 mouse model, *Npc1*^{em1Pav}. *Npc1*$^{em1Pav/em1Pav}$ mutants showed significantly reduced NPC1 protein compared to controls and displayed the pathological and biochemical hallmarks of NPC1. Interestingly, *Npc1*$^{em1Pav/em1Pav}$ mutants on a C57BL/6J genetic background showed more severe visceral pathology, quantified by the presence of CD68+ foam cells, compared to Npc1$^{em1Pav/em1Pav}$ mutants on a BALB/cJ background. Furthermore, C57BL/6J mutants exhibited a significantly shorter lifespan (70 + 4.30 days) than *Npc1*$^{em1Pav/em1Pav}$ mutants on a BALB/cJ genetic background (84 + 7.25 days), suggesting strain-specific modifiers contribute to disease severity and survival. QTL analysis for lifespan of 202 backcross N2 mutants on a mixed C57BL/6J and BALB/cJ background detected significant linkage to markers on chromosomes 1 (LOD=5.57) and 7 (LOD=8.91). The discovery of these modifier regions demonstrates that mouse models are powerful tools for analyzing the genetics underlying rare human disease, which can be used to improve understanding of the variability in NPC1 phenotypes and advance options for patient diagnosis and therapy.

Genomic Analysis of Enhancer Identity and Function in Hair Cell Regeneration

O6
Oral

Erin Jimenez, Ph.D.

Millions of Americans experience a hearing or balance disorder due to permanent hair cell damage/loss. The hair cells are the mechanosensory cells used in the auditory and vestibular organs of all vertebrates and in the lateral line of aquatic vertebrates. In zebrafish and other non-mammalian vertebrates, hair cells turn over during homeostasis and regenerate completely after being destroyed or damaged by acoustic or chemical exposure, while in mammals, destroying or damaging hair cells results in permanent impairments to hearing/balance.

Studies implicate enhancers in response to tissue damage, raising the possibility that enhancer elements may exist in the zebrafish inner ear in response to hair cell death. Our goal is to identify the enhancers involved in repairing a functional vertebrate inner ear. To identify enhancers in response to hair cell damage, we developed a transgenic zebrafish to permit conditional and selective ablation of hair cells in the adult inner ear. This model expresses the *human diphtheria toxin receptor (hDTR)* gene under the control of the *myo6b* promoter, resulting in *hDTR* expressed specifically in hair cells. Cell ablation is achieved by intraperitoneal injection of diphtheria toxin which has a high affinity for the human receptor. On adult zebrafish that have undergone hair cell ablation, we investigated the epigenome and transcriptome of cells from the inner ear at time-points following hair cell ablation.

Because physical accessibility of genomic DNA can be used as a proxy for the “active” genome, we identified open chromatin locations using single-cell Assay for Transposase Accessible Chromatin with high-throughput sequencing (scATAC-seq). scATAC-seq revealed transcription factor motif patterns in specific cell types that become accessible as a consequence of hair cell regeneration. Enhancer accessibility correlates with outputs from downstream genes. Therefore, we used single-cell transcriptomics to identify genes transcriptionally responsive to regeneration. Correlation of RNA expression and chromatin accessibility revealed transcriptomic and epigenomic events that occur in a regenerating inner ear. Future work will employ CRISPR/Cas9 mutagenesis to generate mutants and functionally validate identified enhancers for roles in hair cell regeneration. Results from our approaches will a have an important impact on our understanding of hair cell regeneration mechanisms.

A Novel Approach to Modeling Association in Multi-Layered Networks

O7
Oral

Christopher Marcum, Ph.D.

Networks, which consist of entities and the relationships between them, are of great interest across scientific disciplines. While traditional approaches to studying networks focus on just a single relationship—such as communication between humans or physical proximity among proteins—there are a growing number of studies that ask questions about the structure of networks observed with multiple relationships. Such multi-layered networks are especially important in the social sciences, where multivariate measurement models are commonplace. One challenge of analyzing these networks using contemporary statistical approaches, however, is that the layers may be correlated. When correlation is present in the data, statistical estimation and inference can be difficult to impossible unless it is properly controlled. To address this problem, we've introduced a novel approach to control for underlying association in multi-layered networks. Using the exponential random graph family of models we developed a statistic called the Conway-Maxwell-Binomial parameter that monitors whenever relationships are simultaneously present across layers. The coefficient associated with this statistic represents a marginal effect of correlation in the network. We apply this new method to reveal how cohesion and conflict are structured in 31 family networks coping with the challenge of a history of Lynch Syndrome (LS). Each network consists of 14 layers; 7 each measuring cohesion and conflict, respectively. Our results demonstrate that models incorporating the new statistic more accurately recapitulate underlying association than alternatives. Future work will use this approach to test hypotheses about how cohesion and conflict differ in the support networks of families affected by LS. Additionally, the approach has broad applicability to fields outside of social science that study multi-layered networks.

Towards high quality reference assemblies for all vertebrate genomes

O8
Oral

Arang Rhie, Ph.D.

High quality reference genomes are important to understand functional, structural, or epigenetic effects that may lead to clinically or evolutionary relevant differences. Even in the human genome, until recently, only the euchromatic regions have been studied, leaving the rest in darkness.

Recently, continuing technological improvements and decreases in sequencing costs have allowed the assembly of a multitude of new genomes at unprecedented quality compared to the past. With the goal of building high quality, near gapless, chromosome scale, phased assemblies, the Vertebrate Genomes Project (VGP) has begun to assemble all vertebrate genomes. Initially, the VGP defined a minimum standard for all assemblies, aiming for mega-base scale contigs and assigning >90% of the genome to chromosomes at a base level accuracy of 99.99%.

We found haplotype differences were the primary cause of genome assembly complexity, and developed a method using parental genomes to bin the child's reads by haplotype. Using this approach, we have achieved high quality genome assemblies for diverse species, such as birds, cattle, amphibians, and fishes, including the sex chromosomes.

For genomes where parents are not available, we developed a pipeline using four new technologies which we have found give the best results to date.

Moreover, using related methods, we recently finished the human X chromosome using Nanopore ultra long reads, and successfully assembled the centromere, one of the most difficult to assemble regions in the human genome.

2019 NHGRI Symposium

Poster Abstracts

C G T C G T
A C G A C G
C G T C G T
A C G

Session A
Last Names A - Kat

Humanization of yeast EIF2B for high-throughput variant analysis

A1
Poster

Ilya Andreev [1], Meru Sadhu [1]

One of the roles of genetics is to inform patients of their susceptibility to disease due to mutations in the genome. Vanishing white matter disease (VWM) is a rare genetic disease that is characterized by extensive white matter degradation in the brain. It is caused by loss-of-function mutations in the pentameric eIF2B protein complex, which functions as a guanine nucleotide exchange factor (GEF) for the eIF2 complex that plays an essential role in cellular translation. We will use CRISPR-based high-throughput mutagenesis to generate a large number of novel missense and nonsense mutations in human EIF2B, approximately 15,000 possible mutations that can be reached by a single-nucleotide substitution, and subsequently analyze the degree of their deleteriousness in budding yeast S. cerevisiae. eIF2B function is conserved between humans and yeast and is well studied in the yeast context, which enables us to perform a variety of phenotypic and genetic assays to identify and characterize the effects of eIF2B variants. Using CRISPR, we will first replace the five subunits of eIF2B in yeast with their human orthologs (yeast humanization). Next, we will introduce individual mutations into our yeast, grow them in pool, and then use next-generation sequencing to categorize our mutations as benign, leading to partial loss of function, or to a complete loss of function. Ultimately, we expect that the results of this project will help patients with VWM and clinical geneticists who actively work with these patients and devise disease management plans for them.

1. Systems Biology and Genome Engineering Section, Genetic Disease Research Branch, NHGRI

Exploring Mechanisms of Regeneration Across the Animal Tree: Implications for Human Health

A2
Poster

Sofia Barreira [1], Andy Baxevanis [1]

The animal kingdom is comprised of thousands of incredibly diverse organisms. Despite their very different body forms, these organisms often share many common characteristics at the molecular level. For example, there are numerous organisms such as salamanders that can regenerate lost limbs, and the mechanisms these organisms use to regrow lost or damaged body parts almost always involve the same sets of genes. While these same genes can also be found in the human genome, our own ability to regrow lost tissues is limited for the most part to our livers and skin. We have been studying the genomes of a wide variety of organisms, from simple to complex, to determine what could be responsible for losing much of our own regenerative capacity. For an organism to regenerate, it needs to be able to produce vast quantities of new cells, leading us to study a particular protein called the Upstream Binding Factor (UBF). This protein is responsible for recruiting the cellular machinery that generates all the different types of proteins in a cell. Interestingly, simpler animals that have the ability to regenerate any part of their bodies do not have the same version of the UBF protein that is found in humans and other mammals, possibly providing an important clue as to why we humans have diminished regenerative capacity. Understanding these mechanisms is an important first step towards coming up with therapies that will allow us to repair our own tissues and organs.

1. Computational Genomics Unit, Computational and Statistical Genomics Branch, NHGRI

Developing a Policy for Return of Genetic Results at NIH

A3
Poster

Benjamin Berkman [1,2]

Sequencing is becoming increasingly ubiquitous as a research tool, and there has been a robust debate about the contours of investigators' obligation to return incidental or secondary findings. Much of the existing policy guidance suggests deferring to IRBs to make a protocol-by-protocol determination, but individual IRBs have taken extremely variable approaches resulting in inconsistent and inequitable outcomes. With the advent of a centralized IRB for the NIH Intramural Research Program, there is an opportunity to develop a unified policy for return of genetic results. This poster describes a detailed proposal that is currently under consideration. Specifically, I argue that there is a broad but shallow obligation to return secondary genetic results; broad in the sense that it applies to most research protocols but narrow in the sense that it employs a high threshold for determining which information should be returned. I also argue that the obligation falls to the institution rather than the individual researcher for a number of justice and efficiency reasons.

1. NIH Department of Bioethics
2. NHGRI Bioethics Core

Fetal growth restriction and microcephaly in mouse models of Down syndrome

A4
Poster

Lauren Bishop [1], A. Adams [1], Faycal Guedj [1], Diana W. Bianchi [1]

Objective: We aimed to investigate if fetal growth restriction is associated with reduction in forebrain, hippocampus, and cerebellar volume in Dp(16)1/Yey, Ts65Dn, and Ts1Cje mouse models of DS. Human chromosome 21 genes are orthologous to genes on Mus musculus (Mmu) chromosomes 10, 16 and 17. Each DS model has triplication of different orthologous and non-orthologous genes.

Methods: Dp(16)1/Yey and Ts1Cje males were mated to C57BL/6 females, and Ts65Dn females to C57BL/6XC3Sn F1 males. Pregnant dams were euthanized at day E18.5. Embryos were extracted, weighed and genotyped by PCR. We created a growth chart for all euthanized embryos (51 Dp(16)1/Yey & 68 Euploid (Eup); 44 Ts65Dn & 44 Eup; 51 Ts1Cje & 75 Eup). Trisomic embryos in the lowest 10% were classified as TS, and those above the 10% as TM. Brain volume estimations were performed on Nissl-stained serial coronal sections using the Cavalieri estimator. Unpaired t-tests compared Eup to all trisomic embryos, and one-way ANOVA determined differences in volumes according to growth restriction severity.

Results: Compared with Eup, Ts1Cje and Ts65Dn embryos had reduced cerebellar volumes that positively correlated with the degree of growth restriction. Hippocampal volumes were reduced in Ts1Cje and Ts65Dn trisomic embryos, though there was no correlation with growth restriction severity. Dp(16)1/Yey TM embryos had a larger cerebellum and hippocampus compared to Eup embryos; this was significant for the hippocampus. Forebrain volume was reduced in trisomic embryos across all strains. This was only significant in Dp(16)1/Yey.

Conclusions: Fetal growth restriction in mouse models of DS is associated with microcephaly. Future studies are needed to determine mechanisms underlying growth restriction and its impact on brain development, and whether triplication of specific genes affects certain brain areas.

1. Prenatal Genomics and Therapy Section, Medical Genetics Branch, NHGRI

Aberrant CNS immune activation and signaling in Leigh Syndrome

A5
Poster

Cassidy Burke [1], Peter McGuire [1]

Leigh syndrome (LS, OMIM 25600) is a mitochondrial disorder characterized by the development of bilateral necrotizing CNS lesions. A challenging aspect of managing patients with LS is metabolic decompensation due to infection. This life-threatening deterioration in mitochondrial function oftentimes hastens neurologic disease progression and disability. To define the mechanisms by which infection may promote disease progression, we infected a mouse model of Leigh Syndrome (Ndufs4-/-) with influenza virus. qPCR for inflammatory cytokine expression and viral nucleic acids was performed on selected brain regions. Interestingly, the expression of inflammatory cytokines (e.g. TNF alpha) was elevated in the olfactory bulb of Ndufs4-/- mice at baseline. Infection with influenza resulted in augmented cytokine production in Ndufs4-/- versus wildtype mice. These results suggest that LS may have neuroinflammation at baseline which is enhanced during infection. Virus was detectable in the olfactory bulb and brainstem of Ndufs4-/- mice. Based on the detection of virus in the CNS, we asked whether LS neurons were able to mount an antiviral response. Human LS (SURF1-/-) and control neurons, derived from iPSC, were treated with the viral RNA mimic poly I:C and the expression of interferon beta (IFN beta) was assessed. Preliminary data indicate that IFN beta expression is decreased in LS neurons, implying that the CNS may provide a permissive environment for viral infection. Overall, our results suggest that the CNS in LS displays aberrant innate immune activation and antiviral signaling, both of which may play a role in the exacerbation of neurologic symptoms in LS during infection.

1. Metabolism, Infection and Immunity Section, Medical Genomics and Metabolic Genetics Branch, NHGRI

Checking the Second Box: The Missing History of “Ethnicity” in Genetics Research

A6
Poster

Julia Byeon [1], Christopher Donohue [2], Vence Bonham [1]

The use of race and ethnicity as population descriptors or proxies for genetic variation has been an ongoing challenge for the field of genomics. Much of this challenge is grounded in the desire to move past problematic notions of biological race and racial hierarchies of the 18th, 19th, and 20th centuries. Ethnicity is commonly used interchangeably with race in genomics, and yet our knowledge of the history of this concept is limited in comparison to that of race. This study traces the conceptual history of “ethnicity” and highlights its connections to 18th-20th century racial science by examining the American Journal of Human Genetics from its inception in 1949 to 2018. We calculate the frequency of the use of terms related to “ethnic” and “racial” in this journal and conduct a qualitative content analysis of concepts of “ethnic group” in all articles published from 1949-1969. We found that the use of the term “ethnic” has increased dramatically over the use of “race” from 1949 to 2018. “Ethnic”-related terms were present in 10% of articles in 1949-58 and increased to 32% in 2009-18; on the contrary, “race”-related terms were present in 25% of articles in 1949-58 and decreased to 6% in 2018-2009. Qualitative content analysis indicates that the “ethnic group” was a genetic population which had many continuities with historical biological concepts of race in mid-20th century genetics. This investigation informs current efforts to find consensus on the appropriate use of race and ethnicity in genomic research.

1. Health Disparities Unit, Social and Behavioral Research Branch, NHGRI
2. Communications and Public Liaison Branch, Division of Policy, Communications, and Education, NHGRI

The Technology Development Program at NHGRI

A7
Poster

Eileen Cahill [1], Chris Wellington [1], Shurjo Sen [1], Daniel Gilchrist [1], Anastasia Wise [2], Lisa Chadwick [1], Mike Pazin [1], Carolyn Hutter [1], Michael Smith [1]

The development of genomic technologies for application to basic and clinical research questions is crucial to advancing genomics. NHGRI has a track record of supporting high-impact genomic technologies in opportune areas of significant need. Investing in both new and improved technologies catalyzes answers to valuable scientific questions and opens avenues of inquiry in genomics, biomedical research, and medicine. Consequentially, there are various technology development phases that range from incremental advances in existing platforms to "quantum leap" innovations. Generally, the most effective genomic technologies are characterized by scalability and parallelization. Transformative genomic technologies yield quantitative results of robust value and quality for comprehensive genome-wide analyses of various sample types (e.g., individual humans or cells). Once new technologies are developed as tools for research where ease-of-operation and quickness are increasingly important, many of these technologies can also be applied in clinical settings. Genomic technology advances have revolutionized biomedical and clinical science. However, achieving technological accuracy and completeness, addressing increasing information content, limiting costs, and reducing analyte requirements remain areas of need and opportunity. Research settings and funding approaches for various technology innovation and development projects range from single-lab research grants to multi-lab sustained efforts. The NHGRI Technology Development Program supports a broad suite of genomic technologies with a focus on nucleic acid sequencing and will include a new effort in synthetic nucleic acids. In continued programmatic efforts, NHGRI convenes a yearly grantee meeting where the latest progress is shared and collaborations are facilitated, forming the basis of a productive technology development community.

1. Division of Genome Sciences, NHGRI
2. Division of Genomic Medicine, NHGRI

Decision-making in Mental Health Referral: The Influence of Genetic Counselor Emotion

A8
Poster

Hannah Campbell [1,2], Debra Roter [2], Rebecca Ferrer [3], Amy Turriff [1], Julie Cohen [4], William Klein [3]

To date, little research has focused specifically on the mental health referral practices of genetic counselors (GCs). This study aims to evaluate how the emotional state of GCs might influence their decision to refer clients to mental health services. Study design is based within the appraisal tendency framework of decision-making, which hypothesizes that an individual's emotional state influences the certainty of a decision. This study surveyed practicing GCs recruited from the National Society of Genetic Counselors e-mail listserv. Participants were randomized into three groups to complete a writing exercise meant to induce an emotional state (anger, sadness, or neutral control). GCs were then asked to read three case vignettes. They answered questions about the mental health status of each hypothetical case, and whether they would refer the client to mental health services. Participants rated decision certainty on a 7-point Likert scale. Questions about GCs clinical practice related to mental health assessment and referral were included in the survey. The primary outcome of our study is the level of certainty around the referral decision. To date we have received 30 survey responses. Preliminary results will be shared at the NHGRI symposium.

1. National Human Genome Research Institute
2. Johns Hopkins Bloomberg School of Public Health
3. National Cancer Institute
4. Kennedy Krieger Institute

Exploring the combinatorial fitness landscape of co-evolving human and viral proteins.

A9
Poster

Michael Chambers [1], Thomas Dever [2], Meru Sadhu [1]

We are using deep mutational scanning to explore an evolutionary arms race between the human innate immune system and poxviruses. Protein kinase R (PRK) is a component of the innate immune system that detects the presence of double-stranded RNA (dsRNA), indicating the presence of a foreign entity within the cell. Once activated by dsRNA, PKR phosphorylates the translation initiation factor subunit eIF2α, ultimately halting translation within the cell and preventing viral replication. As a counter defense, poxviruses encode a PKR antagonist denoted as K3L which inhibits the phosphorylation and activation of eIF2α to allow the virus to replicate. Both PKR and K3L are under diversifying selection pressure as PKR must discriminate against K3L while K3L must overcome this discrimination to replicate. The interaction of these two proteins under diversifying selection drives an evolutionary arms race in which superior variants of PKR and K3L are continually pursued. Here we propose a novel approach to generate single-residue variant libraries of both PKR and K3L and simultaneously assess their function with a yeast growth readout. This approach will generate a variant interaction matrix covering a large combinatorial space in a single experiment, highlighting points of constraint and providing a glimpse into the evolutionary landscape in which PKR and K3L are bound.

1. Systems Biology and Genome Engineering Section, Genetic Disease Research Branch, NHGRI
2. Section on Protein Biosynthesis, NICHD

A website for return of negative secondary findings – impacts on understanding and health behavior intentions

A10
Poster

Priscilla Chan [1], Katie Lewis [1], Paul Han [3], William Klein [4], Mark Fredriksen [2], Anh-Dao Nguyen[1], Tyra Wolfsberg [2], Barbara Biesecker [5], Leslie Biesecker [1]

Although genetic counseling is usually conducted in-person, the shortage of counselors creates an opportunity to research alternative delivery models. Returning negative secondary findings (NSF) requires significant resources due to their volume but are low-impact results. The main concern is that NSF should not lead to false reassurance or diminished adherence to healthcare recommendations. Thus, NSF are an optimal target for research on alternative delivery models and to determine if they lead to changes in screening practices.

We developed a website about NSF, including what they are, their limitations and their health implications. Participants came from the ClinSeq project's African-descended cohort. After viewing the website, participants were sent their NSF report and a survey about their subjective and objective understanding of NSF and their health behavior intentions.

Most potential participants agreed to participate (428/483 = 88.6%), viewed the website (341/428 = 79.7%) and completed the survey (285/341 = 83.6%). Most had a college degree or more (71.3%), were female (76.1%), and were not Hispanic or Latino (98.9%). Average scores on the objective understanding of NSF limitations scale were high (mean = 4.0, SD = 0.7, range 1-5) and most self-reported understanding NSF at least "moderately" well (71.8%). Few reported decreased intentions to have mammograms (n = 1), colonoscopies (n = 3), or cholesterol screenings (n = 3), while majority reported no change in screening intentions (81.5%, 74.2% and 71.3%, respectively).

This provides evidence that websites may be effective tools for educating about NSF without decreasing intentions to adhere to health screenings.

1. Clinical Genomics Section, Medical Genomics and Metabolic Genetics Branch, NHGRI
2. Bioinformatics and Scientific Programming Core, Computational and Statistical Genomics Branch, NHGRI
3. Maine Medical Center Research Institute
4. National Cancer Institute
5. Research Triangle Institute

Expanding our menu of human disease models through whole-genome sequencing

A11
Poster

E. Sally Chang [1], Paul Gonzalez [1], Christine Schnitzler [2], Andreas Baxevanis [1]

Model organisms – non-human species that give us insight into human biology – are selected for attributes such as ease of care and relative biological simplicity, allowing researchers to better understand important aspects of human biology. One way to learn from these organisms is to study sets of genes associated with human traits like susceptibility to a disease. Model organisms, like the fruit fly, possess many of these genes, meaning that studying them can tell us about our own genetics. Historically, much of our knowledge of the function of these genes was gained through experimentation on a relatively small number of model organisms. However, the current ease of genome sequencing allows us to evaluate whether a much wider array of potential organisms contain relevant genes in their genomes. We searched the genomes of a diverse set of organisms for about 4,000 genes involved in human disease, such as genes linked to breast cancer. We found that most of these disease genes are present in a wide variety of genomes, even in organisms that are distantly related to humans. In fact, some organisms that do not possess the organs or tissues associated with these diseases possess a similar percentage of disease genes compared with established models. Interestingly, specific categories of genes, like those associated with cancer, have distinct patterns of presence in different organisms. Our work suggests that more organisms should be studied as models of human disease, as seemingly unlikely organisms may hold vital insights within their genomes.

1. Computational Genomics Unit, Computational and Statistical Genomics Branch, NHGRI
2. Whitney Marine Laboratory, University of Florida

A Time and Motion Study from the Clinical Sequencing Evidence-Generating Research (CSER) Consortium

A12
Poster

Joanna Chau [1], Lucia Hindorff [1], Kristen Hassmiller-Lich [2]

As genomic sequencing becomes more prevalent in the clinic, it is necessary to evaluate the resources required for this integration. A "time and motion" study is a scientific health economics method to assess time efficiency in the clinic. By measuring the duration of each step of patient care, researchers can identify bottlenecks in the clinical process. The Clinical Sequencing Evidence-Generating Research (CSER) consortium is an NIH-funded program to incorporate genomic sequencing in the clinic, with a focus on diverse and medically underserved populations. Six extramural project sites enroll patients with various phenotypes, such as fetal structural anomalies and adults at risk for hereditary cancer. CSER is developing a time and motion study to assess the costs and benefits of implementing genomic innovations in the clinic.

The CSER Time and Motion Study Subgroup is currently focused on several aspects of study design. Clinical workflows were compared across the project sites to identify common process steps, which informed the development of standard data collection tools to track the time and resources for each step of the clinical process. Eligible patients for the time and motion study will include a subset of the overall CSER enrollment, focusing on "medically underserved" patients and cases with complex findings. Data collection is projected to start in January 2020. As one of the few time and motion studies examining the cost-effectiveness of incorporating genomic sequencing in healthcare, the CSER Time and Motion Study has the potential to advance understanding of clinical genomics implementation in diverse populations.

1. Division of Genomic Medicine, NHGRI
2. University of North Carolina, Chapel Hill

NHGRI Office of Laboratory Animal Medicine

A13
Poster

Tannia Clark [1], Wendy Pridgen [1], Irene Ginty [1], Stephen Frederickson [1]

The National Human Genome Research Institute (NHGRI) Office of Laboratory Animal Medicine (OLAM) promotes the humane care and use of animals in biomedical and behavioral research, teaching and testing. Animals are an important component of the research conducted at NHGRI. OLAM is comprised of subject matter experts to assist investigators with essential animal research support services such as animal study proposal development, hands-on training in the proper care, use and humane treatment of research animals and the ordering/importing/exporting of animal models. In addition, OLAM is responsible for managing the Animal Care and Use Committee (ACUC), which is responsible for the oversight of the Animal Care and Use Program, the veterinary care, housing and maintenance of research animals and for enhancing their well-being.

1. Office of Laboratory Animal Medicine, Division of Intramural Research, NHGRI

Using whole-genome sequencing to investigate hospital-associated bacterial reservoirs

A14
Poster

Sean Conlan [1], Ryan Johnson [1], Rebecca Weingarten [3], Clay Deming [1], Pamela Thomas [2], Morgan Park [2], NISC Comparative Sequencing Program [2], Tara Palmore [3], Karen Frank [3], Julia Segre [1]

Hospital acquired infections (HAIs) impact 650,000 patients annually, with estimated costs in the billions of dollars. The hospital environment is a reservoir for bacteria that can cause such infections. We have performed two large scale genomic analyses of bacteria associated with hospital plumbing and infrastructure, in order to better characterize this environment. In the first study, we identified hospital plumbing as the reservoir for a cluster of multi-drug resistant Sphingomonas koreensis isolates collected from patients over a decade. Whole genome sequencing of 81 isolates and 49 plate swipes identified a clonal population, suggesting a common source that has persisted over time. We conclude that an S. koreensis reservoir exists within the plumbing system, resulting in persistent recolonization of sinks in patient rooms.

In a second study, sink drains, housekeeping equipment and high-touch surfaces were surveyed for carbapenemase-producing organisms (CPOs). Whole-genome sequencing and analysis of 108 isolates from patients and the environment provided comprehensive characterization of CPOs, enabling an in-depth genetic comparison. Despite a very low prevalence of patient infections with CPOs, all samples from the intensive care unit pipe wastewater and external manholes contained CPOs. Surprisingly, we detected minimal overlap between plasmids in the environment and those associated with patient isolates, indicating that horizontal transmission between environmental and patient isolates is rare. Nonetheless, we did detect examples of connections between patients and the environment. These studies further our understanding of gene-flow between clinically relevant microorganisms and the importance of bacterial reservoirs within the hospital environment.

1. Microbial Genomics Section, Translational and Functional Genomics Branch, NHGRI
2. NIH Intramural Sequencing Center, NHGRI
3. NIH Clinical Center

Clinical Implications of Active Coping on Sleep: John Henryism in a Sickle Cell Disease Cohort

A15
Poster

Kayla E. Cooper [1], Khadijah E. Abdallah [1], Ashley J. Buscetta [1], Vence L. Bonham [1]

Poor sleep quality is often associated with psychosocial and clinical factors contributing to disease burden and stress. One of these psychosocial factors is John Henryism (JH). Defined as a high-effort, active coping style, JH is used by persons with a strong determination to succeed in the face of chronic stressors. Living with SCD is a unique stressor and how persons cope with their disease may impact their health outcomes. The objective of this study is to evaluate the effect of sleep quality on high-effort coping among persons with SCD.

The sample comprised 191 adults aged 19-71 with SCD. Our binary outcome assessed participants' high or low utilization of JH coping style by using the John Henryism Active Coping Scale. Scores range from 12 to 60 with higher scores indicating higher utilization of JH active coping. Predictors included demographic data and psychosocial measures. Sleep quality was assessed via clinical and survey measures.

Multivariable logistic regression was performed to evaluate differences within the cohort. The mean JH score was 52, indicating high utilization of active coping. Poorer sleep quality was associated with higher JH coping (OR:1.14, 95% CI: [1.06-1.22]). There were no significant associations between utilization of JH active coping with the demographic data.

Preliminary findings support a relationship between JH and sleep quality in a SCD cohort.

Future work should focus on how these psychosocial factors impact sleep and clinical presentation. This may provide an avenue for improved quality of life and disease management as well as informing treatment options.

1. Health Disparities Unit, Social and Behavioral Research Branch, NHGRI

Bioinformatics and Scientific Programming Core

A16
Poster

Bioinformatics Core Staff [1]

The Bioinformatics and Scientific Programming Core collaborates with NHGRI investigators on computationally intensive projects and creates Web-based resources to disseminate DIR lab data. Recent projects include:

(1) Developing a high-throughput exome and genome genotyping pipeline that enables efficient incremental re-genotyping of cohorts as new samples are added. We are using this pipeline to genotype additional samples for The Genomic Ascertainment Cohort (TGAC); >6000 genotypes are currently available to the NIH community through a gnomAD-style variant browser.

(2) Investigating the genetics of type 2 diabetes by analyzing single-cell RNA-seq data to uncover differences in cell type composition and gene expression between pancreatic islets from type 2 diabetic and non-diabetic individuals.

(3) Identifying retroviral integration sites in mouse and human genomes and refining methods to characterize the statistical significance of the integration patterns.

To facilitate DIR clinical studies, the Core offers Labmatrix, a HIPAA-compliant web-based database system. Labmatrix manages study data and tracks patient samples, including barcoding and storage. Users benefit from integrated querying and reporting tools, and staff provide support for handling large and complex data sets. Clinical data import from CRIS is also supported.

To address the needs of trainees, we have developed a curriculum of core bioinformatics classes, including courses on the essentials of the Unix operating system and the R programming language.

We offer licensed seats for commercial software, including Partek Genomics Suite and Ingenuity Pathway Analysis (IPA). Most recently, we obtained Partek Flow toolkits for analysis and visualization of bulk and single cell RNA-seq data.

For more information, visit http://dir.nhgri.nih.gov/nhgri_cores/BSPC/.

1. Computational and Statistical Genomics Branch, NHGRI

Cytogenetic and Microscopy Core

A17
Poster

Amalia Dutra [1], Evgenia Pak [1], and Stephen Wincovitch [1]

The mission of the NHGRI Cytogenetic and Microscopy Core is to provide NHGRI Investigators with state-of-the-art molecular cytogenetic services and access and assistance with fluorescence, and confocal microscopy. The Core serves as a source of expertise and collaboration for research projects employing molecular cytogenetic techniques, RNAscope and cellular, molecular and live cell imaging. The Cytogenetic and Microscopy Core is located within the Genetic Disease Research Branch and provides service to NHGRI Investigators throughout the institute free of charge. The Core has two sections: A- Cytogenetic Section and B- Microscopy Section.

The Cytogenetic Section of the Core provides expertise for investigators from sample preparation to analysis and interpretation of the results. This section performs fluorescence in situ hybridization (FISH), Spectral Karyotyping (SKY) on metaphase chromosomes, interphases and extended chromatin fibers in human, mouse, zebrafish, rat and other species. Also karyotyping using G-banding or DAPI-banding. The Core performs RNAscope which is a fluorescent assay based on in situ hybridization to simultaneously visualize up to three different RNA targets per cell in samples mounted on slides. The assay is based on ACD's patented signal amplification and background suppression technology which enable users to study expression as well as positional relationship between multiple genes within a tissue.

The microscopy section provides NHGRI with advice, training and assistance to confocal and optical microscopy tools. This includes laser scanning confocal, super resolution imaging, fast scanning mode Airyscan imaging of thick-cleared samples, AxioScan slide scanning to digitize slides, spinning disk, deconvolution-restoration wide field microscopy, long-term time lapse experiments, 3D imaging and renderings, FRAP (Fluorescent Recovery After Photobleaching), FRET (Fluorescent Resonance Energy Transfer), co-localization, tiling, tracking and extensive fluorescent intensity measurements. We provide detailed quantitative or statistical analysis when required.

Importantly the Core provides an expert knowledge base from which NHGRI investigators can obtain advice on experiment planning or experimental protocols in cell biology and immunostaining protocols. We also provide training to investigators in various software packages for analysis.

1. Genetic Disease Research Branch, NHGRI

NHGRI Embryonic Stem Cell and Transgenic Mouse Core Facility

A18
Poster

Lisa Garrett [1], Jun Cheng [1], Gene Elliott [1], Ceci Rivas [1], Elsa Escobar [1]

The NHGRI Embryonic Stem Cell and Transgenic Mouse Core provides a shared service for our investigators and collaborators to generate genetically engineered mice using site-specific nucleases as well as conventional transgenesis and ES cell targeting. In addition, to providing mouse models, the core cryopreserves important strains and performs rederivation of imported mice by in vitro fertilization. Other services available through the core include ENU injection, teratoma analysis, embryo harvest and dissection, isolation of mouse embryonic fibroblasts and generation of induced pluripotent stem cells. The core further supports NHGRI investigators with consultations for construct design as well by providing advice and training on surgical techniques, embryo manipulations, genotyping, and mouse husbandry.

The core routinely uses gene editing protocols for gene targeting that include both electroporation and microinjection of 1 and 2-cell embryos. Presently, we utilize both SpCas9 and AsCpf1 endonucleases. Our work over the past 6 years to establish these protocols has resulted in significant contributions to NHGRI research including the generation of mouse models for rare disease variants in numerous genes including Clcn7 and Tmem94 identified through the Undiagnosed Disease Program as well as generation of engineered variants of Slc48a1 to study erythrocyte biology and several protein tag knock-ins. The core averages 50 gene editing projects annually using CRISPR and in this poster we will present an overview of the core services and methods.

1. Genetic Disease Research Branch, NHGRI

The NHGRI Flow Cytometry Core Facility

A19
Poster

Stacie Anderson [1], Martha Kirby [1]

The Flow Cytometry Core of the National Human Genome Research Institute has the goal of providing all NHGRI investigators with access to high quality flow cytometry services to enhance the scope and quality of scientific research performed. Services offered include planning, design, execution, and analysis of experiments involving flow cytometry, permitting high quality single cell analyses to be performed by NHGRI investigators. Experiments range from simple fluorescent protein quantitation to complex 21-color analysis. Other analyses include cell survival/apoptosis, proliferation, DNA content, cell cycle, cytolysis, evaluation of biochemical intermediates and gene expression, and many other analyses that can be evaluated on a single cell basis. By flow cytometry sorting, purified cell populations for culture, DNA, RNA and single cell sequencing can be isolated from heterogenous starting populations. Examples of analysis and sorting of samples include cell lines, tissues preparations including, but not limited to blood, bone marrow, lymph node, spleen, thymus, lung, liver and neuronal cells.

The Core is actively involved in a variety of applications including phenotypic and functional analyses of populations on a single cell basis, isolation of tissue and blood cell populations for animal transplantation experiments, isolation of cell sub-populations for analyses of functional and transcriptional profiling and high throughput screening of enhancer regions. The Core has been more heavily involved in Zebrafish analysis and sorting. A new project involves sorting human neuronal cell nuclei for the Brain Iniative. The Core's involvement in scRNA studies continues as a major focus.

1. Genetics and Molecular Biology Branch, NHGRI

NHGRI Genomics Core

A20
Poster

Frank X. Donovan [1], Ursula Harper [1], Aparna Haldipur [1], Settara Chandrasekharappa [1]

The Genomics Core provides genotyping services and analytical support to NHGRI investigators. Genotyping is performed using either of two technologies, Illumina BeadArray for SNPs or ABI capillary electrophoretic sizing of fluorescently tagged PCR products encompassing STRPs or other genomic region(s) of interest. The Core generates genotype data for human, mouse, and zebrafish genomes for a variety of applications and generated more than 3.5 billion genotypes last year. ABI technology can separate fluorescently labeled PCR products at single-base resolution and has been adopted as an efficient screening strategy for identifying germline transmitting founder zebrafish and determining the size of insertion/deletion mutations generated by CRISPR/Cas mutagenesis technologies. Over the past five years, ~95,000 DNA samples were processed to screen for variants induced by CRISPR/Cas mutagenesis in zebrafish. We provide additional services including MLPA (Multiplex ligation-dependent probe amplification) for deletions, mouse speed congenics, human and mouse STRPs, and fragment analysis for a variety of other applications. The methods developed by the Core have been integral in establishing a high-throughput targeted mutagenesis pipeline using CRISPR/Cas technology in zebrafish for large-scale phenotyping efforts. The Core has had a key role in several large-scale SNP projects including studies on cardiovascular disease and Scleroderma, and the genotyping of samples processed by NISC which belong to multiple investigators from other institutes, indicating the Core serves a larger scientific community than just NHGRI. We also provide investigators with analytical support for their genotyped samples including analysis of copy number variation, linkage, association, identification of deletion intervals, and methylation analysis

1. Cancer Genetics and Comparative Genomics Branch, NHGRI

The Microarray, Sequencing and Single-cell Genomics Core

A21
Poster

Abdel Elkahloun [1], Weiwei Wu [1]

The NHGRI Microarray Core (MAC) was established in 1999. The facility represents a consortium between the NHGRI, NIMH and NINDS. It has been providing intramural investigators with full service, cost-effective and time-efficient access to comprehensive state-of the- art genomics and transcriptomics technologies for understanding genome copy number, patterns of gene expression, microRNA profiles, genome wide SNPs analysis and epigenetics.

Requests are “Full Service”, in which the investigator will provide the chips/sequencing reagents, labeling kit and isolated RNA/DNA samples, and the core performs labeling, hybridization, data extraction, data analysis and mining. The core is now equipped with 2 Next-seq system for mid to high-throughput sequencing capabilities for any kind of sequencing projects: gene expression, DNA, exomes, methylation, chip-seq...

The core is also equipped with the 10X Genomics Chromium controller for the generation of single cell in droplets for all genomics applications at a single cell resolution.

Upon project completion, the investigator is then provided with a summary report including the data quality assessment, full analysis and raw data output files.

1. Cancer Genetics and Comparative Genomics Branch, NHGRI

The NIH Intramural Sequencing Center

A22
Poster

Betty Barnabas [1], Sean Black [1], Gerry Bouffard [1], Shelise Brooks [1], Mila Dekhtyar [1], Xiaobin Guan [1], Joel Han [1], Shi-Ling Ho [1], Richelle Legaspi [1], Quino Maduro [1], Holly Marfani [1], Cathy Masiello [1], Jenny McDowell [1], Cassandra Montemayor [1], Morgan Park [1], Nancy Riebow [1], Karen Schandler [1], Brian Schmidt [1], Christina Sison [1], Mal Stantripop [1], Jim Thomas [1], Pam Thomas [1], Meg Vemulapalli [1], Alice Young [1], Jim Mullikin [1]

The NIH Intramural Sequencing Center (NISC), established in 1997, is a multi-disciplinary genomics facility that emphasizes the generation and analysis of DNA sequence. NISC’s role within NHGRI, and more broadly across NIH, aims to advance genome sequencing and its many applications, with a goal not simply to produce sequence data, but to produce the infrastructure required to bring sequence to biology and medicine. We accomplish this by meeting with each investigator to discuss the details of their project to understand which method(s) would work best. The most common types of sequencing projects include whole exome sequencing, RNA sequencing, custom targeted capture sequencing, ChIP-seq and whole genome sequencing. However, we are always interested in exploring new methods and expanding our repertoire in this rapidly changing field. We also work closely with other investigators across the NHGRI IRP to develop novel methods to analyze genomics data with applicability to clinical and basic science questions that were thought to be intractable only a few years ago.

1. NISC Comparative Sequencing Program, NHGRI

A Case of Sialic Acid Storage Disease

A23
Poster

Janice Cousin [1], Mary Hackbarth [2], William Gahl [1,2], David Adams [2], Mary Malicdan [1,2], Marjan Huizing [1], Lynne Wolfe [2]

Background: Sialic acid storage disease (SASD) is an autosomal recessive neurodegenerative disorder caused by mutations in the gene SLC17A5. Severe loss-of-function mutations cause Infantile Sialic Acid Storage Disease (ISSD); milder missense variants cause a milder adult phenotype called Salla Disease, and other variants cause an intermediate disease presentation. The SLC17A5 gene encodes sialin, a protein in the lysosomal membrane that transports free sialic acid out of the lysosome, so free sialic acid accumulates in affected cells' lysosomes in this disease. Sialic acid is a negatively charged sugar that is an important component of the post-translational modification of proteins and lipids called glycosylation.

Methods: A 1.5 year old female patient of Dominican background with compound heterozygous variants, c.406A>G (p.Lys136Glu) and c.533delC (p.Thr178Asnfs*34), in SLC17A5 was enrolled at the NIH Clinical Center under protocol 14-HG-0071 "Clinical and Basic Investigations into Known and Suspected Congenital Disorders of Glycosylation" (NCT02089789).

Results: Clinical investigation included radiographic imaging, neurodevelopmental assessments, and biochemical workup, and confirmed an intermediate phenotype in the patient. Blood, urine, and skin biopsies were also collected from the proband and her parents for further investigation of the cellular defect.

Conclusion: This assessment allows us to further characterize intermediate SASD and expand the range of phenotypes of patients affected with Congenital Disorders of Glycosylation and related disorders.

1. Human Biochemical Genetics Section, Medical Genetics Branch, NHGRI
2. Undiagnosed Diseases Program (UDP), NHGRI

Engendering Support, Gendering Growth: A Gender-Based Analysis of Parents' Positive Aspects of Caregiving and Network Relationships

A24
Poster

Hannah Davidson [1], Chris Marcum [1], Betina Hollstein [1], and Laura Koehly [1]

Gender shapes the kinds of support we seek out and find most useful in caring effectively for those around us. Beyond the self, social supports to caregivers are also informed by the same socializing processes associated with gender, often impacting the kinds of roles and responsibilities that people take on as part of a broader support network. While it is well-understood how gender informs who becomes a caregiver and how people provide care, few studies have examined the intricacies of how caregivers' gender and positive outcomes (e.g., caregiver growth) of the caregiving experience relate to the structure and functions of social interactions within caregivers' networks.

This project will assess how caregivers' gender and positive experiences with caregiving shape the structure and function of relationships in their caregiving networks. Data were collected from parents of normally-developing children and children with inborn errors of metabolism (IEM) and included survey data from the Post-Traumatic Growth Index (PTGI) and social network enumeration, which includes collected data on caregiving tasks and gender of named enumerants. Results from this analysis will help inform the development of personalized interventions adapted to different types of caregivers.

1. Social Network Methods Section, Social and Behavioral Research Branch, NHGRI

Longitudinal Behavioral Characterization of Gaucher-Associated Parkinson Murine Models

A25
Poster

Jenny Do [1], Bahafta Berhe [1], Nahid Tayebi [1], Ellen Sidransky [1]

Mutations in the glucocerebrosidase gene, GBA1, impart an increased risk for Parkinson disease. Murine models created to elucidate the link between GBA1 and Parkinson vary in mutation type and severity and have been evaluated at different ages, complicating comparisons between models. As Parkinson is an age-related disorder, elucidating age-dependent behavioral alterations in mice provides an appropriate timeline for therapeutic studies. We crossed mice overexpressing the mutated human α-synuclein transgene (SNCAA53T) with heterozygous gba knockout mice (gba+/–), termed gba+/–//SNCAA53T mice. Their behavior and the corresponding WT/WT, gba+/+//SNCAA53T, and gba+/– mice underwent evaluation beginning at eight months old, then bimonthly for six months. Phenotypes tested were memory by the novel object recognition test, olfaction by the buried pellet test, and motor coordination by the beam walk test and a tunnel-guided gait walk. Data analyzed in GraphPad Prism 8 revealed age-associated differences between experimental groups on the behavioral tasks. Over-expression of SNCAA53T, regardless of gba genotype, resulted in impaired motor coordination starting from 10 months of age ($p = 0.037$). gba+/–//SNCAA53T mice exhibited a decrease in memory-based performance at 14 months of age ($p = 0.023$). Olfaction and weight were well-preserved throughout this period in all genotypes. This study indicates that prior to symptom onset, gba+/–//SNCAA53T mice model the age-dependent decline in motor coordination and memory seen in Parkinson disease from 10 and 14 months of age onwards, respectively. These results suggest that these phenotypes in gba haploinsufficient mice can be utilized as an efficacy endpoint for testing therapeutics at different timepoints.

1. Section on Molecular Neurogenetics, Medical Genetics Branch, NHGRI

Examining the Feasibility of Olfactory Components in Virtual Reality Research Tools

A26
Poster

Alexander Dolwick [1], Paule Joseph [2,3], Susan Persky [1]

In our research using a virtual reality (VR) buffet environment to measure the effects of genomic risk information on parents' food choices for their children, we implemented a food odor to enhance participants' subjective presence in the virtual environment. Although the olfactory stimulus was administered at a substantial level, few participants consciously perceived it. Given the potential promise of olfactory stimuli for future research related to genomics and eating behavior, we aim to explore whether and when participants in VR environments attend to olfactory stimuli. Past research suggests that people under high visual perceptual load (VPL) can fail to notice odors.

The present study will administer food odors in high- vs. low-VPL VR environments, with interactivity varied in the high-VPL condition. A participant will either enter the VR buffet where they can create a plate of virtual food (high VPL / high interactivity), enter the VR buffet where they cannot use controllers to choose foods (high VPL / low interactivity), or enter a virtual gray room with simple food-related visual stimuli on the walls (low VPL / low interactivity). An olfactory stimulus will be administered during the participant's time in the virtual environment.

We will assess perception of the odor and predictors of odor perception. We will test participants' olfactory ability using the Cross-Cultural Smell Identification Test, a 12-item validated smell identification test. Knowledge gained from this research will inform future VR-based research methods for genomics research.

1. Immersive Virtual Environment Test Unit, Social and Behavioral Research Branch, NHGRI
2. National Institute of Nursing Research
3. National Institute on Alcohol Abuse and Alcoholism

The Anatomy of Friendship

A27
Poster

Patrick D'Onofrio [1], Tonya White [2], Philip Shaw [1]

Homophily refers to people's tendency to like similar others. Most research has examined homophily based on manifest features such as appearance. Here we ask if homophily might extend to neural features, that are 'unobservable'. Specifically: do children who like one another have more similar brain structures? We hypothesize that neuroanatomic similarity tied to friendship is most likely to pertain to brain regions that support social cognition. To test this hypothesis, we collected friendship network data from 1590 children (mean age= 7.5 ± .8 years) in 164 different classrooms. Within each classroom, we identified 'friendship distance'—mutual friends, friends of friends, and more distantly connected children. In total, 161 of these children also had high quality neuroanatomic magnetic resonance imaging from which we extracted properties of the 'social brain network'. We then calculated the similarity (Mahalanobis distance) between the social brain networks of all children within each classroom. This measure of neural similarity was then regressed against friendship distance. We found that neural similarity varied by friendship distance [$F(2, 143)=4.9$, $p=.01$]: mutual friends showed greater similarity in social brain networks than friends of friends ($p=.01$) and even more remotely connected peers ($p=.002$). This finding was not due to concordance between mutual friends based on gender, race/ethnicity, or other demographic features. Importantly, other brain networks supporting general intelligence and attention did not show this relationship with friendship distance. In conclusion, we find that mutual friends have more similar social, but not other, brain networks: birds of a feather flock together.

1. Neurobehavioral Clinical Research Section, Social and Behavioral Research Branch, NHGRI
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Voices Of Individuals: Challenges and Experiences of behavioral variant FTD [VOICE Of bvFTD]

A28
Poster

Laynie Dratch [1,2], Jill Owczarzak [1], Weiyi Mu [3,] Murray Grossman [4], Lori Erby [1,2]

There is limited research considering the personal experiences of individuals living with or at risk for developing behavioral variant frontotemporal dementia (bvFTD). FTD is known to have a significant genetic contribution. With advances in understanding the genetic component of FTD, rates of presymptomatic and symptomatic genetic testing will increase. Though FTD is the second most prevalent cause of early-onset dementia after Alzheimer disease (AD), there is substantially more literature related to AD experiences. In dementia research broadly, care partners rather than persons diagnosed tend to be the informants. This study qualitatively explores the specific experience of living and coping with bvFTD diagnosis and risk from the perspective of the person diagnosed. Semi-structured interviews were conducted to explore how bvFTD may influence an individual's sense of identity, individuals' experiences of loss, how individuals cope and adapt to the challenges they face, and other factors that might be perceived to influence these processes. Participants with a diagnosis of bvFTD (n=6) and with genetic testing results conferring high risk of developing bvFTD (n=14) were recruited through two academic medical centers, the National Institutes of Health, ClinicalTrials.gov, and through bvFTD support resources. The interviews are undergoing thematic content analysis, and preliminary data from these analyses will be presented, with a particular focus on those who have had presymptomatic genetic testing. Results are intended to inform clinical care, development of support resources, and design of future studies.

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2. Medical Genomics and Metabolic Genetics Branch, NHGRI, NIH, Bethesda, MD
3. McKusick-Nathans Institution of Genetic Medicine, Johns Hopkins University, Baltimore, MD
4. Department of Neurology, University of Pennsylvania, Philadelphia, PA

AAV gene therapy intervention for NPC disease

A29
Poster

Benjamin Epstein [1], Cristin Davidson [1], Randy Chandler [2], Alana Gibson [1], Tansy Gu [1], Arturo Incao [1], Charles Venditti [2], William Pavan [1]

Niemann-Pick C (NPC) disease is a fatal, inherited metabolic disease with an estimated prevalence of 1:120,000. Mutations in the NPC1 gene account for ~95% of patients while the remaining 5% are due to NPC2 gene mutations, with indistinguishable phenotypes. NPC1 and NPC2 are found within the lysosome and are involved in egress of unesterified cholesterol. Patients experience progressive decline in intellectual and motor function resulting in an untimely death. The best prospective therapeutic compound, 2-hydroxypropyl-beta-cyclodextrin (2HPβCD), only slows disease progression and entails a rigorous treatment protocol with significant side effects. We are thus evaluating adeno-associated virus (AAV)-based gene therapy to treat NPC.

Our benchmark work in mice involved delivery of AAV9.EF1a(s).hNPC1 viral vector. Mice were tracked for weight loss, behavioral phenotype, and survival, and they were assessed for lipid storage and inflammation in liver and brain and successful gene delivery and expression. We evaluated an evolved AAV serotype with greater CNS transduction (AAV-PHP.B), dose dependence, and synergy with 2HPβCD. Each factor significantly impacts efficacy. We are aiming to optimize and expand the potential of these approaches. We will assess additional serotypes of AAV in conjunction with different delivery avenues to improve transduction of key cell types. In collaboration with StrideBio Inc., we are assessing different promoters as well as novel engineered AAV capsids. In collaboration with Chameleon Biosciences Inc., we are evaluating technology for shielding AAV capsids from an immune response and assessing the potential for treating patients with preexisting antibodies to AAV and for administering multiple doses of AAV.

1. Genomics, Development and Disease Section, Genetic Disease Research Branch, NHGRI
2. Organic Acid Research Section, Medical Genomics and Metabolic Genetics Branch, NHGRI

NIH Participation to USIDNET Registry

A30
Poster

Elizabeth Garabedian [1]

The USIDNET Registry began in 1992 with an NIAID contract with the Immune Deficiency Foundation, which continues today. It aims to provide a resource for clinical and lab research through enrollment of known immunodeficiency patients into a national registry, the USIDNET. NIH is a major national and international referral center for clinical trials on inborn errors of immunity, or primary immunodeficiency diseases. It is a mechanism for depositing NIH data into USIDNET. A Registry of patient information may help us understand how many people have each disease. The information may improve how we diagnose and treat these conditions. The patient Registry is designed to obtain longitudinal data on a large number of patients with primary immunodeficiency diseases who come to NIH to participate in research. The data is collected from the NIH electronic medical record system, CRIS and is deposited into a secure registry with restricted and monitored access. All medical information is anonymized for patient privacy.

1. Office of the Clinical Director, NHGRI

DNA methylome and whole transcriptome analyses identify genes potentially contributing to phenotypic differences in a set of monozygotic twins with Gaucher disease

A31
Poster

Eric Joshua Garcia [1], Shahzeb Hassan [1], Frank Donovan [2], Abdel Elkahloun [2], Mirjam Langeveld [3], Carla Hollak [3], Ellen Sidransky [1], Nahid Tayebi [1]

Epigenetic marks such as DNA methylation result in gene expression modulation without altering the DNA sequence. Gaucher disease (GD), an autosomal recessive lysosomal storage disorder, is hallmarked by vast phenotypic heterogeneity. Epigenetics may contribute to this heterogeneity. We previously described monozygotic twin sisters with GD and divergent phenotypes. One sister exhibited splenomegaly, anemia and thrombocytopenia, severe bone pain, altered saccadic eye movements and complex seizures. The other had no GD manifestations but developed type 1 diabetes. We investigated whether differential methylation may contribute to this phenotypic discrepancy. After Bisulfite conversion, Genomic DNA was ran on an Illumina HumanMethylation450 BeadChip and analyzed using GenomeStudio. This analysis yielded several sites where methylation differed between the twins. One demethylated gene, CACNA1A, a neuronal alpha-1a subunit of a voltage dependent calcium channel, and one methylated gene, MEST, an alpha/beta hydrolase superfamily member, were validated by real-time PCR using RNA extracted from the twin's leukocytes and fibroblasts. Whole exome sequencing revealed no significant differences between sisters. A ClariomTM S transcriptome array, performed on RNA samples from the twins and two aged-matched female controls, confirmed the up- or down- regulation of genes identified from the differential methylation analysis. Differential expression of other genes was observed, some related to MEST or CACNA1A. GeneMANIA pathway and STRING protein networks highlighted potential interactions between proteins encoded by GBA1, MEST, CACNA1A, either directly or through intermediate proteins. Thus, epigenetic analyses can be used to identify genes influencing disease manifestations and contributing to the complexity of genotype-phenotype relationships in Gaucher disease.

1. Section on Molecular Neurogenetics, Medical Genetics Branch, NHGRI
2. Genomics Core, Cancer Genetics and Comparative Genomics Branch, NHGRI
3. Academic Medical Center, Amsterdam, The Netherlands

Differential effects of mtDNA depletion on T-cell function in a mouse model of TFAM deficiency

A32
Poster

Rylee Genner [1], Senta Kapnick [1], Peter McGuire [1]

The immune phenotype in patients with mitochondrial disease is becoming increasingly recognized. However, clinical heterogeneity occurs, suggesting mitochondrial disease subtype specificity. Mitochondrial DNA (mtDNA) depletion syndromes (MDS) are a subtype of mitochondrial disease characterized by a severe reduction in mtDNA content leading to impaired oxidative phosphorylation (OXPHOS) in affected tissues and organs. We hypothesize that due to the reliance of immune cells on OXPHOS and the pleiotropic nature of MDS, T-cells will display perturbations in activation, differentiation and function. To delineate the T-cell immune phenotype in MDS, we utilized a T-cell specific knockout of Transcriptional Mitochondrial Factor A (Tfam-/-)—a protein-encoding gene that is essential for the proper packaging and replication of mtDNA. We found that Tfam-/- CD8+ T-cells exhibited significantly decreased mtDNA copy number, reduced proliferation, increased mitochondrial mass, and a more pronounced glycolytic shift compared to controls. Despite these perturbations, Tfam-/- CD8+ T-cells also demonstrated several enhanced effector capabilities including increased cytokine production, increased effector molecule secretion, and heightened target killing on a per cell basis compared to controls. We propose that the escape of mtDNA fragments into the cytosol leads to T-cell priming and, consequently, enhanced effector function. This is consistent with what has been reported in the literature in other cell types. Our preliminary findings represent promising insights into the effects of reduced mtDNA copy number on T-cell metabolism and will lead to a better understanding of immune function in patients with MDS.

1. Metabolism, Infection and Immunity Section, Medical Genomics and Metabolic Genetics Branch, NHGRI

Reaching Diverse Communities to Improve Genomic Literacy: Mobile App vs. interactive Game

A33
Poster

Fiona Gilpin-Macfoy [1], Calandra Whitted [1], Laura Koehly [1]

Technology and mass media are used to develop and disseminate health risk information through mobile applications (apps), videos, podcasts, and virtual reality simulations. Mobile health technology improves the accessibility of information. Although these technologies are designed to reach a wide range of people, there are limiting factors: Mobile health applications require internet access, adequate wireless service, and basic user knowledge and capability. Some health education programs utilize gamification, an interactive game-like method, to engage participants within a community setting. Families SHARE is a genomic multimedia educational workbook designed to evaluate disease risk based on the family health history of heart disease, diabetes, breast, colon, and prostate cancer. The mobile application includes an embedded version of the Families SHARE workbook and calculates disease risk based upon the user's family health history. The interactive game consists of a powerpoint version of the workbook followed by a family health history trivia-style game.

The purposes of this study are to a) identify perceptions of the Families SHARE workbook mobile app or the interactive game and b) determine which format will most likely encourage family/friend communication about family health and disease risk.

A qualitative thematic approach will be used to analyze participant interviews to elicit user preferences in the feasibility and shareability of the mobile app or the workbook-interactive game.

Results will elucidate the preferred educational method to promote health literacy and health-conscious decisions in an underserved population at risk for heart disease, diabetes, breast, colon, and prostate cancer.

1. Social Networks Methods Section, Social Behavioral Research Branch, NHGRI

Improving CRISPR methodologies using unbiased genome-wide screens in yeast

A34
Poster

Simone M. Giovanetti [1], Meru Sadhu [1]

CRISPR-Cas9 is a widely used and powerful tool for generating specific and targeted genome edits. We are using yeast to perform unbiased genome-wide screens to identify mutants that further expand the reach of CRISPR-Cas9. To function, Cas9 requires a guide RNA (gRNA) with a targeting sequence complementary to the genomic location to be edited. Additionally, the genomic sequence must be adjacent to a PAM sequence. Two limitations of Cas9 are 1) the inability to target genomic regions without a nearby PAM and 2) the variable targeting efficiency of gRNAs. We will find yeast mutants that ameliorate these issues, using pooled and barcoded collections of yeast, including comprehensive collections of knockout and overexpression mutations. Our strategy will be to target an edit that generates a selectable phenotype, using a gRNA or repair template that is suboptimal due to one of the above limitations. After inducing Cas9, we will enrich for cells that were able to make the edit and, via next-generation sequencing of the barcodes, identify genotypes that can utilize the suboptimal gRNA and repair template. To ensure usage of homology-directed repair across all strains in the pooled collections, we have identified expression of the a1 and alpha2 genes as a means to repress the nonhomologous end-joining repair pathway.

1. Systems Biology and Genome Engineering Section, Genetic Disease Research Branch, NHGRI

Heterogeneity of neuroimaging and genotype-phenotype correlations in Leigh Syndrome

A35
Poster

Eliza Gordon-Lipkin[1], Shannon Kruk[1], Peter McGuire[1]

Objective: To understand the spectrum and genotype-phenotype correlations of neuroimaging findings in Leigh Syndrome (LS).

Methods: Individuals were recruited for the Metabolism, Infection and Immunity (MINI) study at the National Institutes of Health and included if they had a mutation in a gene known to be involved in mitochondrial metabolism and associated with LS or LS Spectrum. The NIH MINI Study is the first organized clinical research effort to study infection and immunity in Leigh Syndrome. This ongoing, longitudinal natural history study leverages the resources of the NIH Clinical Center to comprehensively phenotype the pleiotropic effects of LS and its relationship with infection in a multidisciplinary fashion. Imaging was obtained by retrospective record review. If images were unavailable, MRI reports were used for analysis.

Results: 22 children were identified. Affected genes included both mDNA (n=9) and nuclear DNA (n=14) mutations, including one patient with both. 12 (55%) had symmetric basal ganglia lesions and 11(50%) had brainstem lesions. 11 patients had available MR spectroscopy of which 45% had lactate peaks. 18 (82%) had neuroimaging consistent with other cases of the same genotype in literature. New phenotype is described in an SDHA mut with isolated cerebellar atrophy and MICU1 mutation with “MS-like” lesions.

Conclusion: There is broad heterogeneity of neuroimaging findings in patients with LS. Few patients fit a “classic” picture of isolated symmetric basal ganglia and brainstem lesions. As modern genetic testing identifies more individuals with mitochondrial disease, the spectrum of central nervous system (CNS) disease is widening. LS patients more frequently fit a clinical/radiological profile consistent with their genotype rather than a classic LS picture. Reconsideration of LS as a family of mitochondrial diseases with CNS involvement rather than one syndrome may be more appropriate. Better defining genotype-phenotype correlations has the potential to lead to less heterogeneity amongst patients which is helpful for clinical trials and patient care.

1. Metabolism, Infection and Immunity Section, Medical Genomics and Metabolic Genetics Branch, NHGRI

Using RNA-Seq data to identify activation of latent splicing

A36
Poster

Valer Gotea [1], Ayub Boulos [2], Ruth Sperling [2], Laura Elnitski [1]

Gene splicing is a crucial molecular mechanism that determines the proper assembly of gene exons into mature messages, and consequently the sequence of the resulting proteins. During splicing, one important step is the selection of proper splice sites from an abundance of intronically available sequences that resemble consensus splice sites. Suppression of splicing (SOS) is a molecular mechanism whose proposed function is the avoidance of splice sites that would introduce in-frame STOP codons (1,2). Working toward identifying the molecular components of SOS, here we demonstrate how RNA-Seq from siRNA knockdown experiments could be used to identify alterations in gene splicing profiles, which include activation of latent splice sites (LSSs) that are not normally used in the splicing process. We first identified more than 1.3 million LSSs in human intronic regions, which we then queried with a computational pipeline that includes optimization of read alignments, quantification of split reads, and building of expression profiles that allows identification of specific LSS activation events and elimination of spurious signals. This pipeline could be generalized to validate whether specific genes play significant roles in the proper functioning of the SOS mechanism.

1. Genomic Functional Analysis Section, Translational and Functional Genomics Branch, NHGRI
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Roles of unaffected siblings in supporting parent-caregivers of children with inherited metabolic conditions

A37
Poster

Madeleine Granovetter [1], Christopher Marcum [1], Laura Koehly [1]

Children with inherited metabolic conditions often experience serious physical and behavioral challenges such as organ damage, dietary restrictions, and cognitive and developmental delays, which place stress on parent-caregivers. While parents are the primary caregivers of children with inherited metabolic conditions, affected individuals' siblings may also perform critical caregiving tasks that dually support their parents' well-being. Existing literature on sibling caregivers predominantly focuses on the outcomes of children who have siblings with chronic illnesses. Previous research also investigates the burdens experienced by adult siblings caring for aging parents. However, no known studies report the specific roles that unaffected children play in caring for siblings with chronic health conditions, or how their caregiving contributions impact their parents' psychosocial health.

This project uses social network analysis to determine how the presence of unaffected children in caregiving networks relates to parent-caregivers' stress and management of their affected child's activities of daily living (ADLs). The sample consists of parent-caregivers recruited to our Inherited Diseases, Caregiving, and Social Networks Study who identify at least one unaffected child in their own or their affected child's caregiving networks (n=70), and parents that exclude their unaffected children from the networks (n=20). Data were collected through surveys, interviews, and parents' network assessments. Stress, measured with the Perceived Stress Scale, and reported ADL management were compared between these groups to determine whether unaffected siblings in social networks impact parents' psychosocial health and caregiving abilities. Identifying the specific caregiving roles that siblings play may also elucidate whether they mitigate parents' caregiving burden.

1. Social Network Methods Section, Social and Behavioral Research Branch, NHGRI

Methylation Patterns in Genes Related to Stress Susceptibility/Resilience: Comparing Parents of Children with Inborn Errors of Metabolism to Parents of Typically Developing Children

A38
Poster

Meghan Grewal [1], Tracy Swan [1], Faith Pangilinan [2], Aaron Gurayah [1,4], Dawn Lea [1], Hania Petrykowska [3], Laura Elnitski [3], Laura Koehly [1]

The chronic stress of caregiving can increase caregivers' vulnerability to negative health outcomes. One pathway that leads to poorer caregiver health is through the stress response that triggers DNA methylation, which causes certain genes to be activated or silenced. Few studies have investigated the biological impact of stress on the health of parent caregivers of children with inborn errors of metabolism (IEM). We suspect that chronic stress can influence the expression of genes associated with stress susceptibility and resilience in these caregivers; we also hypothesize that the burden of stress is shared among spouses (parent-dyad), in a way that is potentially discernable through DNA methylation patterns.

In this study, we will evaluate the methylation patterns of candidate genes with suspected changes in expression in response to the hypothalamic-pituitary-adrenal axis, neuronal plasticity, and systemic inflammation. We also will compare the parent-dyad response to caregiving burden and assess the extent to which the availability of support resources alleviates the potential health impact of caregiving burden.

Methylation patterns will be examined using peripheral blood of parent caregivers (n=180) who care for children with IEM under the age of 18, along with appropriate controls of parents who care for typically developing children. Participants completed surveys assessing perceived caregiving burden, participated in interviews detailing interpersonal resources, and provided bio-specimen.

This study will enable us to examine pathways through which biological caregiving stress might be buffered by social support, and might allow clinicians and caregivers to establish adaptive support networks and better protect caregivers' health.

1. Social Network Methods Section, Social and Behavioral Research Branch, NHGRI
2. Genetics and Environment Interaction Section, Social and Behavioral Research Branch, NHGRI
3. Genomic Functional Analysis Section, Translational and Functional Genomics Branch, NHGRI
4. University of Miami Miller School of Medicine

Apigenin as a Candidate Prenatal Treatment for Trisomy 21: Effects in Human Amniocytes and the Ts1Cje Mouse Model

A39
Poster

Faycal Guedj [1,2], Jeroen LA Pennings [3], Ashley E Siegel [1,2], Fatimah Alsebaa [2], Umadevi Tantravahi [4], Diana W Bianchi [1,2]

Introduction/Hypothesis: Fetuses with trisomy 21 (T21) have atypical brain development that is apparent sonographically as early as the second trimester. We hypothesize that by integrating dysregulated gene expression and pathways common to humans and mouse models of Down syndrome we can discover novel targets for therapy using repurposed FDA-approved molecules.

Methods: We tested the safety and efficacy of apigenin, previously identified by uploading gene expression profiles from nine different tissues (six human, three mouse) into the Connectivity Map www.broadinstitute.org/connectivity-map-cmap. We cultured human amniocytes from living human fetuses with T21 and euploid karyotypes for three days with apigenin (0-4μM). Effects of apigenin on oxidative stress and antioxidant capacity were analyzed. Apigenin treatment (333-400 mg/kg/day), mixed with chow, was initiated prenatally to Ts1Cje dams and fed to the pups over their entire lifetimes. Effects of apigenin on fetal brain gene expression, neonatal and adult behaviour were analyzed.

Results: In vitro, T21 amniocytes exhibited higher oxidative stress and reduced anti-oxidant capacity compared to euploid amniocytes. Apigenin significantly reduced oxidative stress and improved antioxidant defense response in T21 cells. In the Ts1Cje mouse model, there was no increase in birth defects or pup deaths resulting from antenatal apigenin treatment. In Ts1Cje neonates, apigenin significantly improved several developmental milestones and particularly, spatial olfactory memory. In addition, in adult mice we noted sex-specific effects on exploratory behavior and long-term hippocampal memory, with males showing significantly more improvement than females. Global gene expression analyses demonstrated that apigenin targets similar signaling pathways (G2/M cell cycle transition, G-protein signaling and NFκB signaling) through common upstream regulators (PTGER2, HGF, IFNGR, IKBKB and Forkhead transcription factors) both in vitro and in vivo. Validation of this mechanism of action using live cell imaging is ongoing.

Conclusions: These studies provide proof-of-principle that apigenin has therapeutic effects in preclinical models of Down syndrome.

1. Prenatal Genomics and Therapy Section (PGT), Medical Genetics Branch (MGB), National Institutes of Health (NIH), Bethesda. MD, United States.
2. Mother Infant Research Institute, Tufts Medical Center and the Floating Hospital for Children, Boston, MA, United States.
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Collaborative Development of Therapeutics for Sialic Acid Storage Disease

A40
Poster

Mary Hackbarth [1], Marjan Huzing [1], May Malicdan [1], David Adams [1], Kostantin Dobrenis [2], Melissa Wasserstein [3], William Gahl [1], Steven Walkley [2], SASD Consortium

Lysosomal sialic acid (SA) storage disease (SASD) is an autosomal recessive, neurodegenerative, multisystemic disorder caused by defects in the lysosomal SA membrane carrier SLC17A5 (Sialin). SLC17A5 defects cause free SA and secondary metabolites to accumulate in lysosomes. The clinical spectrum ranges from severe infantile sialic acid storage disease (~40 reported cases), to a milder adult form called Salla disease (~120 cases).

Although sialic acid metabolism, membrane transport, and lysosomal biology have been extensively studied, the pathobiology of SASD remains poorly understood. Moreover, SASD is underdiagnosed; known patients have experienced diagnostic delay due to the rarity of the disorder, non-specific clinical symptoms and absence of routine urine SA testing. There is no approved therapy for SASD.

As is typical for orphan diseases, the small population of patients makes it difficult to motivate industries to invest in performing the pre-clinical and clinical studies necessary to develop therapies. On the other hand, multidisciplinary collaborative efforts involving the NIH, academic clinical scientists, and patient advocacy groups have successfully overcome the scientific, clinical and financial challenges facing the development of new drug treatments for some rare diseases.

Encouraged by these successes, we have initiated a collaborative effort for SASD. This has allowed us to start creating cell and mouse models, perform basic/translational research, initiate a natural history study to aid in the identification of biomarkers and treatment endpoints, raise awareness for SASD, and investigate leads on drug candidates. We aim to collect data that incentivize industry to further develop, obtain approval, and commercialize SASD treatments.

1. Medical Genetics Branch, NHGRI
2. Department of Neuroscience, Albert Einstein College of Medicine
3. Children's Hospital at Montefiore, Albert Einstein College of Medicine

Aberrant post-translational modifications characterize the hepatic proteome of methylmalonic acidemia

A41
Poster

PamelaSara Head [1], Irini Manoli [1], Sam Myung [1], Yong Chen [2], Marjan Gucek [2], Charles Venditti [1]

Organic acidemias (OAs), such as methylmalonic acidemia (MMA), are a group of inborn errors of metabolism that typically arise from defects in the catabolism of amino- and fatty acids. OAs have multisystemic manifestations, leading to increased morbidity and mortality. Accretion of acyl-CoA species is postulated to cause intracellular toxicity. Here, we explore an alternative pathophysiological consequence of impaired acyl-CoA metabolism: the accumulation of aberrant posttranslational modifications (PTMs) that modify enzymes in critical intracellular pathways. Using a mouse model that recapitulates the hepatic mitochondriopathy of MMA (Mut- /-;TgINS-MCK-Mut), we surveyed PTMs in hepatic extracts with propionyl- and malonyl-lysine antibodies. We discovered widespread hyper-acylation in the MMA mice compared to controls, but not in animals with Acsf3 deficiency, a disorder of acyl-CoA synthesis. Next, we prepared anti-PTM antibody columns, purified hepatic extracts from MMA and control mice, and performed mass spectrometry to characterize the PTM proteome. Excessive acylation of enzymes involved in glutathione, urea, arginine, lysine, tryptophan, valine, isoleucine, methionine, threonine, and fatty acid metabolism were detected in the MMA mice but not controls, and further validated via immunoprecipitation analysis and Western blotting. In parallel, we generated, via nonenzymatic acylation reactions, PTM-modified BSA targets for in vitro analyses. We purified, then assayed, SIRT1-7 deacylase activity using BSA-PTM standards to identify the SIRT(s) that most efficiently remove these PTMs. Our observations suggest that hyperacylation of key enzymes in pathways known to be dysregulated in MMA likely contributes to altered metabolism and identifies a new set of targets for therapeutic intervention.

1. Organic Acid Research Section, Medical Genomics and Metabolic Genetics Branch, NHGRI
2. Proteomics Core Facility, NHLBI, NIH

Illness Identity and Psychological Adaptation in Individuals with Hypermobile Ehlers Danlos Syndrome or Hypermobility Spectrum Disorder/Hereditary Disorder of Connective Tissue

A42
Poster

Alexis Heidlebaugh [1,2], Joann Bodurtha [3], Christy Smith [3], Weiyi Mu [3], Debra Roter [2], Lori Erby [1], William Klein [4]

Individuals with Hypermobile Ehlers Danlos Syndrome (hEDS) or Hypermobility Spectrum Disorder (HSD)/ Hereditary Disorder of Connective Tissue (HDCT) experience many challenges including uncertainty in diagnosis, lack of access to knowledgeable healthcare providers, limited treatment and management options, and potential for substantial disability. As a result of these challenges, in addition to chronic pain and fatigue, physical and psychological functioning can be impaired in these individuals. Much of the research literature in the hEDS and HSD/HDCT populations focuses on the description and diagnosis of these conditions and patients' experiences of illness chronicity and its impact on physical and psychological functioning. Further exploration of these challenges is warranted to understand if and how individuals incorporate their illness into their identity and adapt to living with these conditions. This study aims to explore how individuals with hEDS or HSD/HDCT perceive their illness, appraise uncertainty and their ability to cope, integrate their illness into their identity, and adapt to living with a chronic condition. An online survey was distributed by the EDS Society through their research registry, website, newsletter, and social media platforms. The survey consists of several validated measures in addition to questions regarding diagnosis and illness characteristics. Data collection is in progress and preliminary findings will be presented. Understanding if and how illness is incorporated into identity and adaptation to living with these conditions, may provide insight into their medical and psychological needs and help healthcare providers to provide better clinical care to individuals with hEDS or HSD/HDCT.

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4. Division of Cancer Control and Population Sciences, National Cancer Institute

Mouse erythroid commitment occurs in common myeloid progenitors

A43
Poster

Elisabeth F Heuston[1], Cheryl A Keller[2], Belinda Giardine[2], Bethan Psaila[3], Stacie M Anderson[1], NISC Comparative Sequencing Program[4], Ross C. Hardison[2], David M. Bodine[1]

Traditional hematopoiesis models hypothesize that mouse hematopoietic stem and progenitor cells (LSK) produce myeloid progenitors (CMP), which become further restricted to bipotential granulocyte/monocyte progenitors (GMP), or to bipotential megakaryocyte/erythroid progenitors (MEP) that then produce megakaryocytes (Mk) and erythroblasts (Ery). Recent single cell studies have identified lineage-committed Mk cells in progenitor populations thought to be multipotent.

To determine when Mk and Ery cells emerge, we performed single cell RNA-Seq on 36000 hematopoietic progenitors. We used clustering and gene expression analyses to assign candidate identities to hematopoietic subpopulations. In LSK, Mk-associated and lymphoid-associated genes were co-expressed in 56% of cells. No Ery RNA signatures were observed. In CMP, 23% of cells had a Mk RNA signature and 12% had a mixed Ery/Mk RNA signature. Unlike traditional models, 94% of MEP had Ery RNA signatures. We characterized the emergence of erythroid cells by performing pseudotime analysis on the CMP. A CMP subpopulation of cells with an Mk expression profile diverged into one trajectory with cells expressing an early Mk signature, and one with cells decreasing Mk genes and increasing erythroid genes.

We combined index-sorting with single-cell targeted quantitative PCR to further define the CMP subpopulation, "CMP-E". The Assay for Transposase-Accessible Chromatin (ATAC-Seq) showed that 57% of CMP-E ATAC-Seq peaks overlapped with CMP peaks. RNA-Seq showed the highest expressed CMP-E transcripts overlapped with those expressed in Ery. Together, our data suggest that the earliest erythroid-restricted cells exist as a CMP subpopulation, and that the transcriptional change from CMP to Ery precedes chromatin accessibility changes.

1. Hematopoiesis Section, Genetics and Molecular Biology Branch, NHGRI
2. Department of Biochemistry and Molecular Biology, Pennsylvania State University
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4. NISC Comparative Sequencing Program, NHGRI

Aberrant splicing and isoform production of kinase proteins is prevalent in metastatic melanoma

A44
Poster

David Holland [1], Valer Gotea [1], Laura Elnitski [1]

Alternative splicing is a mechanism by which several types of mature messages (isoforms) are created from the same gene locus. Complete exons or exonic fragments can be excluded or included during splicing, which may lead to the removal or addition of functional domains with all their downstream effects. Aberrant splicing has been linked to various cancers. In this study we quantify changes in gene expression and isoform ratios in the kinome of metastatic melanoma cells using RNA-seq data from The Cancer Genome Atlas (TCGA). We contrast 539 total kinases between 103 primary tumor and 368 metastatic samples. We found that 37% of kinases exhibit different expression levels in metastases. Moreover, 60% of the 443 kinases with more than one isoform exhibit significant isoform switching between primary tumors and metastases ($p<0.01$), the nature of which we characterize here. The highest scoring genes are enriched for receptor tyrosine kinases (RTKs), suggesting these receptors play a key role in the development of metastases. Given that RTKs are targetable with therapeutic drugs, our study presents new candidates for combinatorial treatment approaches. Moreover, clustering based on isoform expression identifies four stable clusters, three of which roughly correlate with tumor location (skin, lymph node, distant), suggesting different expression patterns at different stages of metastasis. Sample grouping by genomic subtype reveals that samples with RAS hotspot mutations are enriched in changes to CMGC kinases, which include cell cycle regulators, suggesting that different pathways could be affected in different subtypes.

1. Genomic Functional Analysis Section, Translational and Functional Genomics Branch, NHGRI

The State of Things: Monitoring state legislative activity on issues in genetics

A45
Poster

Rebecca Hong [1], Ketty Bai [2], Elena Ghanaim [1]

NHGRI maintains a database of US state statutes and bills introduced beginning in 2007 through the present. It captures legislation that are grouped into the following categories: health insurance coverage, health insurance nondiscrimination, employment nondiscrimination, nondiscrimination in other lines of insurance, privacy, research, and use of residual newborn screening specimens. For those interested in researching trends in genomic policy at the state level, the database is a unique resource as it is the only collection of all state legislation - both passed and failed - related to genomics. We performed several analyses to demonstrate how the database may be used. First, we found that the number of bills introduced has generally increased, with 2017 showing the highest number of bills so far. We then compared the number of bills that passed, failed, and were pending in each category. Bills regarding the use of residual newborn screening specimens had the highest pass rate, whereas bills regarding other lines of insurance nondiscrimination had the lowest pass rate. Bills about privacy were the most commonly introduced. Finally, those familiar with the Genetic Information Nondiscrimination Act (GINA) know that this law fails to prohibit genetic discrimination in life, long-term, and disability insurance. Nevertheless, several states have passed laws that prohibit discrimination in other lines of insurance. Using the database, we found that few states have passed these types of laws, and that while there is some appreciation for nondiscrimination in disability insurance, very few states have passed laws to prevent discrimination in long-term care insurance.

1. Policy and Program Analysis Branch, Division of Policy, Communications, and Education, NHGRI
2. Duke University

The role of metabolic switching in zebrafish caudal fin regeneration

A46
Poster

David Hoying [1], Jason Sinclair [1], Shawn Burgess [1]

Mammals are generally poor at regenerating tissues, often resulting in complete loss of organs and extremities following damage. In contrast, the zebrafish Danio rerio has the remarkable ability to regenerate fins (limbs), scales, retina, spinal cord and heart. Furthermore, zebrafish can be easily genetically manipulated using the CRISPR/Cas 9 system, have large clutch sizes making them amenable to genetic studies, and are transparent during early development which is ideal for imaging. Together, these properties make the zebrafish a strong model in which to study tissue regeneration. Using the embryonic caudal fin to study early triggers of regeneration, we discovered that a metabolic switch from oxidative phosphorylation to glycolysis in the blastema is essential for fin regeneration. This shift in metabolism is similar to the Warburg effect demonstrated in several types of cancer cells, where glycolysis is the preferred energy generating pathway even in aerobic conditions. It is becoming evident that the Warburg effect is not specific to cancer but a normal physiological process hijacked by cancer cells. To gain a better understanding of how glycolysis regulates regeneration, we performed single-cell RNA sequencing (scRNAseq) on regenerating fins with and without inhibiting glycolysis. Analyses of differential gene expression specifically in blastema cells revealed that Nf-kB signaling is strongly regulated by glycolysis during regeneration. We now aim to further explore the relationship between glycolysis and NF-kB. We believe these studies will provide insight into the mechanisms underlying tissue regeneration and how the Warburg effect is beneficial to highly proliferative cells, including tumor cells.

1. Developmental Genomics Section, Translational and Functional Genomics Branch, NHGRI

Paternal Uniparental Isodisomy on all autosomes, maternal mitochondria and very low level mosaicism in Peripheral Blood Whole Genome Sequencing, a very Uncommon finding in a UDN case.

A47
Poster

Charles Huang [1,2], William Gahl [1,2], Wendy Introne [1,2], Precilla D'Souza [1], Cynthia Tifft [1,2], David Adams [1,2], Ellen MacNamara [1], Thomas Markello [1,2]

UDN_12676 is a 5 yr old female who was referred to the NHGRI UDP program for complex problems beyond her original neonatal diagnosis of Beckwith-Wiedemann Syndrome and uniparental isodisomy of chromosome 11. There are over 116 phenotype ontology findings from all embryonic cell lines. Initial SNP chip findings were remarkable for a peripheral Blood DNA finding of apparent total paternal uniparental isodisomy. This remarkable finding was confirmed on peripheral blood whole genome sequencing, although the mitochondrial markers are exclusively maternal. On close inspection the blood shows approximately 1-2% partial mosaicism for heterodisomy and some maternal mosaicisim suggestive of a trace of post fertilization trisomy rescue. Skin fibroblast and skin melanocyte DNA analyses are pending. A review of the literature found 12 other cases, some with nearly complete findings in blood samples, all with mosaicism in other tissues. In this individual, there are many important variants in homozygosity that explain the complex phenotype. In addition, there is a de novo heterozygous mutation in ENG (engrailed) that could explain the ability for this individual to be viable in embryogenesis since the ENG loci is important in controlling the effects of imprinting, which would be the most important biologic issue with complete uniparental isodisomy.

1. Undiagnosed Diseaease Network
2. Medical Genetics Branch, NHGRI

Modulation of the Gut Microbiome in a Mouse Model of Isolated Methylmalonic Acidemia Induces a Therapeutic Biochemical and Hepatic Response

A48
Poster

Emily Isko [1], Alex Lesser [1], Clayton Deming [2], Sean Conlan [2], Irini Manoli [1], Oleg Shchelochkov [1], Julie Segre [2], Charles Venditti [1]

Methylmalonic acidemia (MMA) is a rare genetic disease of intermediary metabolism. This disorder is characterized by accumulation of toxic metabolites in various tissues as well as multiorgan pathology. Current treatment involves a protein restricted diet and management of disease-related symptoms. Previous reports suggest antibiotic treatment could improve urinary methylmalonic acid excretion in human subjects with MMA by reducing propionate producing gut microbiota, but there is no experimental evidence to prove the beneficial effects on long-term clinical outcomes of MMA. In the current study, we assessed the effects of antibiotic therapy in a mouse model of cblA deficiency, a form of isolated MMA caused by variants in Mmaa. Vancomycin was administered in the drinking water of affected (Mmaa-/-) and unaffected (Mmaa+/-) mice for two weeks and resulted in significant improvements in the mutant phenotype including increased weight (multiple t-test: $p<0.05$) and decreased plasma biomarkers (Mann-Whitney test: MMA - $p<0.01$, Fgf21 – $p<0.05$). To investigate the mechanisms underlying these improvements, we next performed RNA-seq on liver mRNA and bacterial 16S rRNA metagenomic sequencing on stool samples. RNA-seq analysis showed an overall improvement of dysregulated genes in the transcriptome of affected mice on antibiotics. 16S sequencing of stool samples from affected and unaffected mice on antibiotics showed a reduction of Bacteroidetes and Firmicutes, the predominant propionate-producing species in the gut. This study demonstrates the role of anaerobic gut flora in isolated MMA and its association with the liver transcriptome.

1. Organic Acid Research Section, Medical Genomics and Metabolic Genetics Branch, NHGRI
2. Microbial Genomics Section, Translational and Functional Genomics Branch, NHGRI

XMEN Disease

A49
Poster

Shabban Jehangir [1], Juan Ravell [2], William Gahl 1, Lynne Wolfe [1]

XMEN disease is an X-linked immunodeficiency with magnesium transport defect, Epstein-Barr virus infection, and neoplasia caused by a hemizygous loss of function mutations in the X-linked magnesium transporter gene MAGT1. MAGT1 is a subunit of the β oligosaccharyltransferase (OST) complex. MAGT1 loss leads to a well characterized immunodeficiency disorder and has recently been identified more broadly as a systemic congenital disorder of glycosylation manifested by neuro-degeneration with abnormal brain MRI's, splenomegaly, elevated CK's and liver disease.

Methods: We identified 2 male patients with mutations in the X-linked gene MAGT1. Patient 1 was a 32-year old Caucasian male with MAGT1-CDG was enrolled at the NIH Clinical Center under protocol 14-HG-0071, "Clinical and Basic Investigations into Known and Suspected Congenital Disorders of Glycosylation". His MAGT1 variant was identified during an evaluation on protocol 15-HG-0130, Clinical and Genetic Evaluation of Patients with Undiagnosed Disorders Through the Undiagnosed Diseases Network. The patient 2 was a 33-year old Caucasian male with MAGT1-CDG was enrolled at the NIH Clinical Center under protocol 14-HG-0071.

Results: Prospective clinical investigations included radiographic imaging, neurodevelopmental testing, dermatology, ophthalmology, echocardiogram, hepatology and hematology and confirmed an intermediate phenotype in the patient.

Conclusion: This assessment allowed us to fully characterize MGATI-CDG aka XMEN syndrome and expand phenotype as a systemic Congenital Disorders of Glycosylation.

1. Undiagnosed Diseases Program, NHGRI
2. NIAID

Identifying the molecular basis of Staphylococcus lugdunensis antagonism towards human skin associated staphylococci

A50
Poster

Payal Joglekar [1], Clay Deming [1], Sean Conlan [1], NISC Comparative Sequencing Program [2], Heidi H. Kong [3], and Julie Segre [1]

Species belonging to the Staphylococcus genus are important members of the human skin microbiota. These skin associated staphylococci are a rich source of antimicrobials, which play a crucial role in protecting the host against colonization by opportunistic skin pathogens such as methicillin resistant Staphylococcus aureus (MRSA). Currently the role of these antimicrobials in the commensal skin community is poorly understood. Skin staphylococcal community consists of closely related species with overlapping metabolic profiles that compete intensely for colonization advantage in a low nutrient environment. We hypothesize that skin commensal-derived antibacterials reduce colonization of competing bacteria, giving the antibacterial-producer a nutritional boost and a colonization advantage. To test our hypothesis, we conducted pair-wise interactions between staphylococcal isolates from healthy human volunteers and discovered a conserved ability of many Staphylococcus lugdunensis isolates to lyse Staphylococcus epidermidis isolates used in our study. Lysis enhanced the growth of S. lugdunensis during the stationary phase of population growth, indicating a predatory behavior that allowed extracting nutrients from the lysed competitor. We then performed whole genome sequencing of multiple S. lugdunensis isolates using MiSeq, in order to identify candidate genes involved in this interaction. Comparative genome analysis allowed identification of a locus, producing a MRSA-targeting non-ribosomal peptide known as Lugdunin, as a potential candidate involved in this antagonism. Using this analysis, we were able to detect variation in the distribution of this locus between different individuals and at distinct body sites. Current study shows that clinically relevant antimicrobials such as Lugdunin target other commensals in microbial communities. Our work will shed light on the role played by these antimicrobials in the ecology of the human skin microbiota.

1. Microbial Genomics Section, Translational and Functional Genomics Branch, NHGRI
2. NIH Intramural Sequencing Center, NHGRI
3. Dermatology Branch, NIAMS

Toward comprehensive interpretation of RYR1 variants associated with malignant hyperthermia susceptibility

A51
Poster

JJ Johnston [1], D Ng [1], SG Gonsalves [1], RT Dirksen [2], S Riazi [3], LA Saddic III [4], N Sambuughin [5], R Saxena [6], J Weber [7], H Rosenberg [8], LG Biesecker [1]

RYR1 is one of the 59 genes recommended by the American College of Medical Genetics and Genomics (ACMG) for opportunistic screening. Nearly all malignant hyperthermia (MH)-associated variants are rare amino acid substitutions making interpretation challenging. We set out to work toward a complete interpretation of known RYR1 variants, which could be made available to the genetics community. Variants associated with malignant hyperthermia susceptibility (MHS) were identified from the European Malignant Hyperthermia Group's (EMHG) list of "diagnostic mutations", the Human Gene Mutation Database (DM), GeneReviews, and ClinVar (likely pathogenic/pathogenic), (n=302). The ACMG-AMP criteria for variant interpretation were modified based on the biology and genetics of RYR1 and MHS. Proposed rule specifications included: a decision tree for weighting functional data prioritizing in vitro over in vivo data (PS3/BS3); proposed case count requirements for PS4 (case-control data); and reducing the weight for absence in gnomAD (PM2) to supporting. Proposed RYR1-specific rules were piloted on 48 variants from the EMHG list of "diagnostic mutations". Resulting classifications were discussed by the RYR1-MHS ClinGen expert panel to refine the rules. Of the 48 EMHG diagnostic variants, 40 were determined to be pathogenic, 5 to be likely pathogenic and three to be of uncertain significance. Of the remaining 254 variants, 10 were determined to be pathogenic, 38 to be likely pathogenic, 195 to be variants of uncertain significance and 10 to be benign or likely benign. Finalized assessments will be made available through ClinVar after approval by the ClinGen Sequence Variant Interpretation Working Group.

1. Medical Genomics and Metabolic Genetics Branch, NHGRI, NIH
2. University of Rochester Medical School, Rochester, NY
3. University of Toronto, Canada
4. University of California Los Angeles
5. Uniformed Services Univ Health Science, Bethesda, MD
6. Partners Healthcare, Boston, MA
7. Prevention Genetics, Marshfield, WI
8. MH Association of the United States, Sherburne, NY

ASHG Panel Recap: The Policy Implications of Genetics Outside of Research and Medicine

A52
Poster

Samata Katta [1,2], Elena Ghanaim [1], Cristina Kapustij [1]

While the public is acutely aware of the use of DNA as a forensic tool for criminal investigations, rapid decreases in the time and cost of genotyping and sequencing have made these technologies scalable in other non-health applications as well. This new accessibility raises questions about how DNA can or should be used in areas like law enforcement, border security, and disaster response. Whose data is entered into the DNA databases that law enforcement agencies use, how is the data used, and how are these databases regulated? How can genomic information be shared across national borders to help identify the remains of missing migrants? How might officials at the border use genomic data to verify claims of familial relationships for immigration purposes or to stop human trafficking? To consider these societal and policy questions, NHGRI's Policy and Program Analysis Branch co-hosted a panel discussion on the use of genomic information in these human rights and humanitarian contexts at the 2019 Annual Meeting of the American Society for Human Genetics. Moderators polled the session's attendees about their opinions on the issues. This poster gives an overview of the topics discussed by the panelists at this session, as well as the responses from an audience of genetics professionals.

1. Policy and Program Analysis Branch, Division of Policy Communications, and Education, NHGRI
2. American Society of Human Genetics (ASHG)

2019 NHGRI Symposium

Poster Abstracts

C G T C G T
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A C G

Session B
Last Names Kaz - W

Late-onset recurrent aseptic meningitis as the presenting symptom of Cryopyrin-Associated Periodic Syndrome (CAPS) responsive to anakinra

B1
Poster

Megan Kazynski [1], Amanda Ombrello [2], Michele Nehrebecky [2], Rena Godfrey [1], William Gahl [1], Danica Novacic [1], Ivona Aksentijevich [2], Camilo Toro [1]

Recurrent aseptic meningitis (RAM) is a poorly understood disorder with unclear pathophysiology. We report on a 68-year-old male with multiple episodes of sudden neurosensory hearing loss responsive to steroid followed by at least 27 episodes of RAM over a period of six years. Episodes were unprovoked and characterized by fever, malaise, arthralgia, and headache evolving into altered mental status, lymphocytic and monocytic CSF pleocytosis. Trials of IVIG, Rituximab and Azathioprine were ineffective on episode recurrence or severity.

The patient was evaluated at the NIH Clinical Center under the Undiagnosed Diseases Program (UDP). Considering a diagnosis of atypical cryopyrin-associated periodic syndrome (CAPS), a trial of anakinra resulted in nearly complete suppression of episode recurrence for over three years. Whole exome sequencing identified a heterozygous variant in the NLRP3 gene: p.Thr915Met residing in exon 8 of NLRP3 not previously associated with CAPS. Interestingly, most CAPS-associated mutations reside in exon 4 and cause early-onset systemic and CNS inflammation. About 30% of patients diagnosed with CAPS have somatic mutations in immune cells and have late-onset and milder symptoms. Our patient is notable for late-onset manifestations and having a germ-line mutation. NLRP3-associated disorders invoke a "gain-of-function" mechanism leading to unregulated overproduction of interleukin 1 beta (IL-1β), which drives systemic inflammation accounting for the response to IL-1 inhibitor anakinra. An IL-1 β stimulation assay in the patient's primary cells confirmed the pathogenicity of the novel exon 8 mutation. Proper investigation is recommended for RAM regardless of age of onset and can have substantial therapeutic benefits.

1. Undiagnosed Diseases Program (UDP), NHGRI
2. Inflammatory Disease Section, Metabolic, Cardiovascular and Inflammatory Disease Genomics Branch, NHGRI

Neonatal Ultrasonic Vocalization Profiles in Mouse Models of Down Syndrome

B2
Poster

Sabina Khantsis [1], Nicole Reed [1], Diana W. Bianchi [1], Faycal Guedj [1]

Background: Individuals with Down syndrome (DS) have impaired communication abilities and demonstrate delays in the acquisition of expressive language. Here we used analysis of ultrasonic vocalization (USV) patterns to investigate communication differences during neonatal development in mouse models of DS.

Methods: USVs were recorded in five minute sessions from postnatal days (PND) 2-12 in 17 Ts65Dn pups and their 36 euploid (Eup) littermates. Baseline vocalization recordings were collected daily after a 15 minute separation from the dam. For olfactory recognition, USVs were recorded and analyzed at PND 6 in response to three different odorant cues presented sequentially (home cage bedding; male cage bedding; virgin female cage bedding). Statistical analysis was performed using Two-Way ANOVA test, with significance at $p<0.05$.

Results: Under baseline conditions, Ts65Dn neonates emitted fewer total calls during the first postnatal week ($p<0.01$) compared to Eup. USV classification analysis showed that during typical development, the number of short vocalizations was highest at PND 3 and reduced gradually to be replaced with more complex vocalizations (flat, up, down, chevron and step-up) that reached a maximum around the end of the first postnatal week. During this period, Ts65Dn pups showed significantly more short USVs ($p<0.001$) and less complex USVs compared to their Eup littermates ($p<0.05$). Ts65Dn neonates showed similar vocalization profile abnormalities in the presence of different olfactory cues (home cage, male and virgin female odors). Analyses of USVs in Dp(16)1/Yey and Ts1Cje neonates are ongoing.

Conclusions: Ts65Dn pups demonstrated delayed communication abilities. Compared to Eup mice, Ts65Dn produced fewer calls early in neonatal development, and those produced were less complex. Future experiments will determine if these patterns are consistent in other mouse models of DS. Neonatal USVs are a useful behavioral endpoint to evaluate the effects of antenatal treatments of DS.

1. Prenatal Genomics and Therapy Section, Medical Genetics Branch, NHGRI

The Clinical Genome Resource (Clingen): Leveraging the Community to Create Resource of Clinically Relevant Genetic Variants

B3
Poster

Se Julie Kim [1], Erin Ramos [2], Natalie Pino [1]

ClinGen provides expert-curated knowledge on the clinical relevance of genes and variants through a central resource to support genomic medicine and research. The ClinGen consortium comprises 1125 researchers and clinicians from 230 US and international institutions and is formally recognized by the FDA as the first source of valid scientific evidence for germline variants that can be used to support clinical validity in premarket submissions from genetic test developers. Expert Curation Panels curate information on the clinical validity, pathogenicity, and dosage sensitivity of genomic variants using scientific literature and NCBI's ClinVar repository. To date, 811 gene-disease pairs have been evaluated for clinical validity, 984 variant-disease pairs have been evaluated for pathogenicity and submitted to ClinVar, and 1,476 genomic regions have been evaluated for dosage sensitivity. Adult and Pediatric Actionability WGs evaluate whether action can be taken to improve outcomes for those with a specific genetic variant. As of July 31,2019, the working group has curated 127 topics including pediatric and adult conditions. Furthermore, the ClinGen Community Curation Committee (C3) engages, identifies, and communicates with potential volunteers, from high school students to clinical geneticists, and facilitates the organization and placement of volunteers in curation efforts. Since launching their volunteer onboarding survey in August 2018, the C3 has trained 238 volunteers spanning 19 countries. Ultimately, with the help of community curators, ClinGen is working to build a public database of gene variants and their roles in health and disease.

1. Division of Genome Sciences, NHGRI
2. Division of Genomic Medicine, NHGRI

Exploring the neurological pathology in the first viable mouse model of methylmalonic acidemia and homocystinuria cblC type

B4
Poster

Stefanos Koutsoukos [1], Jennifer Sloan [1], Kelsey Murphy [1], Madeline Arnold [1], Nathan Achilly[1], Abdel Elkahloun [2], Weiwei Wu [2], Gene Elliot [3], Patricia Zerfas [4], Victoria Hoffman [4], Charles Venditti [1]

Methylmalonic acidemia and homocystinuria cblC type is the most common inborn error of intracellular cobalamin metabolism caused by pathogenic variants in MMACHC. MMACHC is responsible for the transport and processing of cobalamin (vitamin B12) derivatives into two bioactive cofactors, 5´-adenosylcobalamin and methylcobalamin, which are essential for the enzymatic activity of methylmalonyl-CoA mutase and methionine synthase respectively. Disease manifestations include poor survival, growth failure, heart defects, neurocognitive impairment, progressive maculopathy, and progressive retinopathy. The etiology of underlying disease mechanisms is unknown due to the lack of an animal model. In order to develop a model for cblC, we used genome editing to create pathogenic mutations in Mmachc with detailed characterization of two alleles: 163_164delAC p.Pro56Cysfs*4 (del2) and c.162_164delCAC p.Ser54_Thr55delinsArg (del3). Pathological examination revealed hydrocephalus and hypoplasia of the corpus callosum in Mmachcdel3/del3 mutants. To further explore the molecular characteristics of the model, in the context of these severe pathological findings, we performed a microarray study of whole brain from Mmachc (del2) mutants as compared to wildtype and heterozygous controls (N=6 mutants, N=4 controls) prenatally treated with vitamin B12. Preliminary analysis of the transcriptome revealed an upregulation of genes related to the primary biochemical pathway (Prdx1 and Mthfd2l) and several downregulated genes related to retinal degeneration (Abca4, Nrl, Poc1b, and Prpf4). Ingenuity Pathway Analysis indicated molecular and physiological system abnormalities in RNA damage and repair, embryonic development, and nervous system development and function. These data provide a foundation to further explore the molecular nature of the neurological sequelae of cblC deficiency.

1. Organic Acid Research Section, Medical Genomics and Metabolic Genetics Branch, NHGRI
2. Microarray Core, Cancer Genetics and Comparative Genomics Branch, NHGRI
3. Gene Editing, ES Cell and Transgenic Mouse Core Facility, NHGRI
4. Diagnostics and Research Services Branch, Office of the Director, NIH

The National Human Genome Research Institute (NHGRI) Genomic Data Science Analysis, Visualization, and Informatics Lab-space (AnVIL)

B5
Poster

Nataliya Kucher [1,2], Joanna Chau [1,2], Heidi Sofia [2], Elena Ghanaim [3], Ken Wiley [2], Valentina Di Francesco [1]

The NHGRI Genomic Data Science AnVIL is a cloud-based storage, analysis, and computing platform for data generated by NHGRI-funded research programs. AnVIL is designed based on modular, standards-based data infrastructure to integrate and interoperate with other cloud-based resources relevant to basic and clinical genomics communities. AnVIL is built on Google Cloud Platform and GA4GH standards. AnVIL will provide optimized and reproducible analysis workflows; cloud services that leverage NIH-negotiated discount rates; and harmonized phenotypic data and metadata. All the platform and application components will align with the NIH Security Best Practices for Controlled-Access Data. AnVIL is an integral part of a distributed, federated data ecosystem that facilitates genomic data access, sharing, and computing across similar resources being established by other Institutes at the NIH.

AnVIL is implementing GA4GH standards for data access, workflow development, and data and tools sharing. These include standards from the Cloud Work Stream, such as the Workflow Execution Service, Task Execution Service, and Data Repository Service APIs, as well as standards from the Data Use and Researcher Identity Work Stream. AnVIL is also piloting the Data Use Ontology System and a Library Card program for streamlined data access in collaboration with the NHGRI Data Access Committee.

The AnVIL project development is led by a collaboration of the Broad Institute, Johns Hopkins University, and 11 partner institutions. The link to the portal can be found here: https://anvilproject.org/. The analysis platform is expected to be functional in early 2020.

1. Division of Genome Sciences, NHGRI
2. Division of Genomic Medicine, NHGRI
3. Division of Policy, Communications, and Education, NHGRI

Title: Induced Pluripotent Stem Cells as a Tool to Study Inter-Individual Variability in Down Syndrome

B6
Poster

Sarah Lee [1], Monica Duran-Martinez [1], Diana Bianchi [1], Faycal Guedj [1]

Individuals with Down syndrome (DS) vary in phenotypes and responses to intervention. To better understand molecular and cellular bases of inter-individual variability, we generated a large collection of iPSCs from individuals with trisomy 21 (T21) and age/sex matched euploid (Eup) controls. Live cell imaging was used to measure the proliferation rates. Fibroblasts were transformed to iPSCs using Sendai virus and imaged daily. Measurements included: days (d) needed for iPSC colonies to emerge, number and average colony size (area) over time. T21 fibroblasts were compared to Eup in groups and pairs matched by age and sex and iPSCs were analyzed by group. T21 samples grew slower compared to Eup. This delay did not reach statistical significance. After 138 h of culture, 100% of Eup and 73% OF T21 samples reached >80% confluency. Pairwise analysis revealed significant differences in growth rate at 24 and 48h. Following transduction, iPSCs were obtained from 9 T21 and 9 Eup samples. By day 18 after transduction, 10 or more colonies were obtained from 5/5 (100%) Eup and 3/7 (43%) T21 samples. Similar to fibroblast growth data, significant variability in colony number was observed between individual T21 samples; however, no significant differences were observed in average colony size over time between T21 and Eup samples. Inter-individual variability exists at the cellular level in T21. Our data suggest the need for greater numbers of independent cell lines and novel statistical methods when characterizing phenotypes and responses to treatment.

1. Prenatal Genomics and Therapy Section, Medical Genetics Branch, NHGRI

QC Measurements of Exome Chip Sequence Data in a Family-based Study

B7
Poster

Qing Li [1], Stephen Wank [2], Joan E. Bailey-Wilson [1]

Quality control (QC) is an important step in sequence data analysis. In VCF files, many genotype calling quality measurements are reported at the variant level and the variant-and-sample level. Common practice is to drop variants based on pre-set thresholds of several key QC measurements, including QD, MQ, FS, SOR MQRankSum, and ReadPosRankSum at the variant level. In addition, genotypes at specific loci should be set to missing if the variant-and-sample level measurements are poor. Pedigree information can be used as the third step of data cleaning, eliminating genotypes causing Mendelian inconsistencies.

In a linkage analysis of small intestinal carcinoid tumors, we performed QC on whole exome sequence data from a pedigree of 34 individuals. In this work, we reported the summary statistics on the genotype calling QC measurements of 219,742 variants. We found that the QC measurements have a wide range of variation across different chromosomes. Using hard-filtering based on recommended thresholds, 182,132 (~83%) variants were kept. We found that the current QC thresholds cannot remove all the poorly typed genotypes. Among the retained variants, 21% incurred at least one Mendelian error. Given the 18 informative child-parent trios in the pedigree and the 28,114 variants, this is a rate of ~1.9% per trio, per variant. Extensive and iterative cleaning of Mendelian errors are needed after data filtering by QC measures alone. In conclusion, the current hard filtering thresholds are inadequate and are improved by Mendelian inconsistency checks.

1. Statistical Genetics Section, Computational and Statistical Genomics Branch, NHGRI
2. Digestive Diseases Branch, NIDDK

Systems biology in hematopoietic cell stem and progenitor populations: Integrating multiple *omics datasets to understand differentiation

B8
Poster

Jens Lichtenberg [1], Guanjue Xiang [2], Elisabeth Heuston [1], Belinda Giardine [2], Yu Zhang [2], Cheryl Keller [2], Ross Hardison [2], David Bodine [1]

Systems biology integrates genomic profiles of specific cell types to provide functionally-testable hypotheses of lineage- specificity. Here we compare RNA expression, DNA methylation, chromatin accessibility, DNA binding proteins and histone modification in seven different hematopoietic populations using a Bayesian non-parametric hierarchical latent- class mixed-effect model known as IDEAS to characterize epigenetic changes associated with hematopoietic differentiation.

Previous hematopoietic epigenome segmentation studies have focused on histone modifications, chromatin accessibility and DNA binding protein profiles. DNA methylation has been shown to vary markedly in hematopoietic populations. Inclusion of DNA methylation in these segmentation studies increased the original 36-state model of regulatory interactions to 41 states. These new DNA methylation-related states were associated with repressive marks, RNA transcription, and regulation attributed to DNA methylation alone. Imputing epigenetic models on inputs systematically perturbed for hematopoietic populations resulted in epigenetic models of varying degrees of overlap, which were quantified and set in context with underlying biological processes. We furthermore leveraged these imputation-related differences to infer lineage-specific potential impacts on regulation.

Our data show that methylation has a strong impact on functional genomic modeling and can be used to discern cell type specific epigenetic regulatory behavior by leveraging imputation for missing cell type data.

1. Hematopoiesis Section, Genetics and Molecular Biology Branch, NHGRI
2. Department of Biochemistry and Molecular Biology, The Pennsylvania State University

Vascular abnormalities in murine embryos with ubiquitous expression of Akt1 p.(E17K)

B9
Poster

Marjorie LIndhurst [1], Wenling Li [2], Stephen Wincovitch [3], Xuefei Ma [4], Jasmine Shwetar [1], Yosuke Mukouyama [2], Leslie Biesecker [1]

Mosaic expression of the c.49G>A, p.(E17K) AKT1 variant causes Proteus syndrome. To test the Happle hypothesis which states that the Proteus syndrome variant only survives by mosaicism, we crossed Akt1 p.(E17K) conditional mice with ACTB-Cre mice which express Cre ubiquitously. Embryos from this cross were not viable and died between E11.5 and E17.5, corroborating the Happle hypothesis. Mutant embryos were pale, had fewer visible blood vessels and increased hemorrhage in the skin. Whole-mount immunostaining of E14.5 and E15.5 mutant skin showed no mature arteries or veins in the vasculature, suggesting a vascular remodeling defect. To determine the extent of the vascular defects in these embryos, we used the iDISCO clearing method to perform whole mount immunolabeling and volume imaging on mutant and wild type E11.5 – E14.5 embryos. Examination of E12.5 embryos showed that the vertebral arteries failed to complete formation with heterogeneous, abnormal connections of the basilar artery to the aorta. In E13.5 mutant embryos, several vessels including the thoracic and external carotid arteries were missing, underdeveloped, or had abnormal patterning. Taken together, we conclude that constitutive AKT signaling impairs the remodeling of the developmental vascular capillaries into a hierarchical vascular network and leads to structural vascular anomalies. We speculate that at a cellular level, these vascular anomalies are due to abnormal endothelial cell behaviors such as sprouting, migration, and subsequent endothelial cell fusion, and that disconnection of major arterial networks may cause abnormal circulation, resulting in defective vascular remodeling in peripheral vasculature.

1. Medical Genomics and Metabolic Genetics Branch, NHGRI
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3. Genetic Disease Research Branch, NHGRI
4. Laboratory of Molecular Cardiology, NHLBI

Defining the missing heredity and phenotypic diversity associated with oculocutaneous albinism

B10
Poster

Stacie Loftus [1], Jiyeon Choi [2], Kevin Brown [2], Linnea Lundh [3], NISC Comparative Sequencing Program [4], William Oetting [5], David Adams [3], Wiiliam Pavan [1]

Oculocutaneous Albinism (OCA) is a recessive disorder characterized by reduction of skin, hair and eye pigmentation. OCA individuals are at higher risk for UV-induced skin cancers and a complex set of developmentally-mediated visual impairments of diverse clinical severity. Mutations are predominately found in 2 genes TYR (OCA types 1A and 1B) or OCA2 (OCA type 2), with a small number of cases attributed to mutations in TYRP1, SLC45A2, SLC24A5, C10orf11/LRMDA. Most critically, 20-50% patients remain without identification of biallelic mutations after traditional exon-based screening of known OCA genes. To better define the molecular mechanisms underlying OCA, we are interrogating over 500 OCA proband individuals and family members, using custom capture, short-read sequencing to query coding, intronic, and cis-regulatory regions of 6 OCA and 31 pigmentation GWAS study identified genes. Non-coding cis-regulatory regions include DNase1-HS data (derived from primary and immortalized melanocytes, melanoma tumors, and retinal pigment epithelium), H3K27Ac ChIP binding regions under conditions of MAPK activation and inhibition in 501mel cells and SNPs in linkage disequilibrium with SNVs identified from our melanocyte expression quantitative trait loci (eQTL) dataset. We identified over 73,000 eQTLs for 128 genes associated with visible pigmentation-related phenotypes, including 156 and 684 eQTLs for TYR and OCA2, respectively. Our family, trio-based analysis and interrogation of coding, and non-coding sequence, will better define the molecular spectrum of OCA mutations, facilitate assessment of non-coding DNA variation function, and allow for the subsequent systematic evaluation of the molecular mechanisms underlying both missing heritability and phenotypic variation observed among OCA patients.

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Expectations, Roles, and Experiences of Grandparent-Caregivers of Children with Inherited Metabolic Conditions

B11
Poster

Jasmine Manalel [1], Betina Hollstein [2], Laura Koehly [1]

Grandparenting can be a rewarding and health-promoting experience for older adults. However, grandparent-caregivers often experience greater stress and poorer health relative to non-caregiving grandparents. Further, little is known about grandparents caring for a child with a rare, chronic illness, like inherited metabolic conditions. This study aimed to extend knowledge of the expectations and experiences of grandparents providing care to a child affected with an inherited metabolic condition. The sample included 23 grandparent-mother dyads from the Inherited Diseases, Caregiving, and Social Networks Study. The grandparent sub-sample ranged from 49 to 79 years of age (Mage = 64), the majority were female (83%) and married (74%), and almost half (48%) were retired. Respondents' social network assessments were analyzed to determine concordance between mother- and grandparent-reports of grandparents' role in the child's caregiving network. Fifteen mother-grandparent dyads (65%) agreed on grandparents' role in the child's network, with 14 of those considering the grandparent to be very close and important (versus less close or excluded from the support network). Grandparents were more likely to report spending enough time caregiving if they were in agreement with mothers regarding their caregiving role. Content analysis of grandparents' interviews provided supporting information about the joys and regrets of their grandparenting experience and perspectives on caregiving expectations. This research leverages multi-informant social network and qualitative data to illuminate grandparents' role in the caregiving networks of children with rare, genetic illnesses and adaptation to non-normative grandparenting experiences.

1. Social Network Methods Section, Social and Behavioral Research Branch, NHGRI
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Genetic variants involved in antigen-presenting cell and CD4+ T cell activation increase susceptibility to periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome

B12
Poster

Kalpana Manthiram [1], Silvia Preite [2], Fatma Dedeoglu [3], Selcan Demir [4], Seza Ozen [4], Kathryn Edwards [5], Yuriy Stepanovskiy [6], Alexander Katz [1], Peter Wright [7], Henry Feder [8], Karyl Barron[2], Amanda Ombrello [1], Tina Romeo [1], Beverly Barham [1], Anne Jones [1], Julie Le [1], Pam Schwartzberg [2], Elaine Remmers [1], Daniel Kastner [1]

Periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome is the most common periodic fever syndrome in children. Although the mechanism is unknown, the disease appears to cluster in families. We queried two Caucasian cohorts and one Turkish cohort (total N = 263) for common variants previously associated with other oropharyngeal ulcerative disorders, namely Behçet's disease and recurrent aphthous stomatitis. In a meta-analysis, we found that a variant upstream of IL12A (rs17763641) is strongly associated with PFAPA (OR of 2.05, p=4.8x10-9), and we found that monocytes from individuals who are heterozygous or homozygous for the risk allele produce significantly higher levels of IL-12p70 upon stimulation than those from individuals without the risk allele. We also found that other variants near genes involved in Th1 and Th17 cell activation (IL10, STAT4, IL23R-IL12RB2, and CCR1-CCR3) were significant susceptibility loci for PFAPA, indicating that PFAPA is a disorder of heightened monocyte and Th1 and Th17 activation. Our results illustrate genetic similarities among recurrent aphthous stomatitis, PFAPA, and Behçet's disease, which suggests that these disorders lie on a spectrum of disease. HLA type may be a factor that impacts phenotype along this spectrum as we found HLA class II and class I associations for PFAPA distinct from those reported for Behçet's disease and recurrent aphthous stomatitis. Moreover, HLA type appears to be a more significant risk factor for Behçet's disease than PFAPA and weaker for recurrent aphthous ulcers than PFAPA.

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Circulating biomarkers of mitochondrial dysfunction in methylmalonic and propionic acidemia.

B13
Poster

Samantha McCoy [1], Alexandra Pass [1], Oleg Shchelochkov [1], Jennifer Sloan [1], Susan Ferry [1], Carol Van Ryzin [1], Irini Manoli [1], Charles Venditti [1]

Methylmalonic and propionic acidemias (MMA, PA), caused by mutations in the genes coding for methylmalonyl-CoA mutase (MMUT) and the two subunits of propionyl-CoA carboxylase (PCCA and PCCB), affect mitochondrial enzymes involved in branched-chain amino acid metabolism and share multiorgan complications. Disease-specific metabolites, such as serum methylmalonic and 2-methylcitric acid, depend on dietary protein intake and renal function. Additional biomarkers resistant to these confounds could enable monitoring of organ-specific responses to therapies in these disorders. We recently used MMA murine models treated with AAV gene therapy and liver transplanted patients to show that elevations in plasma fibroblast growth factor 21 (FGF21) correlate with hepatic mitochondrial dysfunction and clinical parameters in MMA and are restored to near-normal post-liver transplantation. FGF21 in MMA patients correlates closely with an additional biomarker, growth differentiation factor 15 (GDF15) that is used in the clinical diagnosis of primary mitochondrial myopathies. We measured plasma FGF21 and GDF15 in 89 individuals with MMA, 36 with PA and 39 controls. MMA patients have significantly higher concentrations in both, FGF21 ($p= 0.001$) and GDF15 ($p<0.001$), compared to PA, while GDF15 was higher in the PCCB compared to PCCA subtype of PA ($p=0.004$). In PA, GDF15 showed stronger correlations with biochemical and clinical parameters, including propionylcarnitine, 2-methylcitrate, propionate oxidation, height z-score and full-scale IQ compared to FGF21. Future studies in PA murine models will clarify how these findings pertain to tissue-specific pathology of the disease.

1. Organic Acid Research Section, Medical Genomics and Metabolic Genetics Branch, NHGRI

Deleterious germline mutations in the BRCA1 gene are associated with increased risk for cancers of the female reproductive system other than breast and ovarian as well as other cancers

B14
Poster

Candace D. Middlebrooks [1], Kenzhane Pantin [2], Mark Stacey [3], Carrie Snyder [3], Trudy Shaw[3], Murray Joseph Casey [3,4], Joan E. Bailey-Wilson [1], Henry T. Lynch [3]

Mutations within the BRCA1 gene have been linked to up to an 80% lifetime risk of breast cancer as well as increased risk for ovarian, pancreatic and melanoma cancers. In this study we examined families with known germline mutations in BRCA1 after long-term follow-up to determine whether carriers experience higher rates of other cancers that have not yet been associated with germline mutations in the BRCA1 gene.

We studied 127 Hereditary Breast and Ovarian Cancer (HBOC) syndrome families (N = 23,078 individuals who have been followed at Creighton University) in which a causal mutation in the BRCA1 gene was identified. We performed survival analysis and a mixed effects cox regression with age at follow-up or cancer event as our time variable and presence or absence of BRCA1-related or other cancers (separate analyses) as our indicator variable. The survival curves showed a significant age effect with carriers having a younger age at cancer onset for BRCA1-related (as expected) as well as other cancers than that of non-carriers. The cox regression models were also highly significant ($P = 1.77E-37$ and $P = 1.04E-07$ for the BRCA1-related and other cancers, respectively). Of the cancers with enough samples to do stratified analyses, cervix, uterine, skin, lymphoma and colon cancers occurred at higher rates and at earlier ages in mutation carriers.

These analyses support the hypothesis that the BRCA1 mutations carriers of HBOC syndrome have increased risk for early onset of several additional cancer types, especially cancers that arise in estrogen-influenced tissues.

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A Methylation Density Binary Classifier for predicting and optimizing the performance of methylation biomarkers in heterogeneous clinical samples.

B15
Poster

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Aberrant DNA methylation is commonly heralded as a promising cancer biomarker; however, intratumoral heterogeneity and natural epigenetic variance often manifests variable intercellular methylation that can complicate the use of methylation biomarkers for clinical diagnostic applications such as blood-based cancer testing. Here, we show development of a statistical method that employs a methylation density binary classifier (MDBC) leveraging cancer-associated differences in intermolecular methylation density distributions to optimize the performance of methylation biomarkers, particularly at dilute concentrations. We developed and tested the classifier using reduced representation bisulfite sequencing (RRBS) data derived from ovarian carcinomas (EOCs) and control tissues, which were assessed by evaluation of methylation density distribution profiles of DNA molecules from ZNF154, a recurrently methylated locus in multiple cancers. While higher mean locus-specific methylation was observed in EOCs compared to controls, mean methylation could not accurately detect tumor DNA at low, clinically-relevant concentrations. In contrast, classification using the intermolecular methylation density outperformed mean methylation at tumor dilutions as low as 0.01%. Assessing the methylation density profiles of cfDNA in an independent set of 26 EOC-positive and 45 control low volume (1-mL) plasma samples achieved a sensitivity/specificity of 73.1%/95.6%. In a direct comparison to CA-125 measurements, the MDBC correctly classified 87% of samples whereas CA-125 classified only 47.8%. Our results indicate that intermolecular methylation densities in cfDNA facilitates methylation biomarkers in clinical applications. Furthermore, MDBC analysis of ZNF154 was able to outperform CA-125 in the detection of etiologically-diverse ovarian carcinomas, indicating potential clinical utility as a broad biomarker for ovarian cancer diagnostics.

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5. Department of Mechanical Engineering, Johns Hopkins University

Bi-allelic variants in the autophagy gene ATG4D are associated with a pediatric neurological disorder characterized by hypotonia, dysarthria, impaired coordination, and gait abnormalities

B16
Poster

Marie Morimoto [1], Mariska Davids [1], Patricia Zerfas [2], Vikas Bhambhani [3], Anna Lehman [4], Jennifer Murphy [1], Ellen Macnamara [1], Camille Wang [1], Undiagnosed Diseases Network [5], Sara Reichert [6], Audrey Thurm [7], Wendy Introne [8], William Gahl [1,8,9], Cynthia Tifft [1,8,9], May Malicdan [1,8,9]

Macroautophagy is a highly conserved process that regulates the degradation and recycling of cellular components. Double-membraned organelles known as autophagosomes deliver cellular components to the lysosomes for degradation. Defective autophagy has been shown to contribute to the pathogenesis of various human diseases. With the aim of identifying a genetic locus underlying the clinical phenotype of two patients with a neurological disorder characterized by hypotonia, dysarthria, impaired coordination, and gait abnormalities, we performed whole exome sequencing. Bi-allelic missense or frameshift variants were identified in ATG4D, which encodes for one of the four ATG4 isoforms that process the LC3 and GABARAP proteins required for autophagosome biogenesis and autophagy substrate selection, in both patients segregating with the disease. Quantitative PCR, western blot, and transmission electron microscopy were used to explore the functional consequences of ATG4D deficiency. Cultured fibroblasts from one proband showed comparable ATG4D mRNA expression compared to controls. Autophagic vacuole formation in response to treatment with Bafilomycin A1 (inhibitor of autophagy) and/or Torin 1 (inducer of autophagy) was comparable to a control. Although no remarkable differences in the levels of the autophagy markers p62, LC3B-II, GABARAP-II, or GABARAPL2-II were identified at baseline or upon treatment with Bafilomycin A1 and/or Torin 1, GABARAPL1-II was not induced after these treatments in the fibroblasts of one patient. In summary, we report two patients with a neurological disorder and hypothesize that bi-allelic variants in ATG4D may underlie the pathogenesis of this disorder. Further functional studies are required to delineate the molecular consequences of ATG4D deficiency.

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An examination of the relationships between symptoms of attention/deficit hyperactivity disorder and resting-state connectivity across time: A longitudinal neuroimaging study

B17
Poster

Luke Norman [1], Marine Bouyssi-Kobar [1], Gustavo Sudre [1], Wendy Sharp [1], Philip Shaw [1,2]

Previous work has demonstrated resting-state connectivity abnormalities within default mode, striato-thalamic and task positive network regions in children and adolescents with attention/deficit hyperactivity disorder (ADHD) relative to typically developing controls. However, existing studies have used cross-sectional designs, and it is unclear to what extent these neural abnormalities represent longitudinal risk factors for and/or outcomes of ADHD symptoms. In the present study, 319 children and adolescents (baseline age=5-17 years old, 116 females) completed baseline resting-state functional magnetic resonance imaging (fMRI) scans and ADHD symptom assessments. Of these, 162 participants (67 females) completed up to two follow-up scanning and assessment sessions at least six months apart (mean duration between scans=2.1 years (SD=1.35 years)). Functional connectivity was quantified using voxelwise global brain connectivity. In line with existing cross-sectional work, inattentive symptoms were associated positively with connectivity within the default mode network and striato-thalamic regions at baseline, but associated negatively with connectivity within regions of cingulo-opercular and fronto-parietal task-positive networks. For the longitudinal analysis, continuous-time cross-lagged models indicated that inattentive symptoms were associated with subsequent resting-state connectivity within a largely overlapping set of regions, although resting-state connectivity was unrelated to subsequent inattentive symptoms (all brain findings $p<0.05$, $k>2000$). Results indicate that, rather than representing a potentially mechanistic risk factor for the development of ADHD, abnormal functioning within default mode, striato-thalamic and task-positive network regions may be a downstream effect of exposure to ADHD symptoms on brain development and functioning.

1. Neurobehavioral Clinical Research Section, Social and Behavioral Research Branch, NHGRI
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Overview of the Encyclopedia of DNA Elements (ENCODE) Project

B18
Poster

Briana Nuñez [1]

The Encyclopedia of DNA Elements (ENCODE) Project creates a public resource of high-quality genomic data with the goal of identifying all of the functional elements in the human and mouse genomes, including elements specific to particular cell types and cell states. Candidate functional elements, such as promoters or enhancers, are identified using transcriptomic and epigenomic assays, genome and epigenomic editing, comparative genomics and integrative bioinformatics methods. ENCODE employs various epigenetic assays on these elements including measurements of chromatin accessibility, histone modifications, interactions of proteins with DNA and RNA, and nuclear architecture.

The ENCODE Consortium consists of mapping and characterization centers that generate primary data, as well as data analysis and coordination centers that carry out data integration and sharing. Together, these groups collect and analyze data to identify candidate regulatory elements, while ensuring these data pass rigorous quality standards. Characterization centers utilize high-throughput assays and computational analyses to elucidate the function, if any, of identified candidate regulatory elements. All data are rapidly released to the public through the ENCODE portal (https://www.encodeproject.org/), where users can access ENCODE's 15,000+ datasets without restriction. To date, the scientific community has used ENCODE data in over 2,100 publications in a range of applications. We continue to see how the scientific community utilizes ENCODE data in new and innovative ways to better understand the human genome and its role in health and disease.

1. Division of Genome Sciences, NHGRI

Rubinstein-Taybi Syndrome in Diverse Populations

B19
Poster

Ugonna Nwannunu [1], Babajide Owosela [1], Cedrik Ngongang [1], Lynne Wolfe [1], William Gahl [1], Maximilian Muenke [1], Paul Kruszka [1]

Background: Rubinstein-Taybi Syndrome (RTS) is a rare autosomal dominant genetic disorder with an estimated prevalence of 1 in 100,000 births and is characterized by intellectual disabilities, distinctive craniofacial dysmorphism, and distal digit abnormalities—as well as other body system anomalies.

Methods: An international group of clinical geneticists, dysmorphologists, and other medical specialists, headed by a team at the National Human Genome Research Institute (NHGRI), have collaborated to create an Atlas of Human Malformation Syndromes in diverse populations and contribute to a series in the American Journal of Medical Genetics on diverse populations. Clinical exam findings such as broad, radially angulated thumbs, distal phalanges, and toes among ethnically and geographically diverse RTS populations are being compiled. Participants are characterized by sex, age and geographical regions (Asia, Africa, North America, South America, and Europe).

Results: We have thus far compiled data from 84 patients. Of the 84, 4 patients are of African descent, 26 are of Asian descent, 8 are of South American descent, 7 are of Middle Eastern descent, and 39 are of Caucasian descent (of either North American or European origin). Patients in each population displayed broad, radially angulated thumbs, distal phalanges, and toes. Discrepancies were observed in the frequency and distribution of each characteristic.

Conclusion: More attention is now being given to rare diseases in diverse populations. Once the cohort is complete, facial analysis using convolutional neural networks will be used to test the hypothesis that this technology can accurately diagnosis RTS.

1. Medical Genetics Branch, NHGRI

Discovery of Microbial Communities in Metagenomic Data Using Topic Modeling

B20
Poster

Brian Ondov [1,2], Angela Wu [3], Adam Phillippy [1]

The microbiome has been linked to many aspects of human health, but much remains unknown about both its interactions with disease and its natural variations among and within individuals. Here we seek to facilitate both basic and targeted research by using machine learning and interactive visualization to organize and classify metagenomic data. Specifically, we make use of Topic Modeling, a technique common in Natural Language Processing that learns coincident cohorts across samples by optimizing a generative statistical model. We leverage this unsupervised approach to create an interactive visualization that reduces high-dimensional taxonomic tables to interpretable structures. We also demonstrate proof of concept for a supervised version of Topic Modeling attempts to discriminate microbiomes associated with disease based on the communities detected.

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Holoprosencephaly and Congenital Heart Defects

B21
Poster

Babajide Owosela [1], Cedrik Ngongang [1], Paul Kruszka [1], Maximilian Muenke [1]

Background: Marked by the incomplete division of the embryonic forebrain, holoprosencephaly (HPE) is a relatively common structural brain defect. Co-occurrence of HPE and congenital heart defects (CHD) has been reported in several individuals with mutations in known HPE-causing genes. Specifically, CHD was reported in 2% of a cohort of 391 individuals with HPE and mutations in SHH. Similarly, in a cohort of 157 individuals with ZIC2-associated HPE, 9% were found to have CHD. Studies in animal models have shown that Hedgehog signaling, a crucial cell differentiation pathway, is involved in both forebrain and cardiac development. Here, we report on the incidence of CHD in a large cohort of individuals with HPE with and without mutations in HPE-related genes.

Methods: A cohort of 450 probands with various types of HPE were carefully phenotyped by means of clinical examination, imaging, and/or autopsy, with special emphasis on cardiac anatomy. Molecular investigations were subsequently performed, including Sanger sequencing of the 4 most common HPE genes (SHH, SIX3, TGIF1, ZIC2) and Exome sequencing.

Results: Thirty probands (6.7%) were found to have a CHD. From these 30 cases, seven had a complex CHD. Only 3 of the thirty cases had variants in known genes: SHH (1 of 28 SHH variants [3.6%]), SIX3 (1 of 26 SIX3 variants [3.8%]), and STAG2 (1 of 2 STAG2 variants). Of interest, of 50 probands with pathogenic variants in ZIC2, none had CHD.

Conclusion: These findings show that CHD infrequently co-occur with HPE, and this co-occurrence may be gene specific.

1. Medical Genetics Branch, NHGRI

A Novel Model of Methylmalonic Acidemia in Zebrafish

B22
Poster

Joel Pardo [1], Katie Ellis [1], Madeline Arnold [1], Raman Sood [2], Blake Carrington [2], Kevin Bishop [2], Jerrel Catlett [1], Victoria Hoffman [3], Patricia Zerfas [3], Nate Achilley [1], Randy Chandler [1], Jennifer Sloan [1], Oleg Shchelochkov [1], Charles Venditti [1]

Methylmalonic acidemia (MMA) is a rare autosomal recessive disorder that impairs the oxidative metabolism of valine, isoleucine, methionine, threonine, and odd-chain fatty acids. The most common form of isolated MMA arises from pathogenic variants in methylmalonyl-CoA mutase (mut), the enzyme responsible for isomerizing methylmalonyl-CoA to succinyl-CoA. A defining characteristic of the disease is the resultant elevation in methylmalonic acid. Patients display multisystemic manifestations, severely affecting development and metabolic stability, often associated with high mortality. Protein restricted diets remains the mainstay of treatment. Some patients undergo liver transplantation to improve metabolic instability and potentially slow the disease progression. Explanted livers harbor evidence of secondary mitochondrial disfunction: megamitochondria and inclusions in the mitochondrial matrix seen on electron microscopy. To understand the nature of the disorder, we generated a piscine model of MMA. Zebrafish mut-/- recapitulated several key findings observed in patients with MMA. Namely, mut-/- had elevated methylmalonic acid at 15 days post fertilization (dpf) (P value <0.0001). They displayed significant developmental delays as compared to unaffected clutchmates (SL: P value <0.0001, HAA: P Value <0.001, SB score: P Value < 0.001). Moreover, mut-/- exhibited 100% mortality after 22 dpf, reflecting the physiological consequence of uncontrolled metabolic impairment. H&E staining in mut-/- zebrafish hepatocytes at 15 dpf showed eosinophilc inclusions suggestive of mitochondrial proliferation. TEM of the zebrafish mut-/- livers demonstrated increased mitochondrial density. Mitochondria had aberrant curved morphology and clearing of mitochondrial matrix. Combined, mut-/- zebrafish reflect key characteristics of elevated metabolites, failure to thrive, and secondary mitochondrial dysfunction in MMA.

1. Organic Acid Research Section, Medical Genomics and Metabolic Genetics Branch, NHGRI
2. Zebrafish Core, Translational and Functional Genomics Branch, NHGRI
3. Office of Research Services, Office of the Director, NIH

A subset of SMN complex members have a specific role in tissue regeneration via ERBB pathway-mediated proliferation

B23
Poster

Wuhong Pei [1], Lisha Xu [1], Zelin Chen [1], Claire C Slevin [1], Kade P Pettie [1], Stephen Wincovitch [2], NISC Comparative Sequencing Program [3], Shawn M Burgess [1]

Spinal Muscular Atrophy (SMA) is the most common genetic disease in children. SMA is generally caused by mutations in the gene SMN1. The Survival of Motor Neurons (SMN) complex consists of SMN1, Gemins (2-8) and Strap/Unrip. We previously demonstrated smn1 and gemin5 inhibited tissue regeneration in zebrafish. Here we investigated each individual SMN complex member and identified gemin3 as another regeneration-essential gene. These three genes are likely pan-regenerative since they affect the regeneration of hair cells, liver and caudal fin. RNA-Seq analysis reveals that smn1, gemin3, and gemin5 are linked to a common set of genetic pathways, including the tp53 and ErbB pathways. Additional studies indicated all three genes facilitate regeneration by inhibiting the ErbB pathway, thereby allowing cell proliferation in the injured neuromasts. This study provides a new understanding of the SMN complex and a potential etiology for SMA and potentially other rare unidentified genetic diseases with similar symptoms.

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Insights from the Sickle Cell Community on Genetics Healthcare: A Qualitative Exploration

B24
Poster

Diana Phan [1,2], Khadijah Abdallah [2], Lisa Thaniel [3], Susan Hannum [1], Barbara Harrison [4], Lori Erby [1,2], Vence Bonham [2], Sophie Lanzkron [1], Debra Roter [1]

Objective: Several studies and professional organizations promote genetic counseling individuals with sickle cell disease and trait. Because genetic testing within the sickle cell context has historically been tensioned by racial discrimination and stigma in the U.S., we argue for the importance of exploring current opinions, experiences, and any concerns of the community to inform ways to enhance the benefits and mitigate the harms of genetic counseling for individuals affected with SCT and SCD. The present study queries perspectives of affected adults and unaffected parents on genetic counseling and related issues as well as perspectives on racialized versus ancestral views of SCD in the medical and broader communities.

Methods: We are conducting qualitative focus group discussions and semi-structured individual interviews with a convenience sample of 20-30 adult participants with SCD and those who have children with SCD. Open-ended questions and probes cover topics including to experiences with and perceptions of genetic counseling, racialized views of SCD in the medical and broader communities, and opinions on ancestral framing of SCD. Transcribed discussions and interviews will be coded deductively by two individuals for inter-coder reliability to derive themes and sub-themes.

Findings: Preliminary focus group data acquired from adults with SCD highlights diverse views on topics raised. Some people expressed the value of genetic counseling but also expressed opinions on the service's limitations and harms and offer suggestions to maximize benefits and address limitations and harms. Participant insights demonstrate the complexity of racial issues in healthcare.

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3. Children's National Medical Center
4. Howard University

Racial and Ethnic Classification in Healthcare Settings: Is it Just?

B25
Poster

Natalie Pino [1], Alice Popejoy [2]

The United States has a long history of institutionally classifying people into categories like race and ethnicity; concepts that are bound to power structures and stereotypical assumptions. Institutions that educate, employ, and deliver services, including health care settings, collect information about race and ethnicity. However, this data collection is often done without questioning the methods used, without examining the impact on patients, and often without offering a justification for why the data are needed.

In this paper, we examine the practice of classifying patients on the basis of race, ethnicity, and ancestry (REA) in a U.S. clinical setting and evaluate whether this practice meets the 'justificatory conditions' for an ethical public health practice as defined by Childress et al. (2002). These conditions include: 1) Effectiveness, 2) Proportionality, 3) Necessity, 4) Least infringement, and 5) Public justification.

Our approach was three-fold; first, we identified case examples of clinical REA classification, using clinical laboratory requisition forms. Second, we reviewed the literature for evidence about the utility and potential harms of categorizing people by REA for clinical care. Finally, we evaluated whether the practice of classifying patients on the basis of REA meets each of the justificatory conditions, using our case examples and evidence from the literature.

Our findings show heterogeneity in the way REA measures are collected, and a lack of clarity and evidence about their utility or effectiveness. As such, further inquiry is required to support the moral and ethical evaluation of this ubiquitous, and often unexamined, clinical practice.

1. Division of Genome Sciences, NHGRI
2. Stanford University

LRRK2 Findings in a Family with GBA1 Mutations: A Case Study and Implications for Genetic Counseling

B26
Poster

Chelsie Poffenberger [1], Jenny Do [1], Emory Ryan [1], Grisel Lopez [1], Ellen Sidransky [1]

Mutations in both glucocerebrosidase (GBA1) and leucine-rich repeat kinase 2 (LRRK2) genes are associated with an increased risk for Parkinson disease (PD). These mutations are more frequent among Ashkenazi Jews, with an allele frequency of ~6-15% for GBA1 mutations and ~2% for LRRK2 mutations. Among Ashkenazi Jews with PD, approximately 17-20% carry a GBA1 mutation and 10-30% carry the LRRK2 p.G2019S mutation. The penetrance of PD for both genes ranges from 10-26%. The increased risk involving both the GBA1 and LRRK2 mutations in the Ashkenazi population creates a compelling investigation into the psychosocial implications and compounded PD risk.

Here, we present a multi-generational Ashkenazi family with both GBA1 and LRRK2 mutations. The family includes two brothers, who both carry the LRRK2 (p.G2019S) and GBA1 (p.N409S/p.N409S) mutations, and their descendants. One of the brothers has PD, one brother does not have PD. Mutation p.G2019S in LRRK2 was discovered as a finding during whole exome sequencing (WES) while looking for other risk or protective alleles. Since multiple family members were either obligate or possible mutation carriers, genetic counseling was performed.

We highlight counseling issues relevant for individuals with both GBA1 and LRRK2 mutations. The potential concerns include the compounded PD risk, incomplete penetrance, perceived PD risk, informing relatives, lifestyle changes, and prospects for the future. It is important to emphasize the complex nature of PD as a multigenic disorder and the difficulty in making phenotypic predictions. The genetic information may prove valuable to family members should neuroprotective therapies or genotype-specific therapies become available. A comprehensive counseling session will enable patients to make educated decisions about informing their descendants and anticipating their future health care needs. As WES is increasingly used in medical practice, counseling regarding unanticipated risk variants and combined risk will be especially relevant.

1. Section on Molecular Neurogenetics, Medical Genetics Branch, NHGRI

Clinical severity in Fanconi anemia correlates with residual function of FANCB missense variants

B27
Poster

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Fanconi anemia (FA) is the most common genetic cause of bone marrow failure, and is caused by inherited pathogenic variants in any of 22 genes. Of these, only FANCB is X-linked. We describe a cohort of 19 children with FANCB variants, from 16 families of the International Fanconi Anemia Registry (IFAR). Those with FANCB deletion or truncation demonstrate earlier than average onset of bone marrow failure, and more severe congenital abnormalities compared to a large series of FA individuals in the published reports. This reflects the indispensable role of FANCB protein in the enzymatic activation of FANCD2 monoubiquitination, an essential step in the repair of DNA interstrand crosslinks. For FANCB missense variants, more variable severity is associated with the extent of residual FANCD2 monoubiquitination activity. We used transcript analysis, genetic complementation, and biochemical reconstitution of FANCD2 monoubiquitination to determine the pathogenicity of each variant. Aberrant splicing and transcript destabilization was associated with two missence variants. Individuals carrying missense variants with drastically reduced FANCD2 monoubiquitination in biochemical and/or cell-based assays showed earlier onset of hematologic disease and shorter survival. Conversely, variants with near-normal FANCD2 monoubiquitination were associated with more favorable outcome. Our study reveals a genotype-phenotype correlation within the FA-B complementation group of FA, where severity is linked to the extent of residual FANCD2 monoubiquitination.

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A Time to Reflect: Revisiting Foundational Work on Genetic Engineering

B28
Poster

Alex Raphael [1], Joy Boyer [1], Dave Kaufman [1], Nicole Lockhart [1], Natalie Pino [1], Lawrence Brody [1]

The possibility of human genetic engineering has been a concern for many in the bioethics community since the start of the Human Genome Project (HGP). The recent birth of two allegedly gene-edited babies in China has brought calls for research on the ethical and societal issues surrounding germline gene editing, one type of genetic engineering. Few calling for this work have cited past scholarship. The National Human Genome Research Institute's (NHGRI) Ethical Legal and Social Implications (ELSI) program has supported foundational work on the ethics of human genetic engineering since 1990. Scholarship in this area is published in many non-traditional venues and can be difficult to find. Here, we used the ELSI Program's public database of funded work to analyze three decades of work on genetic engineering. We found 40 grants and 64 publications spanning 1990 to 2019 covering ethical issues relating to genetic engineering. These issues include human enhancement versus treatment of disease, philosophical and religious discussions on personhood, and policy concerns. We categorized the publications into major themes to analyze how key ethical issues related to human genetic engineering have emerged and re-emerged over the course of almost three decades. A robust analysis of prior scholarship can provide an important foundational resource for researchers, policy makers, educators, and others who are exploring today's issues surrounding gene-editing.

1. Division of Genomics and Society, NHGRI

Parental-Proband Genome Alignment Using Variation Graph Alignment Techniques

B29
Poster

Alex Rodriguez [1,2], Charles Markello [3], William Gahl [1,2], Eric Garrison [3], Jordan Eizenga [3], Adam Novak [3], Glenn Hickey [3], Benedict Paten [3], Wolfgang Resch [4], Thomas Markello [1,2]

When a sequenced genome is aligned with a base human genome reference, traditional linear alignment algorithms will not align accurately to low complexity or repeated regions in a linear canonical human genome reference. This is a problem in rare genetic disease patients, with negative clinical exome sequencing results. Variation graph alignment algorithms can be used to improve alignments to difficult regions that may contain the missing solution these cases. Graph references constructed from the patient's parent's genome sequences are even better than using graph references constructed only from population allele frequencies/haplotypes. This two-stage iteration (parent sequence first, offspring sequences from the parental sequence modified graph) converges to the most probable sequence alignment for the patient. This variant alignment workflow coupled with the previously published NISC VarSifter-based BlackMagicToolKit analysis program have been implemented on the NIH Biowulf cluster for low-PCR whole genome sequence data in the Undiagnosed Disease Program. Like the NISC exome data previously published, the new data shows a difference between the number of variants in probands compared with age-matched siblings.

1. Medical Genetics Branch, NHGRI
2. Undiagnosed Diseases Network
3. University of California Santa Cruz Genomics Institute
4. NIH Center for Information Technology

A Comparison of Different Methods to Generate iPSC-derived Cortical Neurons from Patients with Gaucher Disease

B30
Poster

Richard Sam [1], Yu Chen [1], Barbara Stubblefield [1], Ellen Sidransky [1]

Induced pluripotent stem cell (iPSC) technology, serves as a useful method for understanding the pathogenesis of human diseases. Mutations in the glucocerebrosidase (GBA1) gene causes Gaucher disease (GD), a rare lysosomal storage disorder. GBA1 is also recognized as the most common known risk factor for Parkinson's disease (PD), a debilitating neurodegenerative disorder. In order to elucidate the mechanisms underlying the clinical association between GD and PD, we generated iPSC lines from siblings with GD but discordant for PD.

To characterize transcriptional profiles of cortical neurons derived from these patient iPSC lines, we evaluated neural differentiation protocols for generating cortical neurons from patient iPSC lines. We tested two leading protocols used to generate neural progenitor cells (NPCs), the neuronal precursors that can be further matured into cortical neurons. The embryoid body (EB) protocol utilizes 3D aggregates generated using special culture vessels such as Aggrewells, followed by manual rosettes selection, which may introduce variability between different batches. The second monolayer culture protocol has fewer steps and does not require special culture vessels. We compared NPCs and cortical neurons generated using these two methods by characterizing cell morphology and the expression of cell-specific markers. We plan to use these neurons in further studies aimed at elucidating the association between GD and PD through genomic and biochemical assays.

1. Section on Molecular Neurogenetics, Medical Genetics Branch, NHGRI

Identification of novel cryptic loss-of-function mutations in two independent families with Deficiency of Adenosine Deaminase 2

B31
Poster

Oskar Schnappauf [1], Natalia Sampaio Moura [1], Drew Michael [2], Qing Zhou [1], Natalie Deuitch [1], Daniel Kastner [1], Ivona Aksentijevich [1]

Introduction: Deficiency of adenosine deaminase 2 (DADA2) is an autosomal recessive disorder that manifests with fever, rash, hypocellular bone marrow, early-onset vasculitis, and propensity to ischemic and hemorrhagic stroke. Over 60 pathogenic mutations, mostly missense variants, have been identified to date.

Objectives: This study aimed to identify novel loss-of-function mutations in the ADA2 gene in two unrelated families.

Methods: A variety of molecular and biochemical methods were used to identify novel pathogenic variants in the ADA2 gene and to determine the effect of these variants on RNA and protein expression.

Results: The first index patient is a 5-year-old female, born to a consanguineous Pakistani family, who presented with features of Diamond-Blackfan anemia (DBA). MLPA analysis identified a homozygous duplication of a region comprising exon 7 of ADA2 in the proband, her affected sister, her affected father, and a heterozygous duplication of this region in the proband's mother.

The second index patient is a 17-year-old female who presented with a history of ischemic strokes, livedo rashes and vasculitis. She was found to be heterozygous for a known pathogenic variant in ADA2 (c.1358A>G, p.Y453C). Whole genome sequencing of the mother and the 3 affected children identified a novel canonical splice site variant (ADA2: c.-47+2T>C) in the 5 UTR of ADA2 transcript (NM_001282225.1) present in all four individuals.

Conclusion: The identification of two novel cryptic mutations in ADA2 that were not detected by Sanger sequencing, suggests the incorporation of additional methods in the diagnosis of DADA2.

1. Inflammatory Disease Section, Metabolic, Cardiovascular and Inflammatory Disease Genomics Branch, NHGRI
2. Molecular Physiology and Therapeutics Branch, NIDCR

The moderating effect of family history of cancer on the relationship between patient-provider race concordance and risk perception

B32
Poster

Emma Schopp [1], Susan Persky [1]

Substantial disparities exist in cancer mortality rates between African-American and white patients. Lack of racial diversity among physicians may contribute to these disparities, due in part to less effective clinical communication between patients and physicians of different backgrounds. Previous work has shown cancer risk information provision results in less accurate perceptions of cancer risk among African-American patients when it occurs in an interaction with a racially discordant physician. However, having a family history of cancer may also impact patient-provider communication by making cancer risk information more salient. To examine the impact of patient-provider race concordance on clinical communication, 128 African-American individuals were provided with personalized cancer risk information via a black or white physician in a virtual reality setting. After the interaction, we measured the accuracy and certainty of their estimates of cancer risk. Analysis is currently underway to determine the extent to which family history of cancer impacts risk perception, and whether it moderates the effect of patient-physician race concordance on risk perception accuracy. Findings may illuminate the communication complexities among a population with an elevated cancer risk.

1. Immersive Virtual Environment Test Unit, Social and Behavioral Research Branch, NHGRI

Ensuring Support: Patterns in Utilization of State Services by Caregivers of Relatives with Inherited Errors of Metabolism

B33
Poster

Niyati Shah [1,2], Christopher Marcum [1], Laura Koehly [1]

Individuals with inherited errors of metabolism (IEM) often experience a range of developmental and physical disabilities, including speech delays, cognitive delays, and hypotonia. Thus, caregiving for individuals with IEM demands an elaborate, intense care routine. The caregiving burden associated with this routine involves navigation of state healthcare systems, reliance on informal and formal support services, and exhaustion of financial and emotional resources. In this study, we investigate the patterns of service utilization by caregivers of children with IEM. We examine the types of services caregivers utilize, the barriers that prevent their utilization, and the factors that may improve the caregiving experience.

Our sample consisted of parent caregivers (n = 120) recruited from the Inherited Diseases, Caregiving, and Social Networks Study. In interviews, caregivers answered questions about the types of services they utilize and the services they wish to utilize. Interview responses were coded into service categories based on type (e.g. income-based, nutrition-based). Common barriers, such as lack of insurance coverage, were also identified through this process. To assess utilization and experience with barriers, we conducted descriptive analyses on the quantity of services employed and the frequency of certain barriers.

Our research aims also include quantitatively coding parent caregiver interviews for recommendations to improve service gaps. Examples of such factors include the shortage of social workers, understaffing and underpaying of care providers, lack of funding for services, and specific ineligibility criteria. This qualitative analysis will inform the development of an optimal service model for caregivers of children with IEM.

1. Social Network Methods Section, Social and Behavioral Research Branch, NHGRI
2. Milken Institute School of Public Health

The Clinical and Molecular Spectrum of Patients with Parenchymal Brain Calcification in the NIH Undiagnosed Diseases Program (UDP) Cohort

B34
Poster

Kamyar Sharifi [1], Catherine Groden [1], David Adams [1], Lynne Wolfe [1], William Gahl [1], Camilo Toro [1]

Ectopic intracranial calcifications are often uncovered, sometime incidentally, in the context of imaging studies of patients with a variety of neurological problems. Since its inception, the NIH Undiagnosed Diseases Program (UDP) has evaluated more than 1300 patients who have eluded a diagnosis despite previous detailed evaluations. Ectopic intracranial parenchymal calcifications were identified in 50 of these cases (3.3%). Detailed clinical, imaging and molecular investigations were undertaken in all. Age ranges of patients were 12 to 90 years. Main presenting symptoms in this cohort included neuropsychiatric symptoms, gait abnormalities, parkinsonism, sensory disturbances and headaches. Calcification features included combinations of calcifications of the deep brain nuclei and dentate nuclei and calcification of the white matter. Leukoencephalopathy and brain atrophy were commonly associated with ectopic brain calcifications. Well-known molecular explanations for intracranial calcifications were identified in 15 patients including mutations in SLC20A2 (8 patients), PDGFB (3 patients), Aicardi Gutierrez Syndrome Type VII (AGS-VII) (2 patients), and Lubrune Syndrome due to biallelic mutation in SNORD118 (2 patients). Interestingly, unexpected basal ganglia calcifications were found in the context of other molecular diagnosis, including PKAN, and DYT1 mutations. Three quarters of our patients still lack a molecular diagnosis despite whole exome sequencing and targeted metabolic evaluations. We intend to pursue additional investigations onto possible novel genes and mechanisms of calcification considering the known convergence of metabolic, inflammation and vascular homeostatic signals on various forms of ectopic calcification.

1. Undiagnosed Diseases Program, Common Fund, NIH

Analysis of rare variants in lysosomal pathway genes in patients with Gaucher disease with and without Parkinson disease

B35
Poster

Pankaj Sharma [1], Shruthi Santhanakrishnan [2], Alta Steward [1], Barbara Stubblefield [1], Grisel Lopez [1], Nahid Tayebi [1], Ellen Sidransky [1]

Gaucher disease (GD) is a lysosomal storage disorder (LSD) that results from a deficiency in the enzyme glucocerebrosidase caused by mutations in the GBA1 gene. GD presents with a broad phenotypic spectrum, ranging from lethal to very mild symptoms and is clinically divided into three types: non-neuronopathic (GD1) and acute and chronic neuronopathic (GD2 and GD3, respectively). Mutations in GBA1 are also one of the most common genetic risk factor associated with Parkinson disease (PD), although not all mutation carriers develop PD. Recent literature (Robak et al., 2017) suggests an excessive burden of LSD gene variants in PD. In order to identify possible modifier genes for PD among our cohort, we analyzed whole exome sequencing data for rare variations in 54 LSD genes in 41 patients with both GD and PD (GD/PD) and 32 patients with GD alone. We hypothesize that multiple variants in these genes may collectively impair lysosomal function in GD/PD patients vs GD and contribute to the accumulation of -synuclein in patient's brain. Using T-test and Mann-Whitney U Test our results suggest an increased burden of rare variants in LSD genes in the GD/PD group compared to the GD group. Variants in ATP13A2, CLN6 and CTSD specifically were over-represented in patients with GD/PD vs GD. Since this is a pilot study, replication of the analyses in a larger patient population is needed to validate the findings and well as studies are needed to functionally characterize effects of multiple genetic hits in Parkinson's disease pathogenesis in GD/PD patients.

1. Section on Molecular Neurogenetics, Medical Genetics Branch, NHGRI
2. Rutgers University

The influence of ecological factors and social networks on self-rated health

B36
Poster

Anna Shetler [1], Laura Koehly [1], Christopher Marcum [1]

There is a complex relationship between individual health and well-being and the embedded nature of individuals within personal networks and space. The built environment is associated with the size and geographical distributions of social networks, and with health outcomes. Moreover, social networks have direct and indirect effects on health. While previous studies have explored individual features of the built environment, this study considers ecological factors that span levels of analysis. Using such place-based factors, we explore the associations between self-rated health, network structure and composition, and meso-level environments for the general population. As the importance of place and local resources may be particularly salient for families coping with rare genetic conditions, this study provides a baseline for understanding how individuals with chronic conditions compare to the general population.

The nationally-represented consumer panel utilized in this study collected network data (n=1820) through online survey methods in 2015, including both ego-level (demographics, self-rated health) and alter-level data (demographics, spatial proximity). The informants are majority white (80%), female (54%), span from ages 18 to 91, and report an average network size of 7, made up largely of kin (66%). American Community Survey 2015 Estimates and 2015 County Business Patterns provide geographical variables at ego-level zip codes. Principal component analyses are applied to variables determined by the Social Fragmentation, Social Deprivation, and Social Space Indices to produce the meso-level, ecological factors. Multilevel analyses provide insight into these factors' connections with social networks and individual health, informing both health interventions and urban planning.

1. Social Network Methods Section, Social and Behavioral Research Branch, NHGRI

Identification of polyadenylation signals relevant to Mendelian disease variant interpretation

B37
Poster

Henoke Shiferaw [1], Celine Hong [1], Jennifer Johnston [1], Leslie Biesecker [1]

Polyadenylation is essential in maintaining nascent mRNA stability. Variants in polyadenylation signal (PAS) hexamers can cause reduced polyadenylation at the normal polyA sites and lead to reduced gene expression. Only 26 PAS hexamer variants have been associated with Mendelian disorders. We hypothesize that this is under-representative of this class of mutations. We aimed to comprehensively identify clinically important PAS hexamers, which are most commonly AATAAA. Clusters of polyadenylated 3' UTRs in ESTs were identified from the Alternative Polyadenylation DataBase (APADB) and the Polyadenylation DataBase Version 3 (PADB3) and examined for dominant polyA site usage activity (defined by ≥50% of overall EST representation). We filtered for inclusion of polyA sites with only one canonical AATAAA PAS within 50 bases 5' of the polyadenylation site. Remaining sites from the two databases were intersected to produce the final list of candidate PAS hexamers of interest. To understand constraint in the identified PAS, we compared the number of variants in the PAS hexamers of interest vs. sets of control sequences in gnomAD. We identified 3,242 canonical (AATAAA) PAS hexamers for further examination. Of these PAS hexamers, 1,727 were in genes in HGMD. The 3,242 PAS were at least six times more constrained than hexamers immediately upstream of the signal, and AAT or AAA triplets occurring in upstream 3' UTR regions. A base-specific bias was also seen in middle positions, which favored guanine and cytosine substitutions. We identified 3,242 PAS of potential clinical relevance. We hypothesize that these sites harbor pathogenic variants for mendelian disorders.

1. Clinical Genomics Section, Medical Genomics and Metabolic Genetics Branch, NHGRI

Patient Narratives and the Role of Genetic Counselors in the Undiagnosed Diseases Network

B38
Poster

Catherine Sillari [1], Cecelia Tamburro [1], Anastasia Wise [1], Laurie Findley [1], Heather Colley [1], Jyoti Dayal [1]

Patient narratives are becoming an increasingly important piece of clinical care. Narratives help put patients in context and allow physicians to relate to patients on a personal level. The process of writing a narrative can also be therapeutic for the patients themselves. Arthur Frank classified patient narratives into three types: restitution (expectation of recovery), chaos (suffering and loss), and quest (unexpected positive effect from illness).

The Duke University clinical site of the Undiagnosed Diseases Network collects patient narratives from their applicants to complement the providers' recommendation letters. Early findings from forty of these narratives demonstrate a sense of fear, rejection, and a loss of control. All of them were classified as chaotic. Relatedly, there are higher than normal rates of anxiety and depression in parents of undiagnosed patients.

These findings point to the importance of genetic counselors within the UDN. Genetic counselors play a unique role within a healthcare team as they are trained in both medical genetics and counseling. They serve as a link between patients and physicians, interpreting genetic test results as well as advising patients on risk and treatment options. Genetic Counselors in the UDN play a wide variety of roles, some traditional and others unique to the network.

1. Division of Genomic Medicine, NHGRI

The role of metabolic switching in zebrafish caudal fin regeneration

B39
Poster

Jason Sinclair [1], Erica Bresciani [1], Carli Needle [1], David Hoying [1], Shawn Burgess [1]

Mammals are generally poor at tissue regeneration, often resulting in permanent damage or complete loss of tissues, organs, and extremities following injury or during ageing. However, mammals maintain the ability to regenerate liver and digit tips, suggesting that the pathways governing regeneration are not completely lost. Therefore, it may be possible to improve therapeutic human regeneration if we had a better understanding of the molecular underpinnings guiding these pathways. We utilize the zebrafish Danio rerio, which in contrast to mammals are able to regenerate many tissues and organs such as: fins (limbs), scales, retina, spinal cord and heart. In particular, the embryonic zebrafish caudal fin serves as an ideal regenerative model due to its easy manipulation and superior imaging properties. Using this model, we have discovered that cell types comprising the notochord, which serves as a structural support prior to spine development during embryogenesis, undergo a metabolic switch to glycolysis similar to the Warburg effect as they migrate to the amputation plane and contribute to blastema formation. To gain a better understanding of the molecular pathways that are regulated during metabolic switching, we performed a time series of single cell RNA-seq on the blastema and have detected key signaling events consistent with triggers of regeneration. These studies not only provide new insights into tissue regeneration, but also cancer biology through determining how the Warburg effect is regulated and the pathways it drives in a non-disease state.

1. Developmental Genomics Section, Translational and Functional Genomics Branch, NHGRI

Analysis of DNA Repair Protein Encoding Gene, RFC4, as a Candidate Gene in Two Individuals with a Novel Neurological Disorder

B40
Poster

Benjamin Steger [1], Marie Morimoto [1], Lynne Wolfe [1,2], Alden Huang [3], Peter Steinbach [7], Hane Lee [4,5], Christopher Lau [1], Stanley Nelson [4,5,6], Ariane Soldatos [2], Michele Nehrebecky [8], David Adams [1,2], William Gahl [1], Camilo Toro [1,2,] May Malicdan [1,2]

DNA replication and repair play critical roles in cellular division and tolerance to DNA damage. Multiple accessory proteins are required to facilitate the binding and function of DNA polymerases during these processes. Variants in several genes encoding these DNA repair accessory proteins are associated with neurological disorders such as cerebellar ataxia, neuropathy, Vestibular Areflexia Syndrome (CANVAS) and Ataxia-Telangiectasia (AT). We present probands with an undiagnosed neurological disorder and identify a candidate gene encoding a DNA repair protein that may be associated with this novel disorder.

Two NIH UDP probands presented with cerebellar neurodegeneration, ataxia, photophobia, dysarthria, and other phenotypic similarities to known DNA repair disorders. A UDN-initiated collaborative analysis identified splicing variants from RNA sequencing in one proband. Research re-analysis of genome sequences revealed compound heterozygous variants in RFC4, including likely-deleterious splicing variants, in both probands. RFC4 encodes a subunit of replication factor C (RFC), a DNA repair accessory protein that loads and activates Proliferating Cell Nuclear Antigen (PCNA). RFC4 mRNA expression was either unchanged or slightly increased in patient fibroblasts compared to an unaffected control suggesting possible overcompensation of transcription. cDNA sequencing confirmed splicing variants resulting in deletions of exons 4 or 10, respectively, in fibroblasts from the two probands.

Changes in RFC4 splicing seen in fibroblast cDNA from our probands, in combination with phenotypes similar to those of AT, CANVAS, and other DNA repair disorders, suggest that the bi-allelic variants in RFC4 are pathogenic. Our report implicates RFC4 as a gene involved in a novel DNA repair disorder.

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Redesigning and Revolutionizing the Reference: The Human Genome Reference Program

B41
Poster

Taylorlyn Stephan [1], Baergen Schultz [2], Adam Felsenfeld [1], Adam Phillippy [3], Heidi Sofia [2]

The human genome reference sequence is used for fundamental tasks in genomics, including as a scaffold on which to align genome sequence data and as a consensus genome coordinate system. The current reference –GRCh.38— is useful, but it's clear that it can be improved considerably. The current reference assembly has gaps, is based on information from relatively few genomes, and is a mosaic of multiple haplotypes. Although it does include "alternate paths" (loci with variants that diverge from the consensus coordinates) derived from a number of other genomes, it's not close to fully representing the variation present in the global population. This can lead to failure to accurately and fully assess new genomes using it, leading to population-specific biases. To ensure that as much variation as possible is represented, a "pan-genome" is needed. It will incorporate very high-quality data from populations with diverse ancestry using new sequencing technology, and will improve utility for basic scientists and clinicians.

To build the pan-genome reference, NHGRI issued an announcement for a Human Genome Reference Program (HGRP). It will: maintain, coordinate, and update the reference and provide community training/resources; sequence upwards of 350 individuals of diverse ancestries at reference-quality resolution; explore next-generation pan-genome models/representations; build tools for reference use; and develop technology for complete, telomere-to-telomere sequencing.

This poster will explore the history and science behind the human genome and pan-genome reference; graph and other reference structures; the ethical, legal, and social issues surrounding the reference; NHGRI's HGRP structure and what it aims to accomplish.

1. Division of Genome Sciences, NHGRI
2. Division of Genomic Medicine, NHGRI
3. Genome Informatics Section, Computational and Statistical Genomics Branch, NHGRI

The Intersections of Religiosity, Spirituality, and Sickle Cell Disease Outcomes

B42
Poster

Courtney Stewart [1,2], Khadijah Abdallah [1], Brittany Hollister [1], Vence Bonham [1]

Sickle Cell Disease (SCD) is a blood disorder caused by a single point mutation in the beta-globin gene and is associated with a lifetime of complications affecting one's physical, psychosocial, and emotional health. Previous literature indicates that the spirituality and religiosity of individuals with SCD play a positive role in self-reported coping, pain management, healthcare utilization, and quality of life. However, it is currently unknown how spirituality and religiosity interact with each other and with specific clinical outcomes such as disease severity and depression. Using data from the ongoing INSIGHTS Study, we analyzed the relationship between spirituality and religiosity as they distinctly relate to disease severity and other psychosocial measures. Disease severity was calculated using the published Sickle Cell Disease Severity Measure. Spirituality and religiosity were measured by the Brief Multidimensional Measure of Religiousness/Spirituality. The investigation draws an important distinction between spirituality and religiosity, exploring the intersections and differences between these two measures and specific SCD outcomes. Preliminary results indicate that higher spirituality weakly correlates to higher disease severity (.194, p=.0055), and negative religious coping is positively correlated with higher Beck depression scores (.222, p=.0017). We hypothesize that these correlates may be due to people with more severe disease utilizing spirituality as a coping mechanism. However, in the future we will further explore the unique distinctions of religiosity and spirituality in relation to other psychosocial measures such as coping, locus of control, and global health scores, and how these factors affect disease severity and depression.

1. Health Disparities Unit, Social and Behavioral Research Branch, NHGRI
2. NIH Academy Enrichment Program

The heritability of developmental change in the brain's structural connectivity and its association with change in symptoms of attention deficit hyperactivity disorder

B43
Poster

Gustavo Sudre [1], Wendy Sharp [1,2], Saadia Choudhury [1], Philip Shaw [1,2]

The heritability of the development of many of the neural substrates that underlie attention deficit hyperactivity disorder (ADHD) is unknown; this includes the brain's structural connectivity, made up of white matter tracts. Here, we address this gap using longitudinal data to estimate the heritability of developmental change in the microstructural properties of white matter tracts. We further determine associations with change in ADHD symptoms.

Participants were 252 children drawn from 51 nuclear families; 49% had a diagnosis of ADHD; 63% were male. All children had two assessments from which the annual rate of change in clinician-determined ADHD symptoms was determined. Diffusion tensor imaging estimated two microstructural properties (AD and RD) on twenty major white matter tracts. The total additive genetic heritability (h2r) of the annual rate of change in microstructural tract properties was calculated using SOLAR.

Rates of change of six white matter tract properties emerged as significantly heritable ($p < .05$, FDR $q < .05$): the forceps minor (AD), the right anterior thalamic radiation (AD and RD), the right superior longitudinal fasciculus (RD), the uncinate (RD) and the cingulum (RD). Four of six heritable properties showed an association with change in hyperactivity-impulsivity ($p < .05$, FDR $q < .05$).

We provide the first demonstration of significant heritability in the development of microstructural properties of white matter tracts and highlight the heritable tracts associated with change in ADHD symptoms. Our ultimate goal is to use this relatively small number of developing white matter tracts properties as phenotypes in large scale, collaborative GWAS.

1. Neurobehavioral Clinical Research Section, Social and Behavioral Research Branch, NHGRI
2. National Institute of Mental Health

Exploring Optimal Coping Strategies for Psychological Well-Being Among Caregivers of Children with Rare Genetic Diseases

B44
Poster

Sydney Sumrall [1], Jasmine Manalel [1], Laura Koehly [1]

Children with inherited metabolic conditions (IM) and undiagnosed diseases (UD) require heightened medical attention and often experience physical challenges and cognitive developmental delays. These children depend on parents and other primary caregivers (PCG) to meet the everyday needs required of their conditions. The time and resources this responsibility demands results in stress, burden, and reduction in psychological well-being for caregivers. Psychologically beneficial coping strategies are critical for both caregivers and their children.

The first aim of this project is to explore the associations between caregiving burden, coping strategies and mental health for caregivers of typically developing (TD) or affected children. Coping and psychological impact may manifest differently based on the caregiver's kinship role. Therefore, our second aim is to determine how these associations may differ for parent PCGs versus non-parent PCGs.

The primary caregivers of TD children (n=64), children with IM (n=142), and children with UD (n=23) completed a series of online surveys. We assessed utilization of different coping strategies using the BriefCOPE which contains fourteen coping strategy scales, e.g. self-distraction, denial. Caregiving burden is based on the Pediatric Activities of Daily Living measure, and mental health is based on the CES-D, which measures depressive symptoms, and the Perceived Stress Scale, a measure of personal stress. We will also assess differences in results based on caregiving context and kinship role. This analysis will help us understand how caregivers might establish coping skills that improve their mental health specific to their kinship role and the unique caregiving demands accompanying it.

1. Social Network Methods Section, Social and Behavioral Research Branch, NHGRI

Creating a reporting framework for polygenic risk scores to improve transparency and standardization

B45
Poster

Cecelia Tamburro [1], Hannah Wand [2], Peter Kraft [3], Catherine Sillari [1], Michael Iacocca [2], Iftikhar Kullo [4], Robb Rowley [1], Katherine Vlessis [2], Kim Kinnear [2], Birgitt Schuele [2], Kelly Ormond [2], Alice Popejoy [2], Eric Venner [6], Deanna Brockman [7], Katrina Goddard [2], Genevieve Wojcik [5]

The polygenic risk score (PRS) has recently become a more common tool for bridging the gap between genome wide association studies (GWAS) and clinical disease risk estimation. In contrast with other clinical risk tools, there are no accepted standards for developing, reporting, and applying PRS. To address this gap, the Complex Disease Working Group (CDWG) of the Clinical Genome Resource is assessing PRS reporting criteria by investigating the comprehensiveness and clarity of a pilot reporting framework and conducting a literature review of current PRS reporting practices. To create a preliminary PRS reporting framework, we built upon established guidelines, starting with the GRIPS statement (2011) which recommends a checklist of 25 items when reporting genetic risk estimation studies. We expanded this checklist to include 44 unique items by incorporating expert feedback on anticipated gaps and inconsistencies. We used this framework to assess 30 PRS articles spanning multiple disease domains. Each article was assessed by two independent reviewers, with discrepancies resolved by a third reviewer. Multiple items were found to be under-reported, especially details of statistical analysis, including calibration procedure (37% missing) and validation procedure (33%). Trends in "big data" sample acquisition, including the use of large biobanks, causes incomplete phenotyping of participants. Finally, lack of diversity in PRS studies and differences in reporting ancestry have consequences for reproducibility in non-European populations. The ClinGen CDWG will use the results of this review to inform recommendations for PRS reporting and is seeking community feedback on findings as we iterate on a consensus standard.

1. National Human Genome Research Institute
2. Stanford University
3. Harvard University
4. Mayo Clinic
5. Johns Hopkins University
6. Baylor College of Medicine
7. Mass General Hospital

Pyruvate dehydrogenase deficiency leads to decreased cytolytic activity in CD8+ T cells

B46
Poster

Tatyana N. Tarasenko [1], Julio Gomez-Rodriguez [2], Jessica Sudderth [3], Ralph J. DeBerardinis [3], Peter J. McGuire [1]

T-cells undergo metabolic reprogramming with major changes in cellular energy metabolism upon activation. Following T-cell receptor engagement, glycolysis and OXPHOS are upregulated in the first 24 hours. Previously, our group demonstrated that cytochrome c oxidase (COX) deficiency in T-cells (TCox10-/-) results in OXPHOS deficiency, apoptosis, and impairment of T-cell immunity in vivo. We also found decreased flux through PDH resulting in depressed acetyl-CoA and impairment of the TCA cycle in TCox10-/-. Given the importance of acetyl-CoA and the TCA cycle for bioenergetics and biosynthesis necessary for proliferation and differentiation, we hypothesized that the decreased flux through pyruvate dehydrogenase was accounting for part of the phenotype in TCox10-/-. To answer this question, we created a model of T-cell pyruvate dehydrogenase deficiency via a cre-recombinase system (TPdh-/-). Using [U-13C] glucose in TPdh-/- T-cells to determine metabolic flux, we found intact glycolysis and decreased isotopic labelling of citrate, consistent with PDH deficiency. Probing the TCA cycle with [U-13C] glutamine, we showed that TPdh-/- T-cells had decreased turnover, implying perturbations to bioenergetics. Indeed, extracellular flux analysis was consistent with depressed oxidative phosphorylation in activated TPdh-/- T-cells, indicating that a significant proportion of OXPHOS activity arises from the complete oxidation of glucose. While many aspects of T-cell activation and differentiation remained intact, we found that CD8+ T-cells had reduced cytolytic activity against target cells. Based on these results, we hypothesize that cytolytic activity in CD8+ T-cells is dependent upon acetyl-coA generation.

1. Metabolism, Infection and Immunity Section, Medical Genomics and Metabolic Genetics Branch, NHGRI
2. Cell Signaling and Immunity Section, NIAID, NIH
3. Children's Medical Center Research Institute, University of Texas Southwestern Medical Center, Dallas, TX

Does knowledge of family history influence illness representations among those with Type 1 diabetes?

B47
Poster

Sydney Telaak [1], Kristi Costabile [2], Susan Persky [1]

Illness narratives are an important method to determine how individuals with chronic illnesses personally understand and make meaning from their disease. Knowledge of family history is a significant indicator of one's illness perception and can, in turn, influence how diagnosed individuals understand and educate themselves about their disease. Among individuals with diabetes, there are variations in illness representation based on gender, education, and age. However, the influence of family history on illness representation is not well understood, particularly where it intersects with these demographic variables. We hypothesize that knowledge of family history will mediate the relationships between illness representation and gender, education, and age, such that the presence of family history will result in a more positive and centralized illness narrative across demographic lines.

We are currently analyzing data from the Diabetes, Identity, Attributions, and Health Study. Participants with diabetes were recruited online and asked about their beliefs about the disease. Affected individuals were further questioned on personal attitudes and management of their diabetes. We plan to examine how knowledge of family history influences participants' illness narrative, self-concept, emotional response to disease, and personal definition of disease. We will also explore how these factors differ with regard to gender, education, and age. Results will highlight the multifaceted nature of diabetes-related identities and will have important implications for addressing this chronic illness in a diverse set of families.

1. Immersive Virtual Environment Test Unit, Social and Behavioral Research Branch, NHGRI
2. Department of Psychology, Iowa State University

Mass spectrometry-based proteomic profiling of CRISPR-edited FBXW7-mutant serous endometrial cancer cell lines

B48
Poster

Mary Ellen Urick [1], Daphne Bell [1]

Background: Endometrial cancer arises from cells lining the uterus and affects more than 300,000 women world-wide each year. Although the most common histotype is often treatable through hysterectomy, a disproportionate number of deaths are due to rarer subtypes, the most common of which are serous endometrial cancers. Serous endometrial cancers and some other aggressive subtypes exhibit frequent somatic mutations in the FBXW7 tumor suppressor gene. The purpose of this work is to determine proteomic changes associated with recurrent somatic FBXW7 mutations.

Methods: Two FBXW7 non-mutant serous endometrial cancer cell lines were CRISPR-edited to derive six cell lines, each harboring one recurrent FBXW7 missense mutation. Using liquid chromatography tandem mass spectrometry, we profiled the total and phospho- proteome of parental and CRISPR-edited cell lines from triplicate lysates. Resulting mass spectra were evaluated using SEQUEST and filtered to high-confidence reads. Peptides altered within and among FBXW7 mutant lines were functionally annotated using manual curation, Ingenuity Pathway Analysis, and DAVID.

Results: Differentially expressed or phosphorylated proteins in FBXW7 mutated cell lines compared to matched parental lines ranged from 0.1-2.0% of total proteins and 0.5-5.0% of phosphoproteins. Proteins commonly altered in FBXW7 mutated cells function in potentially druggable signaling pathways and processes.

Conclusions: Discrete recurrent missense mutations in FBXW7 in two biologically distinct serous endometrial cancer cell lines resulted in common proteomic changes that are potentially druggable. Ongoing work includes proteomic profiling of additional FBXW7 mutant endometrial cancer cell lines and interrogation of results via additional biochemical assays.

1. Reproductive Cancer Genetics Section, Cancer Genetics and Comparative Genomics Branch, NHGRI

Treatment of methylmalonic acidemia (MMA) by targeted integration of MMUT into Albumin with a promoterless AAV vector (GeneRide) confers a progressive hepatocellular growth advantage in mice

B49
Poster

Leah Venturoni [1], R J Chandler [1], N Chau [2], J Liao [2], S Gordo [2], M A Kay [3], A Barzel [4], C P Venditti [1]

MMA is a heterogenous inborn error of metabolism most commonly caused by a deficiency of methylmalonyl-CoA mutase (MMUT). Patients suffer from frequent episodes of metabolic instability, severe morbidity, and early mortality. Gene therapy has been explored in MMA mouse models as an alternative therapy to liver transplantation. To minimize the potential of vector-related insertional mutagenesis and preserve MMUT expression after therapeutic gene delivery, we designed a promoterless AAV vector that utilizes homologous recombination to achieve site-specific gene addition of human MMUT into the mouse albumin (Alb) locus. We have previously reported that treatment of different MMA mouse models at birth reduced disease related metabolites and produced durable MMUT expression for more than a year. In older treated mice, RNAscope revealed MMUT positive hepatocytes as distinct and widely dispersed clusters, consistent with a pattern of clonal expansion. Here, we report dose finding studies, biomarker responses, and a time course analysis of MMUT expression after a therapeutic GeneRide. After a latency period of several months, there is a continuous enhancement of MMUT expression accompanied by weight gain, reduced disease metabolites, increased 1-C-13 propionic oxidative capacity, increased Alb-2A levels, increased Alb-MMUT integration events, and a reduction of the mitochondrial-stress biomarker, Fgf21. The progressive clinical and biochemical improvement in the treated mice is consistent with an expansion of corrected hepatocytes, yielding a greater therapeutic benefit with time, and is accompanied by a predictable pattern of biomarker changes that will facilitate clinical translation.

1. Organic Acid Research Section, Medical Genomics and Metabolic Genetics Branch, NHGRI
2. LogicBio Therapeutics
3. Stanford University
4. Tel Aviv University

A comparative analysis of pheomelanin-specific mouse mutants

B50
Poster

Dawn Watkins-Chow [1], Colten Eberhard [1], Andrew Kemal Kirchmeier [1], Gene Elliot [2], Cecilia Rivas [2], Lisa Garrett [2], William Pavan [1]

In humans, the ratio of eumelanin and pheomelanin produced by melanocyte cells in our skin directly impacts skin cancer risk. Studies of animal models have provided a strong foundation for understanding the genes regulating eumelanin synthesis, however, the genetics of pheomelanin synthesis are relatively under studied and of particular interest. Following a genome-wide association study linking human Chr 19 to variation in skin pigmentation among African populations, we utlilized a previously uncloned mouse mutant, grizzled (gr) to confirm Mfsd12 as a novel pigmentation gene in both mouse and human. MFSD12 is a transporter with unknown substrate, and overexpression constructs suggest that MFSD12 localizes to late endolysosomes.

To further extend our knowledge of genes required for pheomelanin synthesis, we have now used exome sequencing and Crispr/Cas9-mediated targeting to confirm a second novel pigmentation gene, Usp32, as causative for the mouse grey intense (gri) phenotype. The pigmentation phenotype of both Mfsd12 and Usp32 is being characterized in the mouse mutants on a defined genetic background for comparison with previously published pheomelanin mutants (Ggt1, Ostm1, Slc7a11, and Clcn7). Interestingly, several of these mutations are associated with overlapping pleiotropic phenotypes, including altered viability, body size, bone density, and tooth eruption. The varied genetic backgrounds of the mutants and lack of comparative studies have made it difficult to distinguish cell-type specific gene function from the influence of genetic background. Thus, we are establishing each mutant on a defined, uniform genetic background to explore the reoccurring association between the pleiotropic phenotypes and pheomelanin disruption.

1. Genomics, Development and Disease Section, Genetic Disease Research Branch, NHGRI
2. Gene Editing, ES Cell and Transgenic Mouse Core Facility, NHGRI

The Human Heredity and Health in Africa (H3Africa) Consortium

B51
Poster

Harry Wedel [1], Baergen Schultz [1], Jennifer Troyer [1], Ebony Madden [1], Ken Wiley [1]

Despite possessing the most genetic variation in the world, African populations are underrepresented in genetics and genomics research. Human Heredity and Health in Africa (H3Africa) serves to address this imbalance by developing a sustainable and collaborative African genomics research enterprise - led by African scientists, for the African people.

H3Africa uses several project types to accomplish its aims: Collaborative Research Centers and Research Projects that investigate the genomics of diseases in pan-African and local contexts; a Biorepository Network that stores high quality biospecimens for future research; Ethical, Legal, and Social Implications (ELSI) Collaborative Centers and Research Projects that explore the ELSI of genomics research in Africa; an Informatics Network that forms the backbone of the consortium, supporting data science and bioinformatics across the continent; and four Bioinformatics Training Programs establishing Masters and Doctorate programs at African universities. Altogether, H3Africa is a robust collaborative network of interdisciplinary African researchers.

So far, H3Africa has provided training for over 2,000 African scientists, established biorepositories and bioinformatics capacity that meets or exceeds international standards, developed an ethical framework for genomic research in Africa, and produced data from over 70,000 study participants. As a result, there are over 200 scientific publications to date describing findings in ELSI research, epidemiology of chronic diseases, infectious disease outbreak and control, and genomic and non-genomic risk factors for several diseases on the continent. Combined, H3Africa's efforts will continue to build research capacity and galvanize the genomics revolution in Africa, benefitting African health for generations to come.

1. National Human Genome Research Institute

Loss of function of zrsr2 leads to hematopoietic defects in zebrafish

B52
Poster

Rachel Weinstein [1], Liesl Broadbridge [1,2], Kevin Bishop [1], Blake Carrington [1], Wuhong Pei [1], Shawn Burgess [1], Paul Liu [1], Raman Sood [1], Erica Bresciani [1]

ZRSR2 is an integral component of the minor spliceosome, which primarily targets U12 type introns. It is involved in the recognition of 3' splice sites during spliceosome assembly. ZRSR2 mutations are associated with myelodysplastic syndrome an acute myeloid leukemia, however the role of ZRSR2 in hematopoiesis is unclear. Zebrafish make an excellent animal model for investigating this gene as there is 78% protein similarity between human ZRSR2 and zebrafish Zrsr2. All functional domains in human ZRSR2 are conserved in zebrafish, and hematopoietic development is well characterized in this species. We used CRISPR/cas9 to generate a zebrafish zrsr2 knockout model with an 11 base pair deletion that results in premature truncation and loss of all functional domains. zrsr2 del11/del11 embryos show aberrant development in the mandible and pharyngeal arches by 4 days post fertilization (dpf), mild to severe edema by 6 dpf, and die by 8 dpf. O-dianisidine staining shows that zrsr2 del11-/- embryos exhibit mild anemia by 2 dpf. Whole mount in situ hybridization shows that zrsr2 del11-/- mutants have normal early hematopoietic stem cell (HSC) emergence (c-myb) at 36 hours post fertilization. However, HSCs fail to develop through 3 and 5 dpf. At 5 dpf, zrsr2 del11-/- mutants lack expression of hematopoietic markers for erythroid cells (hbae1), lymphocytes (rag1), and myeloid cells (mpo). These data suggest that Zrsr2 is important for definitive hematopoiesis in zebrafish. Moving forward, we would like to investigate U12 intron sequences in genes known to effect hematopoietic development in zebrafish in our zrsr2 mutants.

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Chimeragenesis with edit-directing plasmid pools in Saccharomyces cerevisiae

Cory Weller [1], Meru Sadhu [1]

Functional assessment of chimeric proteins, i.e. those generated by combining the primary structure of homologous proteins, can provide insight into which residues or domains interact, how protein structure is constrained, and how proteins evolve. Unfortunately, generating even one single chimeric protein can be labor-intensive despite the ability to make precise edits to a genome, e.g. with CRISPR-Cas9. Here, we describe a method for simultaneously generating a multitude of chimeras for a homologous protein pair in a single Saccharomyces cerevisiae transformation. After inserting a desired homolog and a selectable marker into our yeast strain harboring CRISPR-Cas9, we perform a single transformation with a barcoded plasmid pool. Each plasmid encodes the same guide RNA (to induce a double-strand break between the homologous proteins) and specific repair template that generates a desired chimera via homology-directed repair. We can then screen for successful chimera edits on selective media, determine the specific chimeric identity of transformants by sequencing plasmid barcodes, and assay protein function. Here, we demonstrate the method as a proof-of-principle and consider a computational pipeline for generating all possible chimeras for dozens of homologous genes. Together, these methods allow for high-throughput generation of chimeras for the study of protein structure and function.

1. Systems Biology and Genome Engineering Section, Genetic Disease Research Branch, NHGRI

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