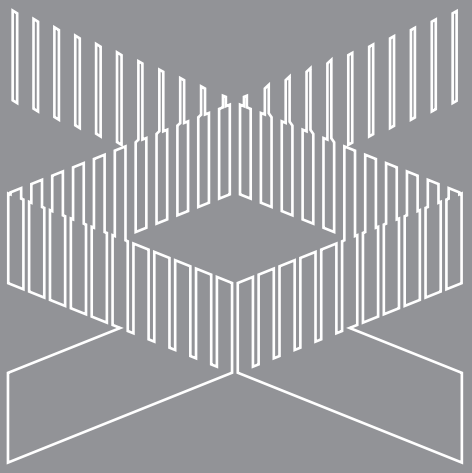
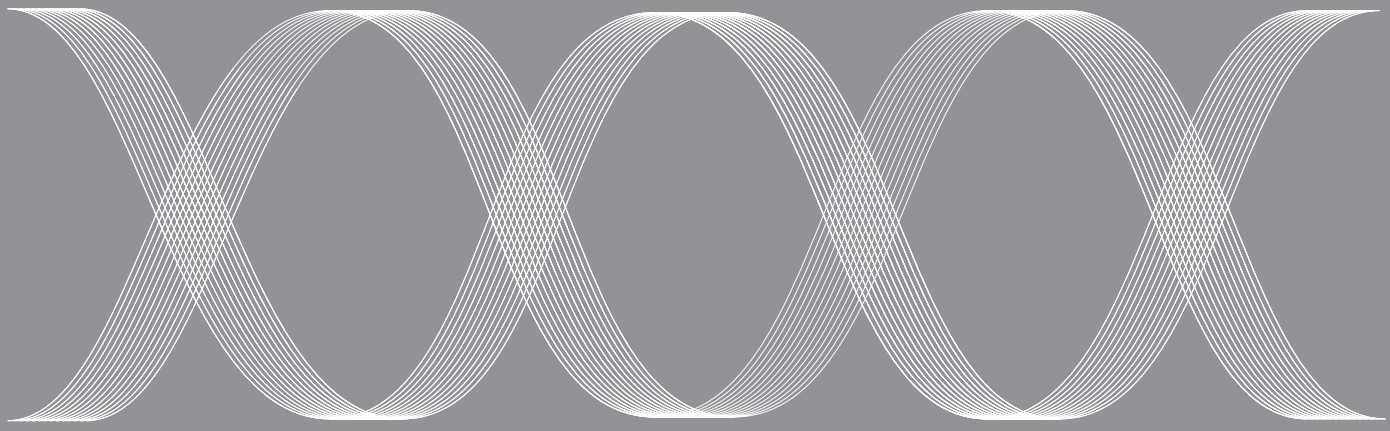


2013 Scientific Symposium

December 12-13

Natcher Conference Center, NIH, Bethesda, MD



NATIONAL HUMAN GENOME RESEARCH INSTITUTE Division of Intramural Research

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES | NATIONAL INSTITUTES OF HEALTH | genome.gov/DIR

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
301-402-2023 Phone
301-480-2634 Fax

December 2013

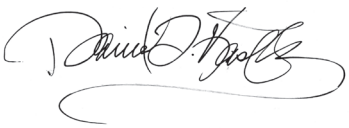
Welcome to the first-ever NHGRI Scientific Symposium!

After two years of not having an NHGRI Retreat, we are delighted to bring back this wonderful tradition as an on-campus NHGRI Scientific Symposium. The Symposium brings together NHGRI Intramural and Extramural scientists and staff for two half-days of science and camaraderie. The Symposium will include an opening plenary lecture by Dr. Ari Patrinos, former Director of the Department of Energy's Human Genome Project and currently Deputy Director for Research at NYU's Center for Urban Science and Progress, as well as poster sessions, scientific talks, breakout sessions, networking, a mystery lunch, and an over-the-top awards ceremony. Although we have been through flat budgets, sequestration, and a government shutdown, the NHGRI is still a vibrant institution that is doing truly great science, and it is an honor to be a part of this wonderful organization. For part of the Symposium we will be joined by our Board of Scientific Counselors, who will hold their annual meeting in parallel with our Symposium.

It is my enormous pleasure to usher in this latest incarnation of a very special NHGRI tradition. I hope that you will all have a great time, forge new friendships and collaborations, learn a lot, and find your next inspiration.

Here's to all of us and to a splendiferous Scientific Symposium.

Warm regards,



Daniel Kastner, M.D., Ph.D.

Scientific Director

2013 NHGRI Scientific Symposium

Program and Abstracts

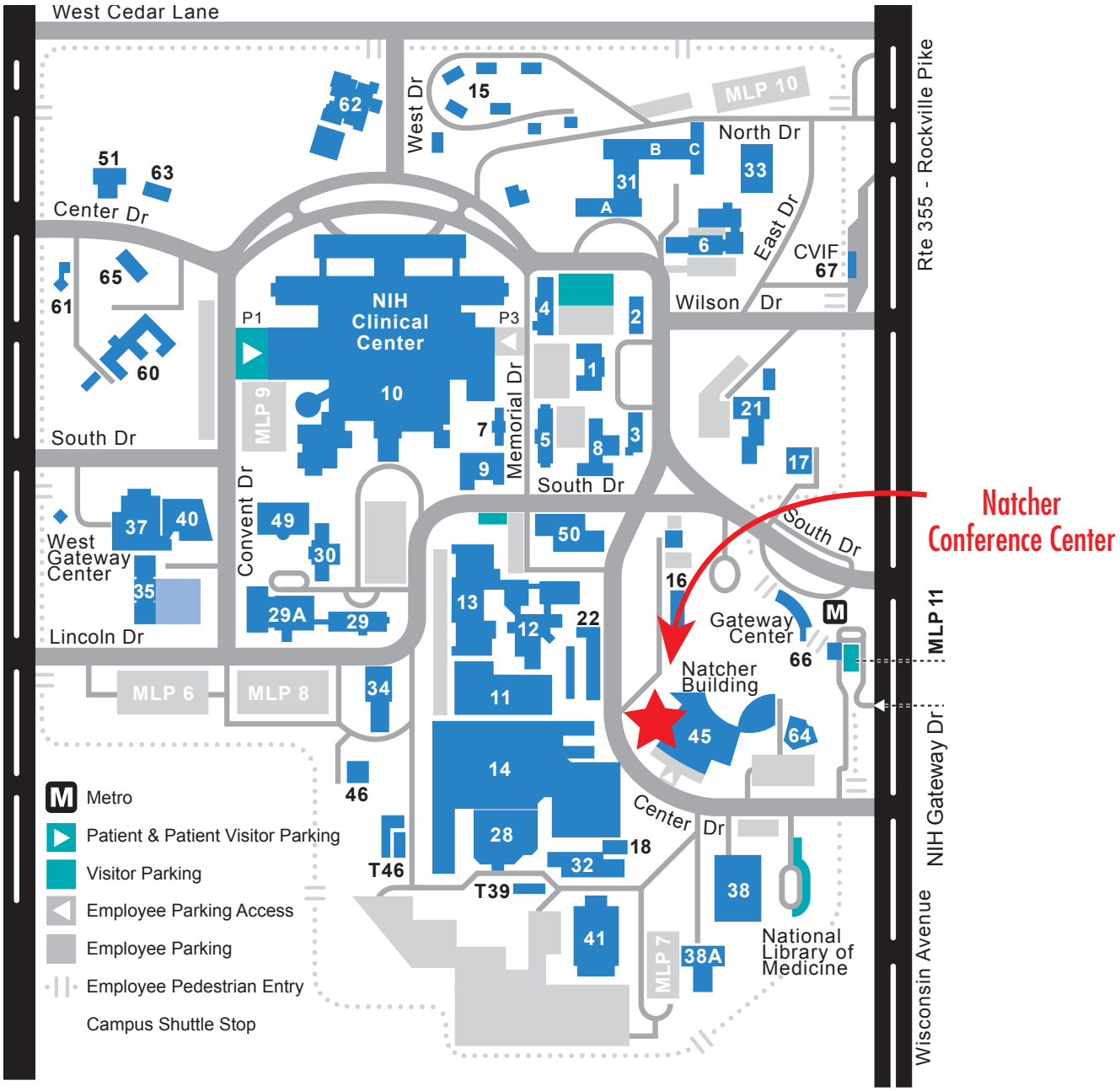
Natcher Conference Center
National Institute of Health
Bethesda, Maryland

December 12-13, 2013

2013 NHGRI Scientific Symposium

NIH Campus Map

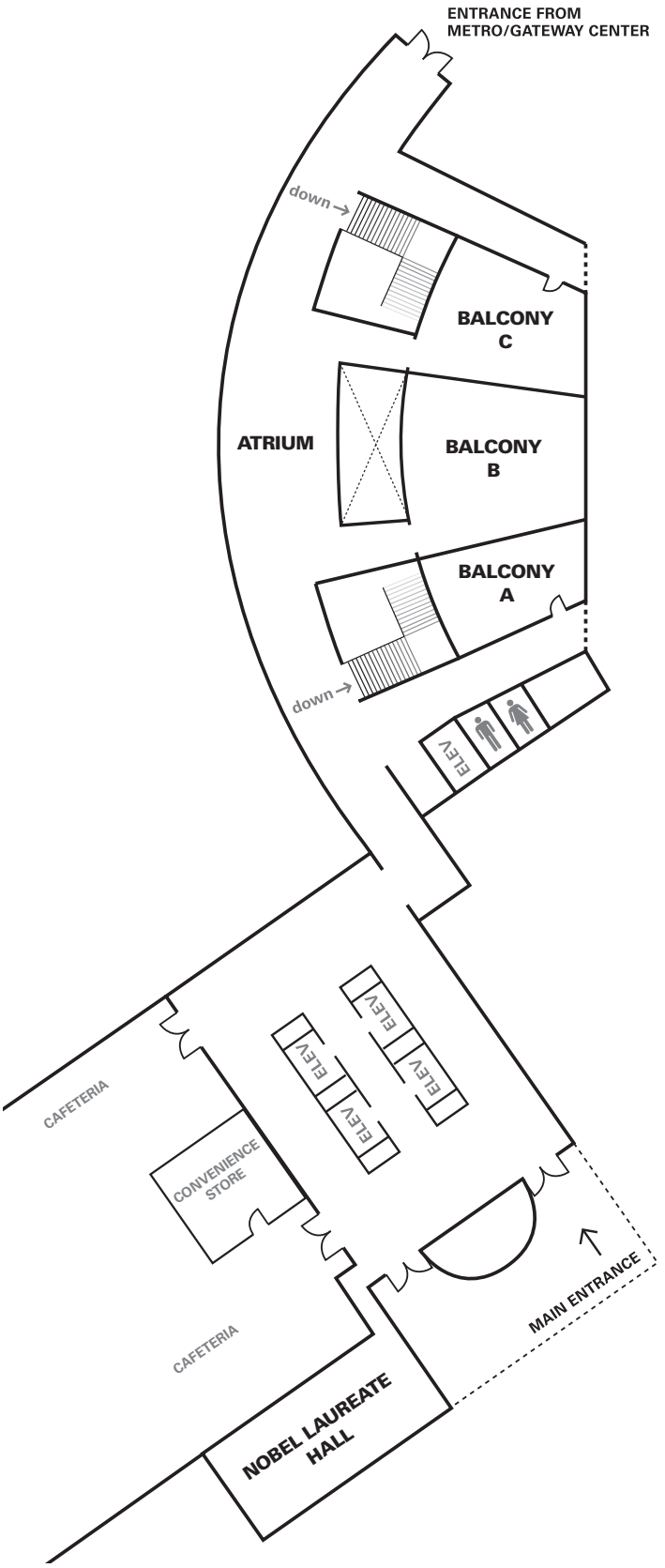
Natcher Conference Center
45 Center Drive
Bethesda, Maryland 20892



2013 NHGRI Scientific Symposium

Natcher Conference Center

MAIN LEVEL



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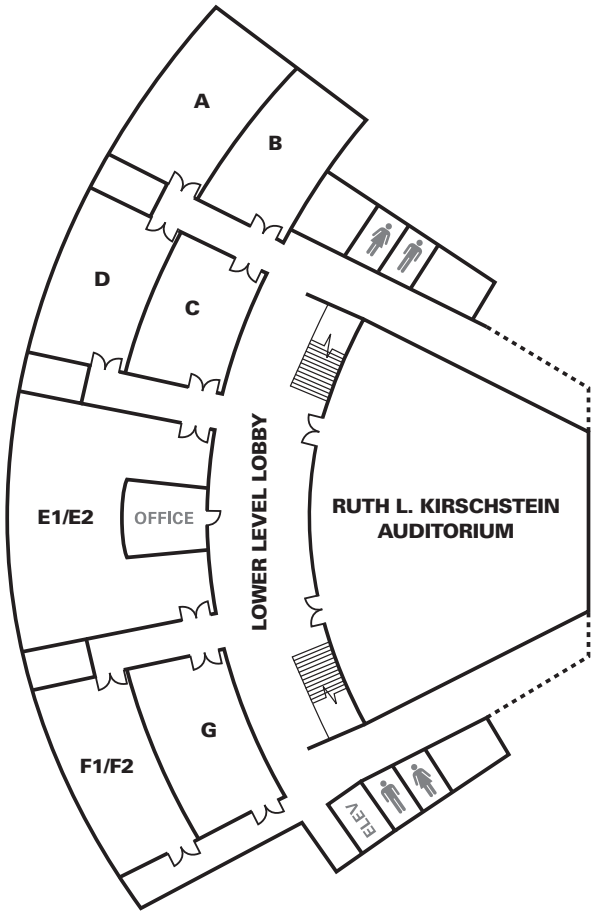

2013 NHGRI Scientific Symposium

Agenda

Thursday, December 12

9:00 am - 1:00 pm	Trainee-Only Session
1:00 - 1:15 pm	Welcome Remarks & Introduction of Keynote Speaker Eric Green, M.D., Ph.D.
1:15 - 2:15 pm	Keynote Address ***Aristides Patrinos, Ph.D.*** **Urban Science: From Genomics to Sensors and Sociology** *Deputy Director for Research, NYU Center for Urban Science & Progress* *Former President & Senior VP Corporate Affairs, Synthetic Genomics Inc.*
2:15 - 4:00 pm	Poster Session A | Concurrent Breakout Sessions
4:00 - 4:15 pm	Break
4:15 - 5:45 pm	Oral Presentations A
5:45 - 7:30 pm	Evening Networking Session

Friday, December 13

8:00 - 9:00 am	Morning Networking Session
9:00 - 10:45 am	Poster Session B | Concurrent Breakout Sessions
10:45 - 11:45 am	Oral Presentations B
11:45 - 12:00 pm	Break
12:00 - 1:00 pm	Oral Presentations C
1:00 - 1:30 pm	Awards Ceremony & Closing Remarks

2013 NHGRI Scientific Symposium

Keynote Speaker

Aristides A.N. Patrinos, Ph.D.

Aristides A.N. Patrinos, Ph.D., is the Deputy Director for Research of the Center for Urban Science and Progress at New York University's Brooklyn Polytechnic Institute (NYU-Poly). He is also a Distinguished Industry Professor of Mechanical Engineering and Chemical and Biomolecular Engineering at NYU-Poly.

Dr. Patrinos joined NYU-Poly from Synthetic Genomics Inc. (SGI), a privately held company founded in 2005 applying genomic-driven commercial solutions that address global energy and environmental challenges. At SGI he served as President and Senior Vice President for Corporate Affairs.

Prior to joining SGI, Dr. Patrinos was instrumental in advancing the scientific and policy framework underpinning key governmental energy and environmental initiatives while serving as associate director of the Office of Biological and Environmental Research in the U.S. Department of Energy's Office of Science. He oversaw the department's research activities in human and microbial genome research, structural biology, nuclear medicine and climate change. Dr. Patrinos played a historic role in the Human Genome Project, the founding of the DOE Joint Genome Institute and the design and launch of the DOE's Genomes to Life Program, a research program dedicated to developing technologies to use microbes for innovative solutions to energy and environmental challenges.

Dr. Patrinos served on several National Academy of Science committees, including America's Energy Future, Providing Strategic Advice to the Climate Science Program, and the Economic and Environmental Impacts of Increasing Biofuels Production. He is a fellow of the American Association for the Advancement of Science and of the American Meteorological Society, and a member of the American Geophysical Union, the American Society of Mechanical Engineers and the Greek Technical Society. Dr. Patrinos is also the recipient of numerous awards and honorary degrees, including three Presidential Rank Awards and two Secretary of Energy Gold Medals, and honorary doctorates from the National Technical University of Athens and the Hellenic American University. A native of Greece, he received an undergraduate degree from the National Technical University of Athens, and a Ph.D. from Northwestern University.

2013 NHGRI Scientific Symposium

Oral Presentations

Oral Presentations A

Thursday, December 12, 4:15 – 5:45 pm

Matthew LaFave, Burgess Group
Minnkyong Lee, Crawford Group
Amy Bentley, Rotimi Group
Melissa Harris, Pavan Group
Irini Manoli, Venditti Group
Thierry Vilboux, Gahl Group

Oral Presentations B

Friday, December 13, 10:45 – 11:45 am

Erica Bresciani, Liu Group
Philipp Andre, Yang Group
Qing Zhou, Kastner Group
Ellen McNamara, B. Biesecker Group

Oral Presentations C

Friday, December 13, 12:00 – 1:00 pm

Arun Unni, Varmus Group
Dina Eliezer, Koehly Group
Claire Simpson, Bailey-Wilson Group
Francisco Sanchez-Vega, Elnitski Group

2013 NHGRI Scientific Symposium

Breakout Sessions

Thursday, December 12, 2013
2:15 – 4:00 pm | Concurrent with Poster Session A

1. **Stress Management | 2:30 – 3:15 pm**
   Rebecca Coca, National Cancer Institute
   Rm E1/E2

2. **Using ENCODE Data to Interpret Disease-associated Genetic Variation | 3:00 – 3:45 pm**
   Mike Pazin, ERP/Division of Genome Sciences
   Balcony B

3. **Women in Science, a Panel Discussion | 3:15 – 4:00 pm**
   Laura Elnitski, DIR/Genome Technology Branch
   Anna Solowiej, DIR/Technology Transfer Office
   Gillian Hooker, DIR/Social and Behavioral Research Branch
   Balcony C

Friday, December 13, 2013
9:00 – 10:45 am | Concurrent with Poster Session B

1. **Dealing with Conflict in the Workplace | 9:00 – 9:45 am**
   David Michael, Deputy Ombudsman, NIH Office of the Ombudsman
   Rm E1/E2

2. **Careers Away from the Bench, a Panel Discussion | 9:15 – 10:00 am**
   Carla Easter, DPCE/Education and Community Involvement Branch
   Claire Driscoll, DIR/Technology Transfer Office
   Lu Wang, ERP/Division of Genome Sciences
   Balcony C

3. **Inside the Genome Studio, an In-Depth Interview | 9:30 – 10:15 am**
   Ellen Rolfes, NHGRI Acting Executive Officer
   Balcony B

4. **It's Not That Simple! Genomic Research & The Consent Process | 9:45 – 10:30 am**
   Bioethics through Theater
   Karen Rothenberg, NHGRI Office of the Director
   Balcony A

2013 NHGRI Scientific Symposium

Information Tables

Information Tables

The following NHGRI offices will be hosting information tables during the poster sessions:

- NHGRI Ethics Office
- NHGRI Division of Policy, Communications and Education (DPCE)
- NHGRI Division of Management (DM) / Combined Federal Campaign (CFC) Bake Sale
- NHGRI Technology Transfer Office (TTO)
- NHGRI Intramural Training Office (ITO)

2013 NHGRI Scientific Symposium

Poster Abstracts

2013 NHGRI Scientific Symposium

Guidelines for Poster 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.

All posters will be on display for both days of the 2013 NHGRI Scientific Symposium.

Authors should be at their displays and available to answer questions according to the following schedule:

- Posters with **EVEN** numbers - Poster Session A | Thursday, December 12 | 2:15 - 4:00 pm
- Posters with **ODD** numbers - Poster Session B | Friday, December 13 | 9:00 - 10:45 pm

Posters may be mounted Thursday, December 12, from 8:00 am - 1:00 pm.
Posters should be removed by Friday, December 13, at 2:00 pm.

Do you agree with Angelina Jolie?

1 Poster

Genetic literacy relates to how men and women think about prophylactic mastectomies

Leah Abrams [1], Laura Koehly [1], Gillian Hooker [1], Joseph Cappella [2], Colleen McBride [1]

The ability to understand genetics is becoming increasingly relevant to everyday life. Genomics recently gained widespread attention when Angelina Jolie chose to have a bilateral prophylactic mastectomy—removing both breasts to decrease cancer risk caused by a BRCA genetic mutation. This report explores whether genetic literacy influences how people think about this difficult health decision.

797 adults, evenly split by gender and over representing African Americans, completed the online survey. Three scales measured genetic literacy— familiarity with terms, objective knowledge, and ability to interpret provided information. Respondents rated their confidence in assessing Jolie's decision and how much they agreed or disagreed with choosing a prophylactic mastectomy in different cases involving a BRCA mutation.

Genetic literacy was positively association with confidence in assessing Angelina Jolie's decision (Pearson's correlation=.329, P<0.001). Increased genetic literacy improved women's confidence more than men's confidence. 45% of respondents remained neutral when asked if they agree with prophylactic mastectomies. Women and those with higher genetic literacy were more likely to make a decision. Considering only those who were decisive (n=436), genetic literacy predicted choosing a mastectomy with confidence as a mediating factor. Respondents who were one unit above the average confidence were almost two times more likely to choose a mastectomy, which objectively reduces cancer risk.

This research shows that higher genetic literacy may help people confidently interpret genomics in non-technical settings and make informed health decisions. These findings highlight the importance of genomics education for the public, especially for women.

1. Social and Behavioral Research Branch, NHGRI, NIH, Bethesda, MD

2. Annenberg School for Communication, U of Pennsylvania, Philadelphia, PA

A zebrafish model of cblC disease displays growth retardation that improves with vitamin B12 therapy

2 Poster

Nathan Achilly [1], Jennifer Sloan [1], Kevin Bishop [2], Marypat Jones [2], Victoria Hoffman [3], Raman Sood [2], Charles Venditti [1]

Cobalamin C disease (cblC) is the most common inborn error of intracellular cobalamin metabolism. It is caused by mutations in MMACHC, a gene responsible for processing and trafficking intracellular cobalamin. Disease manifestations can include growth failure, anemia, heart defects, and blindness. To replicate clinical manifestations of cblC disease, we created loss-of-function alleles in the zebrafish orthologue of MMACHC using zinc-finger nucleases. We chose p.L44PfsX21 (hg12) and p.G32VfsX48 (hg13) for phenotype analysis. mmachc hg12/hg12 and mmachc hg13/hg13 fish survived the embryonic period but displayed growth impairment after 7 days post-fertilization (dpf). By 21 dpf, the standard length (SL) and height at the anterior of the anal fin (HAA) were significantly reduced; mmachc hg12/hg12 fish (SL 6.94 ± 0.07, HAA 0.77 ± 0.03 mm) and mmachc hg13/hg13 (SL 7.40 ± 0.07, HAA 0.86 ± 0.01 mm) fish were smaller than the wild-type fish (SL 10.39 ± 0.18, HAA 1.48 ± 0.03 mm) ($p<0.0001$). Histological examination revealed shortened photoreceptor outer segments. The concentration of methylmalonic acid, a classic biomarker, was elevated by 289-fold in mmachc hg12/hg12 fish. OH-cobalamin (OH-cbl) is administered to patients and ameliorates some of the disease-related complications. When mmachc hg12/hg12 fish were maintained in OH-cbl supplemented water (100 µg/ml) for 21 days, SL increased by 25% ($p<0.05$) and HAA increased by 30% ($p<0.01$). This zebrafish model of cblC disease recapitulates several of the phenotypic and biochemical features of MMACHC deficiency, demonstrates a response to conventional therapy, and should be useful to delineate the pathophysiology in this disorder of cobalamin metabolism.

1. Organic Acid Research Section, Genetics and Molecular Biology Branch

2. Zebrafish Core Facility, Genetics and Molecular Biology Branch

3. Diagnostic and Research Services Branch, Office of the Director

Modified Random Forest Algorithms For Analysis of Matched Case Control Data or Case-parent Trio Data

3
Poster

Joan Bailey-Wilson

Random forests (RF) is a machine-learning method useful to detect complex interactions among genetic markers related to a disease trait based on case-control samples. We propose a new modification of the RF algorithm for matched case-control, or family based (trio) data analysis. RF is an ensemble method, which analyzes data and summarizes results using a large number of classification trees. During the procedure, each classification tree uses a proportion of samples and a subset of predictors. An R package, rpart, has functions implementing classification tree analysis and it can be modified to accommodate different study designs by substituting its functions of classification based on a novel criterion. For ease of implementation, our method utilizes the rpart package to conduct classification tree analysis on a subset of the samples and predictors. Then our ensemble code, also written in R, summarizes results from all trees. For matched case-control, or case-parent trio data, we sample the set of samples (in a matched set, or matched case, pseudo-controls set) to be fit to each classification tree. Different classification criteria are also proposed to accommodate the matched study design. To evaluate our method, we simulated matched case-control, and case-parent trio data, and applied our method to select the top 1% most important predictors. The results are compared with other machine-learning methods applicable for matched case-control data, including RF++ and trio Logic Regression.

1. NHGRI/NIH

Preferences and Understanding: Informed Consent for Genetic Research in Individuals with Sickle Cell Disease

4
Poster

Marci Barr [1], Dominique Diggs [4], Catherine Seamon [2], Mary K. Hall [3], James G. Taylor VI [2]

Informed consent for genetic research is an opportunity to incorporate personal preferences about how participant samples will be used and which types of genetic results they prefer to learn. To investigate preferences about genetic research as recorded during the informed consent process, we reviewed consent forms for two ongoing studies of sickle cell disease conducted through NHLBI, both of which had an auxiliary genetic component. Consent forms from 649 participants were examined for responses to questions about preferences for sample use in genetic research and return of genetic information. We hypothesized that the current consent forms for these two studies were inadequate in facilitating -subject understanding and preference communication. We found that 615 (99.5%) subjects agreed to participate in the genetic portion of a study least once. We reviewed the consent forms for evidence of improper consent, including blank questions and illogical answers and found that 50 (7.7%) of the 649 subjects had at least one element with improper responses. For the 173 participants who were re-consented at additional study follow-up visits using the same consent form, 57.8% (100) answered one or more questions inconsistently across the forms. We concluded that NIH sickle cell research participants are very willing to participate in genetic research. Nonetheless, the consent forms may not adequately facilitate appropriate subject education and decision making as indicated by improper consent responses and inconsistency over time. Additional research may provide further insight into the origins of these inconsistencies and suggest ways to modify the existing consent forms.

1. Genetic Services Research Unit, SBRB, NHGRI, NIH

2. Genomic Medicine Section, Hematology Branch, NHLBI, NIH

3. Critical Care Medicine Department, CC, NIH

4. Howard University College of Medicine

Where does hope fit in? The relationship between hope, uncertainty, and coping efficacy in mothers of children with Duchenne/Becker Muscular Dystrophy

5
Poster

Megan Bell [1,2], Barbara Biesecker [1], Holly Peay [1,3]

The proposed study aims to examine the relationships between uncertainty, trait hope, and coping efficacy in mothers of children with Duchenne/Becker Muscular Dystrophy (DBMD). DBMD is described as a complex chronic condition causing challenges similar to both chronic and terminal illnesses. Parental adaptation to a child's DBMD diagnosis is complex due to the constant evolution of the disease and uncertain timing of progressive losses the child and family will face. In addition to prognostic uncertainties, there can also be uncertainty related to medical management, reproductive planning, the family's social identity, and the existential meaning of the child's life. It is not fully understood how mothers of children with DBMD appraise, cope with, and adapt to their child's condition in light of this uncertainty. While a high level of uncertainty may be seen as a threat to adaptation, there is evidence that caregivers may find benefits in uncertainty. Literature suggests that a person's trait hope may influence the appraisal of uncertainty, as well as have therapeutic value in positively affecting coping and adaptation. This study's conceptual framework is based on Lazarus and Folkman's Transactional Model of Stress and Coping, Mishel's Perceived Uncertainty in Illness Theory, and Dufault and Martocchio's Model of Hope. The proposed study uses a cross-sectional research design (embedded within the second year follow-up survey of a five year longitudinal study) to quantitatively explore the relationships between uncertainty, trait hope, and coping efficacy and will inform interventions aimed at promoting maternal adaptation in the face of uncertainty.

1. Social and Behavioral Research Branch, NHGRI

2. Health, Behavior, and Society, Johns Hopkins School of Public Health

3. Parent Project Muscular Dystrophy

Investigating the phenotypic consequences of frameshift mutations in the vitamin B12 transporter, TCblR, in humans and mice.

6
Poster

David J. Bernard [1], Faith J. Pangilinan [1], Jun Chen [2], Anne M. Molloy [3], Denise M. Kay [4], Lawrence C. Brody [1]

Dietary deficiency and malabsorption of vitamin B12 in humans result in megaloblastic anemia, peripheral neuropathy or other nonspecific neurological symptomology and, if left untreated, can be fatal. A recent study found that newborns with transiently high levels of methylmalonic acid (MMA) were homozygous for a 3bp deletion (E88del, rs0384171) in the vitamin B12 receptor (TCblR) gene. We performed a population-based study on newborns identified with transiently high levels of C3 (propionylcarnitine), a marker for organic acidemia and vitamin B12 deficiencies, and found 7 of 351 cases were homozygous for the E88del genotype compared to none in the control group (n=388). Furthermore, we created a targeted deletion of the TCblR gene in mice. Despite the reduced levels of tissue vitamin B12 resulting in the significantly elevated levels of MMA and homocysteine, the TCblR null mice are normal, viable, fertile, and not anemic. This result suggests the possibility of another mechanism for transporting vitamin B12 in these mice. Upon dietary restriction of vitamin B12, the TCblR knockout females completely fail to reproduce. Ovarian histology indicates normal follicle growth and maturation in the knockout mice. After 3 months on the B12-free diet, knockout mice have elevated levels of plasma MMA compared to control mice. However, after 6 months on the B12-free diet, both control and KO mice have equivalently elevated levels of plasma MMA. Further study of these mice is aimed at determining the mechanisms for the female infertility and elucidating the alternate mechanisms transporting vitamin B12.

1. Molecular Pathogenesis Section, GTB

2. Embryonic Stem Cell and Transgenic Mouse Core, GDRB

3. Department of Clinical Medicine, Trinity College Dublin, Dublin, Ireland

4. Division of Genetics, Wadsworth Center, New York State Department of Health

The NHGRI Bioethics Core

7
Poster

Sara Hull [1,2], Benjamin Berkman [1,2], Victoria Willits [1]

The NHGRI Bioethics Core provides an organizational structure for bioethics service activities within DIR. The mission of the Core is to provide consultation, education, and administrative infrastructure to the Division of Intramural Research in three key areas:

- ethics of research with human subjects
- responsible conduct of research, and
- clinical bioethics

This involves providing infrastructure and training related to the NHGRI Institutional Review Board (IRB), coordinating annual Responsible Conduct of Research educational sessions, moderating bioethics rounds at the clinical genetics case conference, and representing NHGRI within the Clinical Center's ethics consultation service and Ethics Committee. In addition, the Core is available to address emergent bioethics education and consultation needs for the DIR, and it is engaged in a program of evaluation research to help ensure that recommendations are empirically well-grounded and responsive to the various stakeholders involved.

1. Bioethics Core, OCD, NHGRI

2. Department of Bioethics, CC

The Bioinformatics and Scientific Programming Core

8
Poster

Bioinformatics Core [1]

The Bioinformatics and Scientific Programming Core provides expertise and assistance in bioinformatics and computational analysis in support of DIR science.

The Core makes available commonly used commercial and public-domain software; it also develops and maintains Web sites for the dissemination of data compiled by DIR Investigators. In addition, Core staff collaborate with NHGRI investigators on computationally-intensive projects. For example, the Core has developed a computational pipeline for analyzing RNA-seq data, which can perform ab initio and de novo transcript assembly, identify novel genes and transcripts, measure gene expression, and detect alternatively spliced transcripts.

For microarray data analysis, the Core has licensed Partek, which features robust statistical and visualization tools to analyze data from gene/exon expression arrays and DNA copy number studies, as well as MetaCore, which allows for pathway analysis of any type of high-throughput data. For more complex analyses, the Core utilizes the statistical package R.

To facilitate clinical studies, the Core has worked with DIR investigators to implement Labmatrix, a Web-based, HIPAA-compliant clinical database system. Labmatrix provides integrated querying tools and includes functionality for IRB protocol management, biological sample storage management, workflow tracking, and handling of genotyping and phenotyping data. Sample barcoding and import of CRIS clinical laboratory data are supported as well.

DIR researchers needing access to Linux servers can request accounts on NHGRI's Linux cluster.

Finally, the Core provides DIR scientists with educational opportunities in bioinformatics, including a series of hands-on courses. All DIR scientists are encouraged to enroll.

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

1. GTB, NHGRI

Reconstructing the evolution of ancestral gap junction proteins

9
Poster

Stephen Bond [1], Andy Baxevanis [1]

Gap junctions (GJs) are a nearly ubiquitous feature of metazoan life, coupling the cytoplasm of adjacent cells into a partially selective syncytium. The range of physiological and pathophysiological processes that GJs partake in is extensive – a feature that is mirrored by an equally extensive diversity in the primary sequence of GJ-forming proteins. A particular curiosity is the presence of two distinct families of GJ proteins within the animal kingdom. In chordates, connexins (Cxs) are responsible for coupling adjoining cells, while the "invertebrate analogs of connexins", namely innexins (Inxs), perform the function across most of the remaining phyla. While the three-dimensional structures of Inxs and Cxs are thought to be quite similar, they share very little identity at the sequence level, making it difficult to infer homology with any level of statistical rigor. In the current study, we explore the evolutionary relationship between Inxs and Cxs, making use of newly released whole-genome sequence data to include GJ proteins from all major metazoan phyla. Structural features of these proteins are used to guide multiple sequence alignments, and ancestral states are reconstructed as position-specific scoring matrices (PSSMs) from groups of closely related sequences using partial-order sequence graphs, Bayesian inference, and maximum likelihood approaches. Sequential profile-profile alignments then allow us to progressively step down through deeper phylogenetic nodes to the inferred base (i.e., the last common ancestor) of both the Inx and Cx families. It is from these basal PSSMs that we finally calculate the probability of Inx and Cx homology.

1. Genome Technology Branch, Division of Intramural Research, NHGRI

Effect of child's gender on mother's food choices: a virtual reality-based buffet

10
Poster

Sofia Bouhlal [1], Colleen McBride [1], Dianne Ward [2], Susan Persky [1]

National guidelines recommend a higher energy intake for boys than girls beginning as young as 4-5 years. Few studies investigated how mothers apply these recommendations while making food choices for their child, especially when the latter is at risk of becoming overweight. Overweight mothers of a 4-5 year-old participated in a randomized controlled trial assessing their feeding behavior in response to family health history-based (FHH) feedback regarding their child's risk of obesity. Of the 221 overweight mothers recruited, 55% identified their daughter as the child for whom they would be choosing the food. Mothers donned a head-mounted display to be immersed in a virtual buffet restaurant, and selected a lunch for their daughter or son. The caloric content of boys' plates was 43 calories higher than girls' (p=.015). The difference was mainly due to extra calories coming from the less-healthy foods (p=.04). We ran multivariate models with predictors of meal calories, controlling for whether the mother received the FHH risk information or not. More predictors contributed to explaining calories in daughters' meals. Only the mother's weight predicted the boys' meal calories (β=0.20; p=.04). Predicting girls' meal calories were the mothers' obesity genetic causal beliefs (β=0.19; p=.03), mother's weight (β=0.17; p=.05), whether both biological parents are overweight (β=0.26; p=.003), mother's education β=-0.28; p=.001) and her restriction of her daughter's food intake (β=-0.20; p=.02). Differences in dietary choices made for young girls and boys might encourage lifelong gender differences in eating patterns. Gender differences should be considered while designing obesity prevention interventions.

1. Social and Behavioral Research Branch, NIH/NHGRI, Bethesda, MD, USA

2. Department of Nutrition, University of North Carolina, NC, USA

Adhesion GPCR mutations in vibratory urticaria patients

11
Poster

Steven E. Boyden [1], Avanti Desai [2], Colleen L. Satorius [1], Kenneth K. Kidd [3], Dean D. Metcalfe [2], Daniel L. Kastner [1], Hirsh Komarow [2]

Vibratory urticaria (VU) is characterized by development of a localized red, itchy, swollen hive in response to sustained vibration against the skin. Coincident histamine release and a rapid time course suggest aberrant mast cell degranulation as a mediator. Through linkage analysis and exome sequencing we identified a missense mutation in EMR2 as the only candidate variant co-segregating in two Lebanese kindreds with autosomal dominant VU. The mutation is absent from variant databases and 200 sequenced ancestry-matched controls. Exome sequencing in an unrelated VU patient revealed a novel missense mutation in CD97, a closely related paralog of EMR2. Both genes encode adhesion G-protein coupled receptors, characterized by a large N-terminal extracellular domain, a central stalk region, and a C-terminal transmembrane domain. The EMR2:p.C492Y mutation and the CD97:p.D503N mutation both lie in the G-protein proteolytic site (GPS) within the stalk domain. EMR2 and CD97 undergo autocatalytic cleavage just downstream of their GPS motifs, producing an N-terminal alpha subunit and a C-terminal beta subunit that remain non-covalently bound. Patient-derived but not control mast cells undergo a calcium-dependent degranulation in response to vibration, which is accentuated by either dermatan sulfate, the endogenous ligand of EMR2, or an anti-EMR2 antibody that ligates its stalk domain. Prior studies of CD97 and other adhesion GPCRs suggest that the alpha subunit inhibits the beta subunit, which is otherwise constitutively active. We hypothesize a pathologic mechanism whereby the mutations de-stabilize the non-covalent subunit interaction, sensitizing cells to vibration-induced shedding of the alpha subunit and hyperactivation of beta subunit-mediated signaling.

1. Inflammatory Disease Section, Medical Genetics Branch, NHGRI, NIH

2. Mast Cell Biology Section, Laboratory of Allergic Diseases, NIAID, NIH

3. Department of Genetics, Yale University School of Medicine

cbfb Is Required For the Mobilization, but Not the Emergence, of Hematopoietic Stem Cells in Zebrafish Embryos

12
Poster

Erica Bresciani [1], Blake Carrington [2], Stephen Wincovitch [3], MaryPat Jones [4], Aniket V. Gore [5], Brant M. Weinstein [5], Raman Sood [2], Paul P. Liu [1]

CBFβ and RUNX1 form a DNA-binding heterodimer and are both required for hematopoietic stem cell (HSC) generation in mice. However the exact role of CBFβ in the production of HSC remains unclear. The cellular mechanisms and the genetic pathways that drive HSC generation are highly conserved across vertebrates. Thus, we dissected the role of cbfb and the CBF complex using a zebrafish model. We generated two cbfb knockouts, which showed that the function of RUNX1 and CBFβ during HSC development could be uncoupled and revealed a previously unknown role of cbfb during definitive hematopoiesis. The cbfb-/- embryos underwent primitive hematopoiesis and developed transient erythromyeloid progenitors, but they lacked definitive hematopoiesis. Unlike runx1 mutants in which HSCs are not formed, HSCs were formed in cbfb-/- embryos. Rather, the subsequent release of HSCs from the aorta-gonad-mesonephros (AGM) region was blocked, as evidenced by the accumulation of runx1+ HSCs in the AGM that could not enter circulation. Live imaging analysis of cbfb-/-/tg(c-myb:eGFP) embryos confirmed that HSCs egressed from the dorsal aorta but did not enter circulation. Moreover, embryos treated with a specific inhibitor of RUNX1-CBFβ interaction, Ro5-3335, phenocopied the hematopoietic defects observed in cbfb-/- mutants, confirming that the function of RUNX1 and CBFβ during HSC development could be uncoupled. Finally, we found that cbfb was downstream of the Notch pathway during HSC development. Overall our data indicate that CBFβ and functional CBFβ-RUNX1 heterodimers are not required for the emergence of HSCs but are essential for the mobilization of HSCs during early definitive hematopoiesis.

1. Oncogenesis and Development Section, NHGRI, NIH

2. Zebrafish Core, NHGRI, NIH

3. Cytogenetics and Microscopy Core, NHGRI, NIH

4. Genomics Core, NHGRI, NIH

5. Program in Genomics of Differentiation, NICHD, NIH

Genetic Screening of the NIH Hermansky-Pudlak Syndrome Cohort

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Poster

Melanie Bryan [1], Andrew R. Cullinane [1], Bernadette Gochuico [1], Richard A. Hess [1], Gretchen Golas [1], Kevin O'Brien [1], William A. Gahl [1], Marjan Huizing [1]

Hermansky-Pudlak syndrome (HPS) is a rare autosomal recessive disorder of lysosome related organelle (LRO) biogenesis. The syndrome is characterized by oculocutaneous albinism, a bleeding diathesis due to a delta storage pool deficiency in platelets and, in some patients, immunodeficiency, granulomatous colitis and/or pulmonary fibrosis. Nine human HPS subtypes have been identified (HPS-1 through HPS-9). The proteins encoded by the 9 HPS genes belong to one of 4 protein complexes: the Adaptor Protein complex-3 (AP-3) and the Biogenesis of LRO Complexes (BLOC-1, -2, -3). Each HPS subtype has been well phenotyped, and accurate diagnosis of each subtype has important prognostic and therapeutic implications. Extensive molecular studies have revealed that BLOC-3 patients (HPS-1, -4) will develop pulmonary fibrosis and have an increased risk of developing granulomatous colitis. AP-3 patients (HPS-2) have recurrent childhood infections due to neutropenia, but are responsive to rGCSF, and may develop severe pulmonary fibrosis earlier than BLOC-3 patients. BLOC-1 patients (HPS-7, -8, -9) and BLOC-2 patients (HPS-3, -5, -6) have milder clinical manifestations, with no apparent pulmonary fibrosis. Subtyping HPS patients has important genetic counseling implications and can help patients anticipate symptoms, take preventative actions, and optimize their health care. In the current NIH cohort, 285 patients have been subtyped (208 HPS-1, 3 HPS-2, 35 HPS-3, 17 HPS-4, 11 HPS-5, 9 HPS-6, 1 HPS-8, and 1 HPS-9). An additional 18 clinically diagnosed HPS patients do not have mutations in the 9 known HPS genes, suggesting that there are yet to be discovered genes associated with HPS.

1. Section of Human Biochemical Genetics, Medical Genetics Branch, NHGRI, NIH

Establishment of immortalized gba1 mouse cortical neurons - What can we learn from this model?

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Poster

Robert Burnett [1], Marina Siebert [1,2], Rafael Tamargo [1], Bahafta Berhe [1], Nahid Tayebi [1], Wendy Westbroek [1], Ellen Sidransky [1]

Glucocerebrosidase (GCase) is a lysosomal hydrolase, encoded by GBA1, involved in the breakdown of glucosylceramide. GCase deficiency is caused by mutations in GBA1, resulting in Gaucher disease (GD), a recessive lysosomal storage disorder. Mutations in GBA1 are also a common genetic risk factor for Parkinson disease (PD). While primary rodent neurons and neuroblastoma cell lines are used to study the GBA1 and PD link, these models have limited utility because of challenges in culturing cells and/or manipulating levels of GCase expression. To overcome these difficulties, we immortalized GCase deficient neurons from GD mice for further studies. Initially, we immortalized cortical neurons from the null allele gba-/- mouse by infecting differentiated primary cortical neurons with EF1α-SV40T lentivirus. After extensive selection with puromycin, the immortalized neurons were characterized. gba-/- neurons showed no GCase enzyme activity or expression of GCase protein compared to WT neurons. The immortalized neurons were positive for neuronal markers such as TUJ-1 and MAP-2 but negative for GFAP. Immortalized and characterized cortical neurons from gba1 mouse models can be utilized for studies of the pathogenesis of neuronopathic GD, the glucocerebrosidase-PD association, the evaluation of therapeutics, and the validation of GCase-specific reagents.

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

2. Federal University of Rio Grande do Sul, Porto Alegre, Brazil

Dominant-Activating, Germline Mutations in Phosphoinositide 3-Kinase p110 Delta Cause a Novel Human Immunodeficiency

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Poster

Jennifer Cannons [1], Roseanne Zhao [1], Julie Reilley [1], Hye Sun Kuehn [2], Julie Niemela [2], Elissa Deenick [3], Umaimainthan Palendira [3], Danielle Avery [3], Carrie Lucas [4], Michael Lenardo [4], Stuart Tangye [3], Pamela Schwartzberg [1], Gulbu Uzel [4]

The p110δ subunit of phosphoinositide 3-kinase (PI3K) is selectively expressed in leukocytes and is critical for lymphocyte biology. As part of a collaborative study, we have reported germline, heterozygous, gain-of-function mutations in the PIK3CD gene encoding p110δ. Mutations were found in fourteen patients from seven families that presented with sinopulmonary infections, lymphadenopathy, nodular lymphoid hyperplasia, and CMV and/or EBV viremia. This mutation was associated with multiple T and B cell defects. Strikingly, naïve and central memory T cells were severely deficient in these patients, while senescent, effector T cells were over-represented.
To further understand why patients are unable to clear viral infections, we have grown and evaluated the function of patient cytotoxic (killer) CD8 T cells that are specific for autologous EBV-infected B cells (LCLs). Upon encounter with an infected cell, CD8 cells need to polarize their cytotoxic granules to deliver them specifically to the target for effective killing. Consistent with the increased population of effector T cells, we find that EBV-specific CD8 T cells are present in p110δ patients and that these cells express increased levels of effector molecules. However, these cytotoxic T cells are unable to efficiently kill autologous EBV-infected targets. Upon activation, patient T cells show increased release (degranulation) of cytotoxic granules. However, evaluation by microscopy reveals that the patient T cells are unable to polarize their cytolytic granules to the site of contact with the infected cell. Thus, proper regulation of p110δ activity is required for the cell polarization necessary for killing of infected targets.

1. NHGRI, NIH

2. Department of Laboratory Medicine, Clinical Center, NIH

3. Immunology and Immunodeficiency Group, Immunology Program, Garvan Institute

4. NIAID, NIH

The calcium sensing receptor regulates the NLRP3 inflammasome through Ca2+ and cAMP

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Poster

Jae Jin Chae [1], Geun-Shik Lee [1], Naeha Subramanian [2], Ivona Aksentijevich [1], Ronald N Germain [2], Daniel L. Kastner [1]

Mutations in the gene encoding NLRP3 cause a spectrum of autoinflammatory diseases known as the cryopyrin-associated periodic syndromes. NLRP3 is a key component of one of several distinct cytoplasmic multiprotein complexes (inflammasomes) that mediate the maturation of IL-1beta by activating caspase-1. Although several models for inflammasome activation have been proposed, the precise molecular mechanism of NLRP3 inflammasome activation, as well as the mechanism by which CAPS-associated mutations activate NLRP3, remain to be elucidated. Here we show that the calcium sensing receptor (CaSR) activates the NLRP3 inflammasome, mediated by increased intracellular Ca2+ and decreased cellular cAMP. Ca2+ or other CaSR agonists activate the NLRP3 inflammasome in the absence of exogenous ATP. The CaSR activates the NLRP3 inflammasome through phospholipase C, which catalyzes inositol trisphosphate production and thereby induces release of Ca2+ from endoplasmic reticulum stores. The increased cytoplasmic Ca2+ promotes the assembly of inflammasome components, and intracellular Ca2+ is required for spontaneous inflammasome activity in cells from CAPS patients. CaSR stimulation also results in reduced intracellular cAMP, which independently activates the NLRP3 inflammasome. cAMP binds to NLRP3 directly to inhibit inflammasome assembly, and downregulation of cAMP relieves this inhibition. The binding affinity of cAMP for CAPS-associated mutant NLRP3 is substantially lower than for wild-type NLRP3, and the uncontrolled mature IL-1beta production from CAPS patients' PBMCs is attenuated by increasing cAMP. Taken together, these findings suggest that Ca2+ and cAMP are two key molecular regulators of the NLRP3 inflammasome that have critical roles in the molecular pathogenesis of CAPS.

1. Inflammatory Disease Section, Medical Genetics Branch, NHGRI

2. Laboratory of Systems Biology, NIAID

Characterization of small molecules that inhibit ATAD5 induction in response to DNA damage

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Poster

Yong Jun Choi [1], Jennifer Fox [1,2], Menghang Xia [2], Ruili Huang [2], Srilatha Sakamura [2], Sciott Martin [2], Natasha Caplen [3], Kyungjae Myung [1]

Chemotherapeutic and radiation treatments cause a variety of genotoxic insults that lead to cell death in rapidly proliferating cancer cells. The discrimination of potential chemotherapeutic agents acting on impaired DNA repair pathways in cancer cells would result in more personalized treatment to kill cancer cells.

We recently reported that human putative tumor suppressor protein, ATAD5 is stabilized in response to almost all genotoxic insults. We developed a robust cell-based assay to detect genotoxic insults using ATAD5 and identified many putative chemotherapeutic agents. We identified a small molecule named UT2. In addition to inhibition of ATAD5 stabilization, UT2 also blocked general DNA damage responses including RPA32-phosphorylation and CHK1-phosphorylation after UV irradiation. Interestingly, UT2 treatment reduced the expression level of a major protein kinases for DNA damage response, DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and attenuated phosphorylation of Ataxia Telangiectasia Rad3 related (ATR). A whole genome siRNA screen was conducted to identify genes important for the induction of ATAD5 protein in response to DNA damage. 85 genes that reduced ATAD5-luciferase reporter activity greater than 2 median absolute deviation (MAD) while not significantly reducing viability or the control luciferase-only reporter were selected for an initial round of follow-up investigation. 35 genes were confirmed to be active and selective with multiple, additional siRNAs. Among these 35 genes, 4 genes (AHDC1, RAD21, PCID2, and SHFM1) having the highest MAD difference were chosen for further investigation.

1. GMBB, NHGRI, NIH

2. NCATS, NIH

3. NCI, NIH

The Genotype-Tissue Expression (GTEx) Project: an atlas of human gene expression

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Poster

Deborah Colantuoni [1]

Genome-wide association studies have identified thousands of novel loci for common diseases, but most are not associated with protein-coding changes and the mechanisms underlying the disease susceptibility remain unknown. The careful examination of gene expression and its relationship to genetic variation has thus become a critical next step in the elucidation of the genetic basis of common disease. Cell context is a key determinant of gene regulation, but to date, the challenge of collecting large numbers of diverse tissues in humans has largely precluded such studies outside of a few easily sampled cell types. The Genotype-Tissue Expression (GTEx) project will create a public atlas of human gene expression and its relationship with genetic variation in multiple reference tissues, and an associated tissue bank to allow external investigators to perform additional assays on the samples. After a pilot period was completed in January 2013, the resource is scaling up to include approximately 900 post-mortem donors by the end of 2015. Nearly 30 tissues on average are collected from each donor, and each sample undergoes expert pathology review and gene expression analysis by deep RNA-Seq. Results indicate high quality nucleic acids from a wide range of tissues that yield robust gene expression profiles. Genome-wide analysis to detect cis-eQTLs, and to evaluate allele and tissue-specific expression patterns, has shown to validate known eQTLs and reveal novel ones. Preliminary data suggest that the GTEx resource will be a powerful tool to unravel patterns of genetic variation and gene regulation across diverse human tissue types.

1. National Human Genome Research Institute, NIH

Analysis of plasmid diversity amongst hospital-associated carbapenem-resistant Enterobactericeae

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Poster

Sean Conlan [1], Clayton Deming [1], Evan Snitkin [1], NISC Comparative Sequencing Program [3], Jonas Korlach [4], Tara Palmore [2], Karen Frank [2], Julia Segre [1]

We previously reported tracking the transmission of carbapenem-resistant Klebsiella pneumoniae amongst patients at our institute. That analysis highlighted the importance of integrating epidemiological data with whole genome sequencing. Here we describe another layer of complexity to shape our understanding of outbreak surveillance and infection control; the repertoire of carbapenem-resistance encoding plasmids amongst the patient population and the hospital environment. In addition to the primary strain, other carbapenem-resistance Enterobactericeae were isolated concurrently, including additional Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter cloacae and Citrobacter freundii.

Full genome sequencing revealed that these organisms carry as many as five plasmids each with carbapenem-resistance genes on eight different plasmids; challenging initial assumptions about horizontal gene transfer. Specifically, we demonstrate that Enterobactericeae isolated from a single patient harbor two different carbapenem resistance-encoding plasmids. We could rule out transmission of the carbapenem resistance-encoding plasmids between patients, but find evidence for horizontal gene transfer of these plasmids between Enterobacteriaceae within the hospital environment. The complex nature of plasmid biology, where isolates carry many different plasmids which are themselves made up of mobile elements and gene cassettes, required improved tools for sequencing, finishing and annotation that exceed draft assembly pipelines.

This detailed picture of virulence factor distribution could not have been achieved using conventional typing methods, like targeted gene sequencing, and demonstrates a role for full genome sequencing in hospital infection control. We show it is possible to track transmission of bacterial strains and plasmids encoding antibiotic resistance with full genome sequencing of isolates from patients and the hospital environment.

1. National Human Genome Research Institute, Bethesda, MD 20892, USA
2. National Institutes of Health Clinical Center, Bethesda, MD 20892, USA
3. NIH Intramural Sequencing Center (NISC), Bethesda, MD 20892, USA
4. Pacific Biosciences, Menlo Park, CA, 94025, USA

TCGA Pan-Cancer: A Cross-Tumor Analysis Project

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Poster

Catherine Crawford [1], Carolyn Hutter [1], Heidi Sofia [1]

Since its inception in 2006, The Cancer Genome Atlas (TCGA) Research Network has characterized and catalogued molecular aberrations in DNA, RNA, proteins, and other genetic markers that lead to the uncontrolled growth of cells, otherwise known as cancer. The data generated from analyzing and profiling thousands of human tumors created a ripe opportunity to develop an integrated picture of genomic abnormalities and characteristics across tumor types. The TCGA PanCancer project, a cross-tumor analysis, examined the similarities and differences among molecular indicators of tumor formation across 12 tumor types (breast, bladder, colon, endometrial, glioblastoma, head and neck, kidney, leukemia, lung adenocarcinoma, lung squamous, ovarian and rectum). This poster presents an overview of the TCGA PanCancer initiative, highlighting major themes and findings ensuing from the project. The PanCancer project has identified several examples of shared molecular patterns among tumor subtypes from different organs, including 127 significantly mutated genes (SMGs) across cellular processes implicated in cancer development. These PanCancer findings offer researchers new insights into the relevance of somatic molecular changes and the biology of cancer. Ultimately this work may inform clinical decision-making, expand the use of existing cancer therapies, and promote research and development of novel, individualized therapeutic agents. This poster will also include a discussion of future directions in Pan-Cancer research, including emerging collaborations with the International Cancer Genomics Consortium.

1. National Human Genome Research Institute

Reduction of SOX10 results in arrested melanoma cell cycle, senescence and suppression of melanomagenesis in Grm1Tg mouse model

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Poster

Julia C. Cronin [1], Dawn E. Watkins-Chow [1], Art Incao [1], Joanne H. Hasskamp [2], Nicola Schönewolf [3], Nicolas K. Hayward [4], Boris C. Bastian [5], Reinhard Dummer [3], Stacie K. Loftus [1], William J. Pavan [1]

The transcription factor SOX10 is essential for proper survival and differentiation of neural crest cell lineages, and plays an important role in the establishment and maintenance of melanocytes. SOX10 is also highly expressed in melanoma tumors, however its role in the progression of the disease is not yet understood. Here, we report that melanoma tumor cell lines require wild-type SOX10 expression for proliferation, and SOX10 haploinsufficiency reduces melanoma initiation in the metabotropic glutamate receptor 1 (Grm1Tg) transgenic mouse model. Stable SOX10 knockdown in human melanoma cells results in arrested growth, altered cellular morphology, and senescence. Cells with stable loss of SOX10 are arrested in the G1 phase of the cell cycle and exhibit reduced expression of Microphthalmia-associated transcription factor (MITF), elevated cyclin-dependent kinase inhibitor 1A and 1B (p21 and p27) expression, hypophosphorylated retinoblastoma protein (RB) and reduced expression of its binding partner E2F1. One of the main events driving cellular transformation is the dysregulation of the cell cycle, therefore the role that SOX10 has in maintaining this process provides great promise for targeted interventions.

1. Genetic Disease Research Branch, NHGRI, NIH, Bethesda, MD

2. MD Melanoma Center at Medstar Franklin Square Medical Center, Baltimore, MD

3. Department of Dermatology, University Hospital of Zurich, Switzerland

4. Queensland Institute of Medical Research, Oncogenomics, Australia

5. Helen Diller Family Comprehensive Cancer Center, UCSF, San Francisco, CA

8q24 Risk Alleles and Prostate Cancer in African-Barbadian Men

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Poster

Cheryl D. Cropp [1,6], Christiane M. Robbins [2,6], Xin Sheng [3], Anselm J.M. Hennis [4], John D. Carpten [2], Lyndon Waterman [4], Ronald Worrell [4], Jeffrey M. Trent [2], Christopher A. Haiman [3], M. Cristina Leske [5], Suh-Yuh Wu [5], Joan E. Bailey-Wilson [1], Barbara Nemesure [5]

African American men (AA) exhibit a disproportionate share of prostate cancer (PC) incidence, morbidity and mortality compared to other groups. Several genetic association studies have implicated select loci in the 8q24 region as increasing PC risk in AA. We evaluated the association between previously reported 8q24 risk alleles and PC in African-Barbadian (AB) men, also known to have high rates of PC. Ten previously reported tag SNPs were genotyped in 447 AB men with PC and 385 AB controls from the Prostate Cancer in a Black Population (PCBP) study. Only rs2124036 was nominally significant in AB men, (OR = 2.0, 95% CI (1.0-4.3), P=0.06) for the homozygous C/C genotype after correction for multiple testing. We also conducted a meta-analysis including our AB population along with two additional African-Caribbean populations from Tobago and Jamaica for SNPs rs16901979 and rs1447295. A significant association resulted for the rs16901979 A allele (Z score 2.75; p=0.006; summary OR= 1.21 (95% CI: 1.01-1.46)). Our findings may indicate: i) the presence of a founder effect; ii) the selected SNPs not being tagged to an ancestral haplotype bearing the 8q24 risk allele(s) in this population; or iii) inadequate power to detect a true association. Additional GWAS and sequencing studies are underway to further interrogate any potential contribution of the 8q24 region to PC in this West African-derived population.

1. Inherited Disease Research Branch, National Human Genome Research Institute

2. Integrated Cancer Genomics Div, Translational Genomics Research Institute

3. Dept of Preventive Medicine, Keck School of Medicine, Univ of Southern Cal

4. Chronic Disease Research Ctr & Faculty of Med Sci, Univ of West Indies

5. Dept of Preventive Medicine, Stony Brook Univ Medical Center

6. These authors contributed equally to this work

Cytogenetics and Microscopy Core

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Poster

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

The mission of the NHGRI Cytogenetics and Microscopy Core is to provide NHGRI Investigators with state-of-the-art molecular cytogenetics services and access, and assistance with fluorescence, and confocal microscopy. In addition, the Core serves as a source of expertise and collaboration for research projects employing molecular cytogenetics techniques and cellular, molecular and live cell imaging. The Cytogenetics and Microscopy Core is located within Genetic Disease Research Branch and provides service to NHGRI Investigators throughout the institute free of charge.

Over the last year, the Core has served a total of 27 NHGRI Investigators currently on the Faculty, as well as 4 Adjunct NHGRI Investigators.

1. GDRB, NHGRI

Using multiple "-omics" in the understanding of the pathogenic mechanism of mutations in the calcium independent phospholipase (PLA2G6)

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Poster

Mariska Davids [1], Megan Kane [1], Taylor Davis [1], Charles Markello [1], Cornelius Boerkoel [1], Miao He [2,3], William Gahl [1]

To date >50% of the patients entered in the Undiagnosed Diseases Program (UDP) have changes in their glycosylation profiles in serum and/or urine compared to healthy controls. We hypothesize therefore that integration of glycomics with clinical, biochemical and genomic data improves identification of unknown disorders and understanding of pathogenic pathways. To test this hypothesis, we focused on the glycomic abnormalities observed in two individuals with Phospholipase A2-Associated Neurodegeneration (PLAN) and mutations in calcium-independent phospholipase, PLA2G6. The first patient presented as a young child with muscular symptoms, neurodegeneration and brain iron accumulation. The second patient presented as an adult with left shoulder dystonia and Parkinsonism. Glycomics analysis of serum from both patients showed similar abnormalities in their O-linked glycosylation. Suggesting that these changes are intrinsic to patient tissue and not simply markers of disease, studies of cultured fibroblasts also detect abnormalities of glycosylation. In support of this, recent studies have shown PLA2G6 regulates the dynamics and organization of ER-Golgi-Intermediate Compartment complex through phospholipid remodeling. After rescue by reintroduction of the wild type PLA2G6, these observations will be discussed in the context of their implications for the pathophysiology of PLAN.

1. NIH Undiagnosed Diseases Program, NHGRI, National Institutes of Health, Bethesda

2. Department of Pathology and Laboratory of Medicine, University of Pennsylvania

3. The Michael J Palmieri Metabolic Laboratory, Children's Hospital of Philadelphia

Center for Inherited Disease Research (CIDR): NHGRI Intramural-Extramural Collaboration

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Poster

Camilla Day [1], Lawrence Brody [1], Kim Doheny [2], Alan Scott [2], Dave Valle [2], Jane Romm [2], Lee Watkins [2], Elizabeth Pugh [2], Kim Kutchins [2], Hua Ling [2], Michelle Zilka [2], Marcia Adams [2], Janet Goldstein [2,] Elvin Hsu [2]

The Center for Inherited Disease Research was established in 1997 with the goal of creating a high throughput genotyping laboratory supported by several NIH Institutes. The program now provides state-of-the-art genotyping and DNA sequencing services to 13 NIH Institutes via a contract to Johns Hopkins University. Since inception, the Center has completed over 750 projects involving 800,000 samples. The data from these projects have appeared in over 600 peer-reviewed publications. Intramural and external investigators can obtain access to CIDR services is via a peer-review process coordinated by Dr. Camilla Day in the NHGRI Office of Scientific Review.

CIDR currently offers whole exome, whole genome and custom DNA sequencing using Illumina and Ion Torrent platforms. A wide array of genotyping options are also offered including multiple GWAS chips, custom genotyping and methylations arrays. A hallmark of CIDR is the strong laboratory expertise and on-site statistical genetics and IT support. CIDR also provides expert data cleaning support via a consulting agreement with the University of Washington Genetics Coordinating Center. This data cleaning process also allows all CIDR-supported projects to be deposited in the appropriate databases for data sharing.

1. NHGRI

2. Johns Hopkins University

Development of Novel CBF Leukemia Inhibitors

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Poster

Jamie Diemer [1], Lea Cunningham [1], Steven Finckbeiner [1], Wei Zheng [2], Ching-Shih Chen [3], Paul Liu [1]

A subset of acute myeloid leukemias (AMLs) exists that are marked by chromosomal abnormalities such as inversions and translocations affecting the transcription factor RUNX1 or CBFβ. These are termed core binding factor (CBF) leukemias and although the prognosis is generally better for CBF leukemias than other AMLs, the current treatments present with significant morbidity and mortality and the 5-year survival rate is only ~50%.

RUNX1 and CBFβ normally form a heterodimer to regulate downstream gene expression. Work from several labs, including our own, have shown that RUNX1 and CBFβ fusion proteins associated with CBF leukemia require normal CBFβ and RUNX1, respectively, for leukemogenesis which underscore the importance of this interaction. We therefore hypothesized that compounds targeting the interaction of RUNX1 and CBFβ would serve as potential treatments for CBF leukemias. A chemical library screen performed by our lab and the NIH Chemical Genomics Center (NCGC) led to the discovery of the anti-leukemic activity of a benzodiazepine Ro5-3335. We found that Ro5-3335 repressed the gene expression of RUNX1/CBFβ targets and was also active in both a zebrafish and a mouse model of CBF leukemias.

We are currently working to understand the mechanism of action of Ro5-3335 and we are actively engaged in screening analogs of Ro5-3335 that may be more potent. The ultimate goal of these experiments is to move Ro5-3335 and/or its analogs to pre-clinical and then clinical trials in the hopes of providing better treatments to CBF leukemia patients.

1. Oncogenesis and Development Section, NHGRI, NIH

2. Therapeutics for Rare and Neglected Diseases, NCATS, NIH

3. College of Pharmacy, Ohio State University

RAD001 and Lonafarnib treatment of a transgenic mouse model of HGPS

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Poster

Amanda DuBose [1], Stephen Lichtenstein [1], Urraca Tavarez [1], Michael Erdos [1], Francis Collins [1]

Hutchinson Gilford Progeria Syndrome (HGPS) is a rare genetic disorder in which a mutant form of the nuclear lamina protein lamin A (progerin) accumulates in the nuclear envelope, disrupting normal cell function. HGPS patients display a segmental premature aging phenotype with features including growth retardation, sclerotic skin, alopecia and skeletal dysplasia. Patients develop atherosclerosis leading to premature death due to stroke or myocardial infarction. In ~90% of HGPS cases, a C to T substitution in exon 11 of LMNA activates a cryptic splice site causing aberrant mRNA splicing.

Our lab has developed a transgenic mouse model that expresses human progerin, and displays many aspects of HGPS. These mice show growth impairment, kyphosis, reduced joint mobility, atherosclerosis and early death at 5-6 months for females and 6-7 months for males.

Rapamycin increases longevity in mice, and improves the phenotype of HGPS fibroblasts by reducing progerin levels. Rapamycin activates the mTORC1 pathway, leading to an increase in autophagy. An analog of Rapamycin, RAD001 (Everolimus) has been shown to have a similar effect on HGPS fibroblasts.

We are evaluating the effect of RAD001 on our transgenic mouse model of HGPS, alone and with the farnesyltransferase inhibitor Lonafarnib. We are measuring kyphosis, longevity, vessel stiffness, weight gain and changes in the major vessels in treated and untreated mice. We hypothesize that RAD001, alone and synergistically with Lonafarnib, will reduce the accumulation of progerin, leading to increased longevity and improved health.

1. Molecular Genetics Section, Genome Technology Branch, NHGRI, NIH, Bethesda

Exploring Health Disparities in Genetic Conditions through the Lens of Sickle Cell Disease and Sickle Cell Trait

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Poster

Keisha Findley [1], Vence Bonham [1]

We seek to study health disparities from the lens of a single gene condition, sickle cell disease (SCD). Within the United States, SCD is a model hemoglobinopathy to study a disparity population. It is estimated that SCD affects 90,000 to 100,000 Americans and individuals with SCD have less access to comprehensive medical care than individuals with other genetic conditions such as cystic fibrosis. One of the most debilitating SCD complications is a leg ulcer. The cause of which is unknown and limited research has been conducted. We present a framework to study the association of an individual's microbes and the social and physical environment on the formation and healing of leg ulcers.

The carrier status of sickle cell disease is described as sickle cell trait (SCT). These individuals are heterozygous for a single mutation in their beta-globin gene (HBB). Within the United States (U.S.) more than 2.5 to 3 million people live with SCT, accounting for an estimated 6% to 9% of the African-American population and 0.01% to 0.05% of the remaining population, primarily those of Arab, Southeast Asian, Hispanic, and Mediterranean descent. Although SCT is generally regarded as an asymptomatic carrier state, numerous studies over several decades have reported possible clinical manifestations of SCT. SCT has been reported to be associated with sudden death of athletes and other clinical complications. We report the findings of a systematic review on clinical complications associated with sickle trait in the literature from 1970-2012.

1. Social and Behavioral Research Branch, Public Health Genomics Section

Piloting the Families SHARE educational risk assessment tool among African-Americans in under-resourced communities

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Poster

Cynthia Fiorino, Andrea Goerge, Laura Koehly

Use of family health history is important in becoming aware of potential health issues, as it can be a contributing factor to risk for diseases like heart disease, diabetes, breast cancer and colorectal cancer. The Families SHARE Family Health Package was designed as an educational tool for families to share and discuss their family history based risk for diseases. The initial version of the tool was developed using a user-centered design approach based on the feedback of 82 mothers, the majority of whom were white, well educated, and from middle to upper middle class families. Given the homogeneity of these initial participants, it was unclear whether the Families SHARE tool would be equally accepted and useful to more diverse populations from under-resourced communities. To address this issue, we are currently conducting a second evaluation of the tool within a more diverse community. Approximately 44 male and female African-American participants of varying ages from low socioeconomic backgrounds have been enrolled. Participants complete a baseline phone interview and are sent the Family Health Package. After approximately four weeks, participants are contacted for follow up information and feedback, and are recruited for focus group interest. Preliminary results show significant differences between the two samples' baseline characteristics, including family structures and sizes; barriers to making healthy changes; and education levels, all of which could impact acceptability and utility of the Families SHARE tool. Results of this project will be valuable in further development of the tool for future use in interventions among diverse populations.

NHGRI Flow Cytometry Core Facility

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Poster

Martha Kirby [1]

The goal of the Flow Cytometry Core is to provide NHGRI investigators access to flow cytometry resources which enhance the scope and quality of their research. The Core supports the spectrum of research from bench to bedside. Core personnel are available for training as well as development and implementation of protocols.

1. GMBB

Data Sharing and Access Policy and Practices:

The NHGRI and NCI/NHGRI TCGA Data Access Committee Experiences

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Poster

Brandon Floyd [1], Vivian Ota Wang [2]

By examining relationships between genomic phenotypic and exposure data, genomic data sharing enhances scientific progress by accelerating understandings and applications of genomic research that benefit public health. The NIH Genomic Data Sharing policy encourages data access and sharing unencumbered by intellectual property claims, discourages premature claims on pre-competitive information, and increases data availability to secondary data users

Data sharing and access oversight are implemented by an NIH governance structure that includes senior leadership and staff who are responsible for the ongoing management, stewardship, and operating procedures across NIH Institutes/Center (NIH I/C). A central data repository, the database of Genotypes and Phenotypes (dbGaP) facilitates broad and consistent access to genotype and phenotype data. NIH Data Access Committees (DACs) ensure procedural standards for data access that reflect shared principles and procedural standards related to data management, security, privacy, and research participant protections.

Researchers' Data Access Requests (DARs) are reviewed by DACs who oversee and review researcher credentials, terms of data use and access, compliance with the Approved User Code of Conduct and research proposals for closed access dbGaP data. Since its inception in 2007, the NHGRI DAC has reviewed over 3,450 data access requests, while the TCGA DAC has reviewed over 1,170 data access requests. This poster will report data sharing experiences including data access procedures, studies and consent groups, and data usage including approval rates, and review times of the NHGRI and TCGA Data Access Committees.

Genome-wide Analysis of SOX10 Reveals its Dual Functions in Transcriptional Regulation in Melanocytes

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Poster

Temesgen D. Fufa [1], Dawn Watkins-Cho , David U. Gorkin [2], Andrew S. McCallion [2], Stacie K. Loftus [1], William J. Pavan [1]

SOX10 plays important roles in the development and maintenance of neural crest-derived melanocytes. Recently SOX10 expression has also been shown to be necessary for melanoma cell growth, as deficiency results in G1 arrest and senescence. Despite these critical roles, SOX10 genomic targets and key genes directly controlled by its activity have not been comprehensively defined. To address this, we performed global analysis of SOX10 DNA binding and gene expression in melanocytes. Chromatin immunoprecipitation coupled to high-throughput DNA sequencing (ChIP-seq) identified 4,085 SOX10 binding sites genome-wide that exhibit significant evolutionary conservation and strong association with an extensive set of genes, including those that play critical roles in neural crest developmental processes and the pathogenesis of cancers. Moreover, SOX10 occupies more than 50% of previously identified melanocyte enhancers indicating its extensive involvement in lineage-specific enhancer regulation. As expected, SOX10 deficiency in melanocytes leads to downregulation of genes essential for pigmentation, cell survival and proliferation. Remarkably, depletion of SOX10 also results in significant transcriptional upregulation of the epithelial-to-mesenchymal transition (EMT) gene signature, a molecular hallmark of highly invasive and metastatic cancers such as malignant melanoma. We find that SOX10 localizes to the regulatory regions of a number of EMT and metastasis promoting genes, and thereby transcriptionally suppresses their expression. The identification of SOX10 as a direct regulator of the EMT gene expression program provides a framework for understanding the molecular basis of SOX10 function in melanoma pathogenesis.

1. Genetic Disease Research Branch, NHGRI, NIH, Bethesda, MD 20892

2. Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Genomics Core

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Poster

Marypat Jones [1], Ursula Harper [1], Frank Donovan [1], Settara Chandrasekharappa [1]

The Genomics Core provides routine access to a central set of services related to genomics technologies: genotyping, sequencing, physical mapping, and access to DNA panels. While genotyping is the main activity of the Core, services related to physical mapping (access to genomic clones from human, mouse and zebrafish BAC libraries), sequencing (running the investigator-prepared sequencing reactions on ABI3130 sequencer) and access to DNA panels (HD200CAU, HD100AA, HD100MEX, HAPMAPPT01, HAPMAPPT02 and HAPMAPPT03) are also provided. Both SNP- and STRP-based genotyping services are available from the Core. A panel of >400 human STRP markers are used for genome-wide scans, and nearly 500 markers are available for scanning mouse genome. SNP-based services are provided using both the GoldenGate and Infinium technologies from Illumina. The Core is prepared to process any type of array that is supported by the Illumina SNP technology, and currently the SNP chips processed at the Core include HumanOmni2.5-8v1, HumanCore, HumanExome, HumanCoreExome, Methylation450K, HumanOmniExpress and HumanOmniExpressExome. The genotyping services are used for a variety of applications, such as linkage, association, scanning focus regions for fine mapping of linked loci, copy number variation, identification of deletion intervals, methylation, variations introduced by the iPS technology, mosaicism, uniparental disomy, homozygosity mapping, among others. The ABI sequencer-based analysis of human, mouse and zebrafish samples were for a variety of studies such as deletion mapping by MLPA, microsatellite instability, speed congenics, cell line characterization, parent-of-origin studies, alternate splice site verification, haplotype analysis, and screening for mutations.

1. Genomics Core, NHGRI

Creation of a genome-wide zebrafish gene knockout collection as a resource for investigating gene function in a model organism

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Poster

Derek Gildea [1], Gaurav Varshney [2], Jing Lu [3,4], Suiyuan Zhang [1], Haigen Huang [4], Wuhong Pei [2], Sunny Huang [2], David Schoenfeld [4], Nam Pho [2], Deborah Mosbrook-Davis [2], Li-En Jao [5], Matteo Pellegrini [4], Shuo Lin [3,4,] Shawn Burgess [2], Tyra Wolfsberg [1]

Although the zebrafish is a widely used model organism that is well suited for whole-genome reverse genetics screens, there is, at present, no publicly available collection that contains a knockout of every gene. Therefore, we aim to create a zebrafish knockout library where every gene is inactivated by retroviral mutagenesis. In order to devise a high-throughput means to identify proviral insertion events with a high probability of severely disrupting gene function, we developed a retroviral mutagenesis pipeline that incorporates the ligation of an oligonucleotide linker to DNA fragments that contain genomic sequence adjacent to the insertion site. The insertion site DNA tags are amplified by linker-mediated PCR (LM-PCR) and then sequenced on the Illumina platform. Coupling linker ligation and LM-PCR to this pipeline allows for the introduction of a six-nucleotide "barcode" that enables multiplexed-sample sequencing of insertion site DNA tags. To identify the genomic location of proviral insertions and those mutagenic lesions that result in putative gene knockouts, we developed a customized bioinformatics pipeline that performs the following: trimming of non-zebrafish sequence from insertion site DNA tags, identification of the sample origin through the nucleotide "barcode," mapping the insertion tags to the zebrafish genome, and identification of those insertion events that occur within annotated zebrafish genes. Currently, 6,144 mutagenized F1 zebrafish embryos have been screened. 5,164 integrations occurred within ENSEMBL-annotated genes, 3,776 of which are predicted to cause gene inactivation. All knockout lines are searchable through a Web-accessible database called the Zebrafish Insertion Collection (ZInC; http://research.nhgri.nih.gov/zinc/).

1. Bioinformatics and Scientific Programming Core, GTB, NHGRI, NIH
2. Developmental Genomics Section, GTB, NHGRI, NIH
3. Shenzhen Graduate School of Peking University, Shenzhen, China
4. Department of Molecular, Cell, and Developmental Biology, UCLA
5. Department of Cell and Developmental Biology, Vanderbilt University

Itk-mediated integration of T Cell Receptor and Cytokine signaling regulates the balance between Th17 and regulatory T cells

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Poster

Julio Gomez-Rodriguez [1], Elizabeth Wohlfert [2], Robin Handon [1], Françoise Meylan [3], Julie Wu [4], Stacie Anderson [1], Martha Kirby [1], Yasmine Belkaid [2], Pamela Schwartzberg [1]

A major function of the immune system is to mount specific responses to pathogens, while minimizing self-reactivity. To help orchestrate this, CD4+ T cells differentiate into distinct effector cell populations. Among these, Th17 cells drive inflammatory responses against bacteria and fungi–however excessive Th17 responses can cause immunopathology. This immune activation is countered by another CD4+ subset, regulatory T cells (Tregs). A proper balance of Th17 and Tregs is critical for generating protective immunity while minimizing autoimmunity. We show that the Tec kinase Itk, a component of T Cell Receptor (TCR) signaling, influences this balance via crosstalk between TCR and cytokine signaling. Under both Th17 and Treg differentiation conditions, Itk-/- CD4 cells developed higher percentages of functional FoxP3+ Treg cells, associated with increased sensitivity to IL-2. Itk-/- cells also preferentially developed into Tregs in vivo. Itk-deficient cells exhibited reduced TCR-induced activation of the PI3K target mTOR, a critical regulator of Treg differentiation, accompanied by downstream metabolic alterations. Itk-/- cells also exhibited reduced IL-2-induced mTOR activation, despite increased IL-2-induced FoxP3 expression. We demonstrate that in WT CD4+ T cells, TCR stimulation led to a dose-dependent repression of Pten, permitting effective mTOR activation; however, in the absence of Itk, Pten was not repressed, thereby uncoupling STAT5 phosphorylation and PI3K/mTOR pathways. We link this to impaired TCR-mediated induction of Myc and miR-19b, known repressors of Pten. Itk therefore helps orchestrate feedback mechanisms integrating multiple T cell signaling pathways, suggesting Itk as a potential target for altering the balance between Th17 and Treg cells.

1. NHGRI

2. NIAID

3. NIAMS

4. NHLBI

The link between Gaucher and Parkinson diseases: a whole genome transcriptome analysis using mouse models

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Poster

Ashley Gonzalez [1], Niraj Trivedi [1], Nahid Tayebi [1], Bahafta Berhe [1], Ellen Sidransky [1]

Gaucher disease (GD) is a rare, autosomal recessive disease caused by a deficiency of the lysosomal enzyme glucocerebrosidase (GCase). Mutations in GBA1, the gene encoding GCase, were found to be the most frequent genetic risk factor for developing Parkinson disease (PD), although the cause for this association is not fully understood. To elucidate the connection between GD and PD, we utilized transgenic mice overexpressing human mutant A53T alpha-synuclein (α-syn) as well as mice that are carriers for a null gba allele (wt//ΔMGC). By crossing these lines, we generated mice that overexpress α-syn and carry a null gba allele (wt/ΔMGC//α-syn). Neurological symptoms and weight loss were monitored regularly as indicators of illness, and showed that wt/ΔDMGC//α-syn mice exhibit symptoms on average 15 weeks earlier than α-syn mice.

Preliminary data show that GCase RNA and protein levels are similar in wt//ΔMGC and wt/ΔMGC//α-syn mice, yet increased α-syn aggregation is observed in the brains of wt/ΔMGC//α-syn mice. These data suggest that haploinsufficiency of GCase coupled with α-syn overexpression impact disease onset. To evaluate potential modifiers involved in this association, a whole transcriptome array was performed on mice with the four genotypes. The analysis has identified several candidate genes that could impact PD and GD, including PRNP (p-value: 3.22*10-12; fold change: 3.6), SERPINA3 (1.71*10-6; 4.7), SNORD13 (0.021; 2.60), SNORA62 (0.023; 2.57), Rnu1b1 (0.023; 2.60), and an undefined probe set that shares homology with histocompatibility complex 2 (3.37*10-12; 6.98). These candidate genes and the networks are being pursued further.

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

Mutational Profiles of Tumor Samples with Methylator Phenotype

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Poster

Valer Gotea [1], Francisco Sánchez-Vega [1], Gennady Margolin [1], Hanna Petrykowska [1], Laura Elnitski [1]

Increased DNA methylation across many CpG islands, usually referred to as CpG island methylator phenotype (CIMP), has been widely recognized as a defining characteristic for tumor subsets in a wide array of cancer types. CIMP tumors have been associated with different outcome prognoses in different cancer types, but CIMP classification follows different standards across different research groups. Here we attempt to clarify and unify criteria for sample classification into a methylator status by linking methylation levels to molecular characteristics, including mutational spectra, specific mutations, and gene expression. For this purpose we used samples of ovarian and uterine endometrioid carcinoma, as well as data for other cancer types collected by the TCGA consortium. We found that methylator phenotype (MEP) samples are characterized by higher mutation rates than non-methylator phenotype (NMP) samples. MEP samples exhibit a significantly increased proportion of C>T mutations (consistent with higher levels of 5-methylcytosine, which spontaneously deaminates to thymine) both within and outside of CpG islands, suggesting that the impact of increased methylation extends beyond CpG islands. By analyzing mutation profiles at mutation, gene, and pathway levels, we find that MEP samples are preferentially mutated in genes previously known to be involved in carcinogenesis (e.g. PTEN, KRAS, targets of MIR-27), as well as genes involved in related epigenetic processes (e.g. NSD1). Through the analysis of MEP samples in several cancer types, we hope to find a core set of features that are responsible for DNA hypermethylation, and could potentially lead to customized treatment with improved outcome.

1. NIH / NHGRI / GTB

The Immersive Virtual Environment Testing Area

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Poster

Peter Hanna [1], Stephanie Browning [2], Rachel N. Ullah [3], Susan Persky [4]

The Immersive Virtual Environment Testing Area (IVETA) is a state of the art social and behavioral research laboratory situated within the Social and Behavioral Research Branch. Using the latest immersive virtual reality tools, we conduct experiments to investigate an array of social and behavioral processes relevant to applying emerging genomic knowledge. We will present information about IVETA resources, characteristics of immersive virtual reality technology for research, and past and current studies that are being supported by the IVETA.

1. Social and Behavioral Research Branch, NHGRI

Physicians' experiences with and beliefs about non-medical sex selection through preimplantation genetic diagnosis

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Poster

Nina Harkavy [1], Lori Erby [1,2], Barbara Biesecker [1,3]

Preimplantation genetic diagnosis (PGD) is an assisted reproductive technology (ART) by which embryos, created through in vitro fertilization (IVF), can be screened for genetic conditions or traits before they are implanted into a woman's uterus. Recently, a relatively new use of PGD has gained recognition for its potential ethical implications at the individual and societal levels. This controversial practice, termed non-medical sex selection (NMSS), describes the use of PGD technology to choose the sex of a child for social, as opposed to medical, reasons. In the United States and a select few other countries, it is legal to use NMSS for "family balancing," the intentional selection of embryos of one sex in a family with existing children of the other sex. NMSS via PGD is divisive because of concerns around sexism broadly, as well as parenting norms, commodification of children, and resource allocation, among others. In the US and other countries, physicians are often the gatekeepers to PGD, and consequently, NMSS. This qualitative study will endeavor to explore physicians' experiences with NMSS and their ethical concerns, if any, by interviewing practicing OBGYNs and reproductive endocrinologists. Also of interest are physicians' preferences regarding their role in decision-making and regulation of NMSS. Finally, because of the concern that NMSS is the beginning of a "slippery slope" toward designer babies, we are interested in eliciting traits that physicians believe are hypothetically appropriate or inappropriate for PGD and how they make the distinction.

1. JHU/NHGRI Genetic Counseling Training Program

2. Johns Hopkins School of Public Health

3. Social & Behavioral Research Branch, NHGRI

Stable Isotope Breath Tests to Assess Metabolite Flux in Methylmalonic Acidemia (MMA)

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Poster

Elizabeth A. Harrington, Yiouli Ktena, Julien Senac, Irini Manoli, Charles P. Venditti

Isolated methylmalonic acidemia is caused primarily by a defect in the mitochondrial enzyme methylmalonyl-CoA mutase (MUT). Secondary mitochondrial dysfunction, characterized by aberrant ultrastructure and decreased complex IV activity, is observed in patient- and murine model-derived tissues. Oxidation of 13C-labeled propionate and substrates, such as methionine, a-ketoisocaproic acid and octanoate that depend on mitochondrial function, could prove useful as prognostic or outcome measures in this disease. We used mice that express the Mut gene under the control of a muscle-specific creatine kinase promoter (Mut-/-;TgINS-MCK-Mut). These mice are rescued from the neonatal lethality observed in the Mut-/- mice but develop a hepatorenal mitochondriopathy that resembles findings in MMA patients. Mut-/-;TgINS-MCK-Mut mice were injected intraperitoneally with 1-13C-isotopomers of sodium propionate, sodium acetate, sodium pyruvate, sodium octanoate, sodium a-ketoisocaproic acid, glycine, and L-methionine. Analysis of breath 13CO3 enrichment was made using isotope ratio mass spectroscopy and the cumulative percentage of total isotopomer dose metabolized was calculated. Mut-/-;TgINS-MCK-Mut mice displayed a decreased propionate, methionine and glycine but normal acetate, pyruvate, octanoate and a-ketoisocaproic acid oxidation. In addition, we performed 1-13C-propionate breathing studies in 3 controls and 9 patients with isolated MMA. Delayed release of label was observed in all MMA patients vs. controls, while liver transplant recipients showed improved oxidation rates.13C breath testing could prove helpful in determining the whole body in vivo MUT activity, identify a number of perturbation in secondary pathways and test the response to therapeutic interventions, such as B12 supplementation, transplantation or gene therapy for methylmalonic acidemia.

Aging and stem cells; what our gray hairs are telling us.

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Poster

Melissa Harris [1], William Pavan [1]

The median age of the world population is rising, and age-related degenerative disease is becoming the reality for much of our society. In adults, stem cells replenish tissues over time and are found in places like the skin and gut. As we age, our stem cells function less efficiently and in some cases are lost completely. We do not understand why stem cells fail and how much stem cell failure contributes to the aging process. My research focuses on using the melanocyte stem cells to address these questions.

Melanocyte stem cells exist within each hair follicle, and they produce the melanocytes that make the pigment for newly growing hairs. Gray hair is associated with age and is a result of melanocyte stem cell depletion. We are beginning to appreciate that this depletion is caused by the failure of melanocyte stem cells to renew and their inappropriate transformation into pigmented melanocytes. My research focuses on understanding how our genes and proteins coordinate this process. I am also exploring if all forms of hair graying (environmental and genetic) are caused by this same mechanism. And finally, using the melanocyte stem cells as my example, I am employing gene sequencing and mouse genetic screening technology to identify new genes that are important in protecting stem cells from age-related loss.

Continuing to understand how stem cells are made and maintained will open new avenues for investigating the role of stem cells in disease and aging and provide novel basis for regenerative research.

1. Genetic Disease Research Branch, NHGRI, NIH

Differences in the Genome-Wide Epigenetic Profiles of Mouse Erythroblasts and Megakaryocytes

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Poster

Elisabeth F. Heuston [1], Jens Lichtenberg [1], Stacie M. Anderson [2], Martha Kirby [2], Cheryl Keller Capone [3], Vikram Paralkar [4], Ross C. Hardison [3], Mitchell Weiss [4], David M. Bodine [1]

Hematopoietic differentiation occurs via tightly controlled epigenetic events that regulate lineage commitment and cell maturation, including DNA methylation and transcription factor binding. Our goal is to define the epigenetic events associated with terminal differentiation of primary erythrocytes (Erys) and megakaryocytes (Megs) using Methyl-Seq, ChIP-Seq, and RNA-Seq. We identified 100,000 distinct regions of methylation in Erys and Megs. Over 50% are located in intergenic regions, and the majority of the remainder are within gene bodies. In each cell type, approximately 50% of peaks are unique and 50% are shared between Ery and Meg. We also identified 9,287 sites of GATA1 occupancy in Erys compared with 1,355 peaks in Megs, and 5,980 peaks of NFE2 occupancy in Erys compared with 4,415 peaks in Megs. Approximately 13% of Ery GATA1 sites are found in Megs, and over 42% of Ery-specific GATA1 occupied sites are co-occupied by NFE2. In contrast, NFE2 occupancy is more pronounced in Megs compared to Erys. Of the 4,415 NFE2 peaks in Megs, 72% are Meg-specific. Less than 1% of Meg-specific NFE2 sites are co-occupied by GATA1, compared with 71% of Ery-specific NFE2 sites co-occupied by GATA1. Finally, we compared these profiles to RNA-Seq data. Within gene bodies, over 60% of sites occupied by GATA1 with or without NFE2 are also methylated in Erys. These genes are four times more likely to be than expressed. In contrast, NFE2 is most often associated with actively expressed genes in Megs, and over 75% of these genes also show gene body methylation.

1. Hematopoiesis Section, Genetics and Molecular Biology Branch

2. The NHGRI Flow Cytometry Core Facility

3. The Pennsylvania State University

4. Childrens Hospital of Philadelphia

Functional characterization of FBXW7 mutations in endometrial cancer

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Poster

Bo Hong [1], Meghan L. Rudd [1], Matthieu Le Gallo [1], Jessica Price [1], Suiyuan Zhang [2], Maria J. Merino [3], Daphne W. Bell [1]

Endometrial cancer (EC) is the eighth leading cause of cancer death amongst American women. We recently identified somatic mutations in the FBXW7 tumor suppressor gene in 29% of serous, 13% of clear cell, and 10% of endometrioid ECs. FBXW7 is a component of a ubiquitin ligase complex that mediates the proteasomal degradation of oncogenic proteins. FBXW7 mutations correlate with elevated levels of cyclin E in EC. The purpose of this study is to determine whether additional oncogenic proteins are deregulated by FBXW7 mutations in EC. We mined The Cancer Genome Atlas to determine the frequency and pattern of genomic alterations in FBXW7 and genes that encode FBXW7 substrates, in serous EC. We performed a mutational analysis of FBXW7 in 14 EC cell lines, using PCR and Sanger sequencing. The stability of FBXW7 substrates was measured by Western blotting in FBXW7-mutant and FBXW7-wildtype EC cell lines, following cyclohexamide treatment. MCL1, MYC and CCNE, which encode FBXW7 substrates, undergo putative genomic amplification in 20%, 23% and 23% of serous ECs, indicating that the dyregulation of MCL1, MYC and CCNE may be important in these tumors. In preliminary functional studies, we observed prolonged stabilization of c-Myc and cyclin E1, and possibly of MCL1, in FBXW7-mutant compared to FBXW7-wildtype endometrial cancer cell lines. In ongoing studies, we aim to determine whether the prolonged stabilization of these substrates is caused by the underlying endogenous FBXW7 mutations, and whether the FBXW7 hotspot mutations identified in EC result in dysregulation of MCL1, MYC, and cyclin E.

1. Reproductive Cancer Genetics Section, Cancer Genetics Branch, NHGRI, NIH

2. Genome Technology Branch, NHGRI, NIH

3. National Cancer Institute, NIH, Bethesda MD

Adaptation to Living with a BRCA1/2 mutation in previvors and their partners

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Poster

Gillian Hooker [1], Lori Erby [2], Bill Klein [3], Lindsay Hoskins [3]

The proposed study aims to describe adaptation and dyadic adjustment in unaffected BRCA1/2 carriers and their partners. It is not fully understood how women and their partners adapt to high-risk status over time, nor how different aspects of living at risk relate to this process. Neither psychological adaptation nor dyadic adjustment has been systematically measured in this population. This study is informed by Lazarus & Folkman's Transactional Model of Stress and Coping and modifications made to this model for use in studying dyadic relationships. A cross-sectional research design quantitatively explores the relationships between the appraisals and timing of risk-related stressors, dyadic coping, and the outcomes of individual adaptation and dyadic adjustment.

1. NHGRI

2. JHSPH

3. NCI

A genome-wide association study identifies susceptibility loci for non-syndromic sagittal craniosynostosis on chromosomes 20 and 7

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Poster

Cristina M. Justice [1], Blake Carrington [2], Garima Yagnik [3], Yoonhee Kim [1], Inga Peter [4], ICC [5], Ethylin W. Jabs [4], Jinoh Kim [3], Raman Sood [2], Simeon A. Boyadjiev [3], Alexander F. Wilson [1], et al.

Sagittal craniosynostosis is a common congenital malformation, affecting approximately one out of 5,000 newborns. We conducted the first genome-wide association study (GWAS) for non-syndromic sagittal craniosynostosis (sNSC) using 130 non-Hispanic white (NHW) case-parent trios. Highly significant associations were observed in a 120 kb region downstream of BMP2 on chromosome 20p.12.3, flanked by rs1884302 ($P \leq 1.13 \times 10\text{-}14$) and rs6140226 ($P \leq 3.4 \times 10\text{-}11$) and within a 167 kb region of BBS9 on chromosome 7p14.3 between rs10262453 ($P \leq 1.61 \times 10\text{-}10$) and rs17724206 ($P \leq 1.50 \times 10\text{-}8$). The associations for rs1884302 were replicated ($P \leq 4.39 \times 10\text{-}31$ and $P \leq 3.50 \times 10\text{-}14$, respectively) in an independent NHW population of 172 unrelated sNSC probands and 548 unaffected controls. After observing that a 715 bp fragment placed in front of the BMP2 promoter drove expression of Renilla luciferase, and that the fragment with the risk allele (C allele) resulted in increased expression, as compared to the reference allele (T allele), we performed zebrafish transgenesis by subcloning these two 715bp fragments into a ZED vector. Preliminary data using transient transgenesis suggested a difference in the pattern of GFP expression driven by the C versus T alleles. We are currently analyzing stable lines for both alleles.

1. Genometrics Section, IDRB, NHGRI

2. Zebrafish Core, GMBB, NHGRI

3. Department of Pediatrics, Section of Genetics, UC Davis

4. Department of Genetics and Genomic Sciences, Mount Sinai School of Medicine

5. International Craniosynostosis Consortium

Integration of Glycomics, Genomics and Cell Biology in a study of Joubert Syndrome with OFD1 mutation

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Poster

Megan Kane [1], Mariska Davids [1], Taylor Davis [1], Charles Markello [1], Miao He [2], William Gahl [1], Cornelius Boerkoel [1]

The National Institutes of Health Undiagnosed Diseases Program (NIH UDP) has begun an effort to integrate a multi-omics, agnostic approach to the study of rare and unknown diseases. The association of abnormal glycomics findings in patients with known diseases raises questions regarding the impact of glycosylation on disease pathogenesis or as a disease biomarker. One NIH UDP patient with abnormal glycosylation was found to have Joubert syndrome attributed to a genetic defect in OFD1. Analysis of N-linked glycosylation in the patient's blood indicated below-average levels of galactosylation and sialylation, suggestive of a defect of N-linked glycosylation in the Golgi. Abnormal glycosylation was also seen in cultured primary fibroblasts indicating that the abnormal glycosylation was a nonspecific marker for illness. OFD1 protein is a core component of the human centrosome and may play roles in both microtubule organization and primary ciliary function. It is conceivable that a defect in OFD1 may affect cellular protein transporting to Golgi through its effects on microtubule dynamics and a number of genes in vesicle trafficking are known cause glycosylation disorders in human. Therefore, we next transduced patient fibroblasts with a lentivirus expressing wild-type OFD1 to attempt to rescue the defect in glycosylation. These observations will be discussed in the context of their implications for how OFD1 mutations may impact Golgi function and protein glycosylation.

1. Undiagnosed Diseases Program, NHGRI, National Institutes of Health, Bethesda

2. The Michael J Palmieri Metabolic Laboratory, Children's Hospital of Philadelphia

Investigating the role of inducible T-cell kinase in CD8+ T-lymphocyte cytolytic effector function

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Poster

Senta Kapnick [1,2], Pamela Schwartzberg [1]

During the adaptive immune response, CD8+ cytotoxic T-lymphocytes (CTLs) are critical for eliminating virally infected cells, and defects in CTL responses are implicated in lymphoproliferative diseases. Patients with mutations in inducible T-cell kinase (Itk), a tyrosine kinase that serves as an amplifier of T-cell receptor (TCR) signaling, develop lymphoproliferative disorders associated with ineffective responses against Epstein-Barr virus (EBV), a virus that infects B-cells. This phenotype is similar to X-linked lymphoproliferative syndrome, in which mutations affecting the adaptor, SAP, lead to fatal EBV infections associated with specific defects in the cytolysis of B-cells, despite normal cytolysis of other targets. Unlike SAP-deficient CTLs, however, we found that Itk-deficient CTLs exhibit impaired cytotoxicity against multiple targets, suggesting that Itk-deficiency leads to global defects in cytolysis. To understand this phenotype, we examined how Itk-deficiency affects discrete stages of CTL function. Cytolysis by CTLs is initiated when the TCR triggers activation and adherence to targets, leading to centrosome polarization toward the target contact site, and release of secretory granules that induce cytolysis of targets. Although early events in killing, such as adhesion, actin ring formation, and polarization of lytic machinery were intact in Itk-deficient CTLs, we found defects in granule secretion suggesting that Itk may play a previously unappreciated role in the final stages of cytolytic effector function. Exploration of the role of Itk in CTL function will provide clues as to how early TCR signaling contributes to cytolytic activity, and why mutations in Itk lead to defective cytolysis and the development of lymphoproliferative disorders.

1. NHGRI

2. JHU

Essential role of G protein Gαs in regulating liver size

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Poster

Sanjoy Khan [1], Yingzi Yang [1]

The G protein Gαs is essential for G protein coupled receptor mediated cAMP production for intercellular signaling and is an important metabolic regulator. Previously, it has been shown that liver specific Gαs-signaling pathways play an important role in glucose and lipid metabolism. Using liver specific Gαs knock out (LGsKO) mouse model, it was shown that LGsKO mice had increased glucose tolerance and insulin sensitivity. Moreover, these mice also exhibit increase liver weight. Though the mechanism for glucose tolerance and insulin sensitivity has been described for these mice, the underlying mechanism for increased liver size remains elusive. Using this LGsKO model, our aim is to dissect the molecular signaling mechanisms governed by liver Gαs-signaling to regulate liver size. Our lab previously showed that, in skeletal system, Gαs plays a key role in bone formation by maintaining a proper balance between the Wnt–β-catenin and Hedgehog pathways. Here in this LGsKO model, our preliminary data indicate an upregulation of hedgehog signaling in the liver in absence of Gαs. Therefore, under normal physiological condition, Gαs may negatively regulate Hedgehog signaling in the liver to maintain proper liver size. Further studies will confirm the essential role of Gαs in regulating liver size in mammals.

1. GDRB, NHGRI, NIH

The Clinical Course of Patients with Gaucher Disease and Parkinsonism

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Poster

Jenny Kim [1], Catherine Groden [1], Edythe Wiggs [2], Ellen Sidransky [1], Grisel Lopez [1]

Mutations in the GBA gene, which encodes the enzyme glucocerebrosidase, result in Gaucher disease (GD), an autosomal recessive disorder characterized by glycolipid accumulation and lysosomal dysfunction. During the past decade, it was discovered that homozygosity and heterozygosity for GBA mutations is the most common known genetic risk factor for developing Parkinson disease (PD), a neurodegenerative disorder involving loss of dopaminergic neurons in the substantia nigra. In this study, we reviewed clinical data on seventeen patients with GD and parkinsonian manifestations to assess their PD course and outcome. Clinical data evaluated included laboratory data, family history, neurological evaluations, neurocognitive evaluations, olfactory testing, pulmonary function testing, echocardiograms, transcranial ultrasounds, positron emission tomography, magnetic resonance imaging, dual-energy X-ray absorptiometry, and other tests. Questionnaires regarding potential non-motor manifestations were also collected. Most of the subjects studied had longitudinal follow-up. Compared to published findings on the natural history of sporadic PD, we generally found that this cohort had a poorer clinical prognosis, including an earlier age of onset, faster disease progression, and more significant cognitive decline. However there was considerable variation from case to case. In comparing the clinical course of this cohort with patients with sporadic PD or the related disorder dementia with Lewy bodies (DLB), we hope to discern whether GBA mutations may serve as a predictor of a more aggressive form of parkinsonism. This could help to elucidate pathophysiological mechanisms underlying parkinsonism and potentially inform discussion about preventative treatments for PD.

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

2. Office of the Clinical Director, NINDS

Wnt/β-catenin signaling suppresses the positive feedback between YAP/TAZ and Notch pathway to restrain organ size and cancer development.

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Poster

Wantae Kim [1], Jelena Gvozdenovic-Jeremic [1], Sanjoy Kumar Khan [1], Ogyi Park [2], Bin Gao [2], Yingzi Yang [1]

Hippo signaling pathway has essential roles in embryonic development as well as tumor formation and is highly conserved from insect to mammal. Our previous studies showed that liver specific loss of Mst1 and Mst2 (Double Knockout, DKO), the mammalian homologues of the hippo kinase, results in tissue enlargement and hepatocellular carcinoma by activating the YAP/TAZ oncogenes. However, the underlying molecular mechanism still remains elusive. Cross-talks between Wnt and Hippo signaling has been reported, but it remains unclear how much the activation of Wnt/β-catenin signaling contributes to liver enlargement and tumorigenesis. Here, we have identified a novel regulatory mechanism that Wnt/β-catenin suppresses the positive feedback loop between YAP/TAZ and Notch signaling. YAP/TAZ up-regulates a Notch signaling ligand, Jagged, to activate the Notch pathway. As a result, NICD (Notch intracellular domain) promotes YAP/TAZ activation by stabilizing TAZ, but not YAP. NICD does so by regulating the interaction of TAZ with β-TrCP, which controls TAZ ubiquitination. Pharmacological inhibition of Notch signaling further supports the findings that Notch signaling suppression can mediate the liver defects of Mst1 and Mst2 double knockout mice. In contrast, loss of β-catenin in Mst1/2 DKO enhances the liver phenotype via inhibition of Numb, an inhibitor of Notch signaling. Therefore, β-catenin, a protein known for oncogenic properties, has an unexpected function in restricting YAP/TAZ activity during liver enlargement and cancer development. Taken together, these findings identify novel cross-talks between Hippo, Notch and Wnt/β-catenin pathways as well as new clinical insights for potential therapeutic targets for liver cancer treatment.

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Identification and characterization of biallelic pathogenic variants in 162 FANCA families

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Poster

Danielle Kimble [1], Elizabeth Flynn [1], Aparna Kamat [1], Siobhan Gregg [2], Uma Veturi [2], Supawat Thongthip [2], Erica Sanborn [2], Francis Lach [2], Frank Donovan [1], Elaine Ostrander [1], Arleen Auerbach [3], Agata Smogorzewska [2], Settara Chandrasekharappa [1]

Fanconi anemia (FA) is a rare recessive disorder resulting from pathogenic variants in one of at least 16 different genes. Variant type and phenotypic manifestations of FA are highly heterogeneous. We intend to identify the variants in ~300 patients from the International Fanconi Anemia Registry (IFAR) in order to gain a comprehensive understanding of genotype-phenotype correlations. Since ~60% of families harbor variants in FANCA, our initial effort was to screen for variants in this gene. We identified biallelic variants in 162 FANCA families through sequencing of the exons and adjacent intronic regions, as well as identified deletions through Comparative Genome Hybridization (CGH) analysis. Fifty-five novel FANCA variants were discovered. The 18 novel missense variants were analyzed for their pathogenicity by expressing them in a FANCA null cell line and measuring the survival of the cells with exposure to a low dose of MMC. Through immunofluorescence, we found that the FANCA protein resulting from the pathogenic variants, unlike those from the nonpathogenic variants, was localized to the cytoplasm. Confirmation of the pathogenicity of a synonymous variant, p.K522K, and a missense variant, p.Q174H, required analysis of RNA from cells harboring these variants. CGH revealed large deletions, and these deletions (81/324) accounted for 25% of the FANCA mutations. Twenty-four deletions extended beyond the 5′ of the gene and 10 beyond the 3′ end of the gene, with some of these eliminating neighboring gene(s).

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Developmental requirements of PLZF expressing innate-like CD4+ thymocytes in Itk-/- mice

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Poster

Zachary Kraus [1], Pamela Schwartzberg [1]

T lymphocytes develop in the thymus where immature T cells undergo selective processes to ensure a diverse and largely non-self reactive pool of mature T cells. These conventional T cells expand in response to foreign substances to protect against infection and develop immunological memory. Thymic selection also results in the development of small numbers of “innate-like” T cells that rapidly respond to T cell receptor (TCR) stimulation and secrete copious amounts of cytokines. Innate T cells can profoundly influence early immune responses, yet regulation of innate T lymphocyte development remains poorly understood. Studies of gene-targeted mice demonstrate that SAP, an adaptor that is mutated in X-linked Lymphoproliferative Syndrome and is required for signaling from SLAM family receptors, is required for innate T cell development. Interestingly, mice with certain defects in TCR signaling (eg. Itk-/- mice) develop more innate lymphocytes. We found that costimulation of thymocytes through the TCR plus the SLAM family member Ly108 potently enhances expression of PLZF, a transcription factor that drives the activated phenotypes of many innate CD4+ T cell populations. Indeed, global analyses of gene expression revealed that Zbtb16, (encoding PLZF), is one of the most highly induced genes in thymocytes which received Ly108 costimulation. Conversely, Ly108-deficient mice develop fewer PLZF+ T cells. In contrast, Itk-/- thymocytes respond to Ly108 costimulation, but fail to undergo TCR-induced negative selection, resulting in an increase in innate lymphocytes. Our results suggest that a balance between TCR and SLAM family receptor signals is critical for regulating innate T cell development.

1. NHGRI

Modeling FPD/AML with human iPSCs and identification of RUNX1 expression inducers in hematopoietic differentiation

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Poster

Erika Kwon [1], Jon Connelly [1,2], Yongxing Gao [3], Linzhao Cheng [3], Wei Zheng [4], Marshall Horwitz [5], Paul Liu [1]

Familial platelet disorder with propensity to acute myeloid leukemia (FPD/AML) is an autosomal dominant disorder caused by heterozygous germline mutations in RUNX1. FPD/AML patients have a bleeding disorder and a tendency to develop AML. Currently no suitable animal models exist to study FPD. We hypothesize that induced pluripotent stem cells (iPSCs) derived from patients can serve as a model for the disease. We derived iPSCs from two FPD patients and found that the FPD iPSCs display defects in megakaryocyte differentiation in vitro. We achieved gene targeting to correct the RUNX1 mutation in one FPD iPSC line, which led to normalization of megakaryopoiesis of the iPSCs. Our results demonstrate successful in vitro modeling of FPD with patient-specific iPSCs and confirm that RUNX1 mutations are responsible for megakaryocytic defects in FPD cells. Moreover, in order to make it possible to treat patients suffering from blood disorders using patient-specific hematopoietic stem cells (HSCs), there is a great need to establish efficient methods to differentiate human iPSCs to transplantable HSCs. We have developed human RUNX1 reporter iPSC lines in which either a Firefly or a Renilla luciferase cassette is targeted to the RUNX1 locus using ZFNs. We will verify that the RUNX1 reporters can be used to test and monitor HSC production from human iPSCs. We will then perform chemical library screening with the RUNX1 reporter iPSC lines to identify novel compounds that induce hematopoietic differentiation and further characterize their effects on the cultured iPSCs.

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Systematic identification of somatically mutated genes in endometrial malignant mixed Müllerian tumors using whole exome sequencing

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Poster

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Malignant mixed Müllerian tumors (MMMTs) of the endometrium account for less than 5% of uterine cancers. The 5-year survival rate for MMMTs is only 30-39%. They are mix of two histological components: sarcoma and carcinoma. The latter component is considered to be responsible for the aggressiveness of the tumor. To date, no comprehensive screens of the genetic alterations in MMMTs have been reported.
To identify somatic mutations that drive the development of MMMTs, we whole exome sequenced 14 primary MMMT tumor DNAs and the matched normal DNAs in a mutation discovery screen. The short sequence reads were aligned to a human reference sequence using NovoAlign and nucleotide variants were called using the MPV algorithm. Nonpathogenic SNPs and private variants were excluded from further analysis. We compared the variants called in tumors and matched normal exomes to identify somatic variant calls. Nonsynonymous and splice site variants were evaluated in paired tumor-normal DNAs, using Sanger sequencing, to confirm high-confidence somatic mutations.
We identified 507 somatic nonsynonymous and splice site mutations in 470 protein-encoding genes. We selected 17 genes for a mutation prevalence screen, based on their mutation frequency in MMMTs, and/or the fact that they are altered in other histological subtypes of endometrial carcinoma. We are currently sequencing the coding exons of these 17 candidate driver genes in an additional set of 80 MMMTs to determine the frequency of mutations and whether they are statistically significantly mutated above the background mutation rate, indicating potential pathogenic driver genes for MMMTs.

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SHPRH is recruited to the nucleoli in an mTOR-dependent manner and regulates rRNA transcription by recognizing histone code

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Poster

Deokjae Lee [1], Hungjiun Liaw [2], Roger Woodgate [3], Kyungjae Myung [1]

Several DNA repair proteins have an additional function besides their role in DNA repair. In addition to its role in DNA repair by catalyzing PCNA polyubiquitylation responding to the stalling of DNA replication, we found that SHPRH facilitates rRNA transcription in the nucleoli. SHPRH was localized in the nucleoli and enriched at the ribosomal DNA (rDNA) promoter, which is dependent on the Plant Homeo Domain (PHD) of SHPRH. The PHD of SHPRH bound to various modified histone H3 except when the fourth lysine residue of H3 was tri-methylated, which was induced upon starvation. Consistently, SHPRH enrichment at the rDNA promoter was inhibited when cells were treated with rapamycin that blocks the mTOR pathway or starvation. Facilitation of rDNA transcription by SHPRH was mediated through physical interaction between SHPRH and RNA polymerase I complex. Taken together, we provide the first evidence that SHPRH functions in rRNA transcription through its direct interaction with histone H3 and RNA polymerase I, which requires the active mTOR pathway.

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A germline polymorphism in Necdin causes changes in gene expression and mammary tumor metastasis

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Poster

Minnkyong Lee [1], Sarah Beggs [1,3], Jens Lichtenberg [1], Sujata Bupp [1], Niraj Trivedi [2], Nigel Crawford [1]

Necdin (Ndn) is a transcription factor that regulates gene expression by cooperating with chromatin modifying factors. In humans, NDN is a paternally imprinted gene which is encompassed by chromosome 15q11-q13, the region deleted in Prader-Willi Syndrome. Earlier work using mouse models of mammary tumorigenesis implicated Ndn as a metastasis efficiency modifier that exerts its influence at the germline level. Differential Ndn expression is associated with the dysregulation of extracellular matrix genes, which display abnormal expression in highly metastatic tumors.

Mouse Ndn contains a non-synonymous coding germline single-nucleotide polymorphism (SNP) (T50C; V17A) that distinguishes metastasis-prone AKR/J mice from metastasis-resistant DBA/2J mice. We hypothesize that Ndn induces the observed prognostic changes in gene expression through direct transcriptional regulation, and that the differences in metastasis efficiency between the AKR/J and DBA/2J mouse strains is in part due to the V17A Ndn SNP. Over-expression of either the wildtype 17V or the variant 17A Ndn alleles in highly metastatic mouse mammary tumor cells demonstrated that the variant 17A allele is associated with increased cell invasiveness in vitro, as well as increased pulmonary metastases in animal studies. Furthermore, microarray analyses demonstrated that substantial differences in gene expression exist between cells expressing the two different Ndn allelic variants.

These data collectively demonstrate that Ndn functions as a metastasis modifier, most likely by regulating gene expression, and that the V17A SNP plays a significant role in this process. We are currently examining this by analyzing ChIP-seq data generated from cells overexpressing either one of the Ndn SNPs.

1. Genetics and Molecular Biology Branch, NIH/NHGRI

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3. NIH Summer Internship Program

Sialylation of Thomsen-Friedenreich antigen is a noninvasive blood-based biomarker for GNE myopathy

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Poster

Petcharat Leoyklang [1], May Christin Malicdan [1], Frank Celeste [2], Carla Ciccone [1], William Gahl [1], Miao He [3], Nuria Carrillo-Carrasco [2], Marjan Huizing [1]

GNE myopathy is an adult-onset progressive myopathy resulting from mutations in GNE. The pathomechanism of GNE myopathy likely involves aberrant sialylation, since administration of sialic acid itself or its precursor, N-acetylmannosamine (ManNAc), rescued the hyposialylation of GNE myopathy mice. Recently, clinical trials for GNE myopathy patients were initiated. A robust, noninvasive biomarker is highly desirable for diagnosis of GNE myopathy and for evaluating response to therapy. Since GNE myopathy muscle biopsies demonstrated hyposialylation of predominantly O-linked glycans, we analyzed the O-linked glycome of patients' plasma proteins using mass spectrometry. Most patients showed increased plasma levels of the O-linked Thomsen-Friedenreich (T)-antigen and/or decreased amounts of its sialylated form, ST-antigen. Moreover, every patient we analyzed had an increased ratio of T-antigen to ST-antigen when compared to unaffected individuals. Importantly, the T/ST ratios were normalized in GNE myopathy patients treated with intravenous immunoglobulin (which are highly sialylated proteins) or with self-administered ManNAc (off-label use), indicating response to therapy. Natural history and clinical trial data will reveal whether T/ST ratios correlate with clinical outcomes. These findings not only highlight plasma T/ST ratios as a robust blood-based biomarker for GNE myopathy, but also help explain the pathology and adult onset of the disease. Specifically, a shortage of total sialic acid occurs in GNE myopathy, likely later in life. Some glycans (N-linked) may be preferably sialylated over others (O-linked). Proteins with significant O-linked glycosylation will predominantly be affected and contribute to the phenotype, many aspects of which remain to be identified.

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Development of next-generation sequencing analysis tools and databases to study hematopoiesis

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Poster

Jens Lichtenberg [1], Ross Hardison [2], Mitchell Weiss [3], David Bodine [1]

Our goal is to develop a model for how gene regulatory processes facilitate different mature blood cell types. Previous research into the formation and maturation of blood cells has generated large expression and epigenetic datasets for multiple blood cell types in a variety of species. The study of regulatory profiles is highly dependent on the discovery of enriched expression and epigenetic profiles with a high degree of confidence.

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The genomic landscape of lung adenocarcinomas in individuals without significant exposure to tobacco

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Poster

William Lockwood [1], Kelsie Thu [2], National Intramural Sequencing Center [3], Jim Mullikin [3], Stephen Lam [2], Wan Lam [2], Harold Varmus [1]

A significant fraction of lung adenocarcinomas (LAC) in never smokers (NS) are not associated with mutations in known oncogenic driver genes. In addition, mutations in these genes appear to be insufficient for tumorigenesis, suggesting that additional alterations are required. To address these issues, we have comprehensively studied a panel of 15 LACs from NS - seven "triple negative" tumors (with normal EGFR, ALK, and KRAS genes) and eight EGFR mutant tumors - with the goal of identifying novel mutant genes in these subsets. Whole-exome sequencing revealed a median of 46 coding mutations/tumor with a median mutation density of 2.69 mutations/Mb. To identify mutated genes that confer a selective growth advantage we used a multistep approach incorporating the SMG function in Genomic MuSIC. 32 unique genes demonstrated significant evidence of conferring a selective advantage, including known oncogenes (EGFR, ERBB2 and MET) and tumor suppressor genes (p53, RB1 and ATM). In addition, sequencing of tumor RNA revealed fusions involving RET or ROS1 in two tumors. As a result, 14/15 tumors displayed an alteration in a known oncogene, with no more than one such mutation in any tumor. The variations in MET consisted of truncating and splice-site mutations and have not been previously characterized in this context. We also observed mutation, hemizygous and homozygous loss of multiple genes from the chromosome arm 6q. Pathway analysis indicated frequent mutation in genes implicated in PI3-kinase signaling, RNA splicing and histone modification. Together, this work will expand our understanding of LACs arising in NS.

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Difficulty and Positive Growth in Alzheimer's Disease Caregiving Networks

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Poster

Amanda Ludden [1], Sato Ashida [2], Christopher Marcum [1], Laura Koehly [1]

Using a Network Approach to Identify Missed Opportunities in Caregiving Networks

Alzheimer's disease and related dementia (ADRD) affects 5.2 million people, with 1% to 5% of cases (52,000–260,000) attributed to genetic factors. These people increasingly receive care from informal caregivers, such as spouses and adult children, in addition to formal, institutional caregivers. Previous research has generally relied on one caregiver (an index informant) to describe their role and other family caregivers' (alters) roles. This single informant approach may be limited by participant bias and the inability to capture the flow information, such as genetic risk or support resources, which can affect the quality of care provided to patients.

Using data from 24 networks of caregivers to ADRD patients, we demonstrate the added value to caregiving research by using a multiple informant social network approach. On average, each additional informant beyond the index added 6.2 new members, resulting in about 10 new members per family network. Among these new members were a significant proportion of people who provide direct care (85% increase) and decision making support (48% increase) to the ADRD patient. In addition to identifying these other integral network members, this approach also allows for the comparison of informants' reports about caregiving contributions. For families affected by conditions like ADRD which have genetic risk factors , the information gained from the multiple informant approach can inform future interventions and facilitate adaptation.

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The effect of N-acetylmannosamine on Adriamycin-induced podocytopathy

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Poster

May Christine V. Malicdan [1], Veeraya Tanawattanacharoen [2], Shashi Shrivastav Shrivastav [2], Taichi Murakami [2], Petcharat Leoyklang [1], Avi Rosenberg [3], Marjan Huizing [2], Jeffrey B. Kopp [1]

The epidemic of chronic kidney disease (CKD) and end-stage renal failure represents a crisis for healthcare world-wide. More than 10% of people, or more than 20 million, aged 20 years or older in the United States have CKD. The most common forms of chronic kidney disease (CKD) are diabetic kidney disease, hypertensive kidney disease, and glomerulonephritis. . Given the presence of unwanted side effects of the current treatment modality consisting of corticosteroids, in addition to high morbidity of dialysis, its cost and the shortage of donor kidneys, there is an urgent need for further therapeutic options. We have studied a novel therapy for one form of glomerulonephritis, focal segmental glomerulosclerosis (FSGS). We used an established mouse model of FSGS, in which a single dose of doxorubicin (Adriamycin) damages the glomerular podocytes. Prior work, in part by our group, has shown that podocyte proteins in FSGS patients and animal models are hyposialyted. We hypothesized that N-acetylmannosamine (ManNAc), a precursor to sialic acid, might prevent or repair hyposialyation of glomerular proteins. We found that ManNAc, administered orally, can prevent and also can ameliorate Adriamycin-induced proteinuria and FSGS. Since ManNAc appears to have minimal toxicity and is orally administered, this may be a promising therapy for FSGS. ManNAc is currently tested in Phase 1 clinical trial for the treatment of the rare hyposialylation disorder GNE myopathy, and could be repurposed for patients with glomerular hyposialylation.

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Informing model organism selection for human disease research through evolutionary profiling

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Poster

Evan Maxwell [1,2], Christine Schnitzler [1], Paul Havlak [3], Nicholas Putnam [3], Anh-Dao Nguyen [1], Travis Moreland [1], Andreas Baxevanis [1]

While the standardization of methods for studying human diseases in traditional animal models has yielded many clinically actionable results, it has effectively narrowed the breadth of species in which we look for insights. The recent expansion of whole-genome sequence data available from diverse animal lineages provides an opportunity to investigate the feasibility of using non-traditional model organisms to advance human disease research. Cases in which traditional animal models have led to conclusions that are not applicable to humans are becoming more commonplace, calling for a re-evaluation of how appropriate models are selected. To that end, we have used a comparative genomics approach encompassing a broad phylogenetic range of animals to determine which could serve as viable models for studying various classes of human diseases. We show that some non-bilaterians have surprisingly high proportions of human disease gene homologs despite their great evolutionary distance from humans; these organisms may confer advantages as animal models in terms of their ease of use, short generation times and cost-effectiveness. Conversely, while previous evolutionary surveys have shown that most genes implicated in the causation of human diseases are of ancient origin, our results demonstrate that some disease classes involve a significantly large proportion of genes that emerged relatively recently within the Metazoa. These disease classes, having a more recent evolutionary history, may be difficult to replicate phenotypically outside of our closest animal relatives. Taken together, these findings emphasize why model organism selection should be done on a disease-by-disease basis, with evolutionary profiles in mind.

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Genomics of CpG Methylation in Developing and Developed Zebrafish

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Poster

David McGaughey [1], Hatice Ozel Abaan [1], Ryan Miller [1], Peter Kropp [1], Lawrence Brody [1]

DNA methylation is a dynamic process through which specific chromatin modifications can be stably transmitted from parent to daughter cells. A large body of work has suggested that DNA methylation influences gene expression by silencing gene promoters. However, these conclusions were drawn from data focused mostly on promoter regions. With regards to the entire genome, it is unclear how methylation and gene transcription patterns are related during vertebrate development. To identify the genome-wide distribution of CpG methylation we created series of high-resolution methylome maps of Danio rerio embryos during development and in mature, differentiated tissues. We find that embryonic and terminal tissues have unique methylation signatures in CpG islands and repetitive sequences. Fully differentiated tissues have increased CpG and LTR methylation and decreased SINE methylation relative to embryonic tissues. Unsupervised clustering analyses reveal that the embryonic and terminal tissues can be classified solely by their methylation patterning. Novel analyses also identify a previously undescribed genome-wide exon methylation signature. We also compared whole genome methylation with genome-wide mRNA expression levels using publicly available RNA-seq datasets. These comparisons revealed previously unrecognized relationships between gene-expression, alternative splicing and exon methylation. Surprisingly, we find that exonic methylation is a better predictor of mRNA expression level than promoter methylation. We also found that transcriptionally skipped exons have significantly less methylation than retained exons. Our integrative analyses reveal highly complex interplay between gene expression, alternative splicing, development, and methylation patterning in zebrafish.

1. Molecular Pathogenesis Section, Genome Technology Branch

Obstetric and Gynecologic Issues in patients with Chediak-Higashi Disease

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Poster

Melissa A. Merideth [1], Vikas Bhambhani [2], Catherine Groden [2], Camilo Toro [2], Wendy J. Introne [2]

Objective: To evaluate ob/gyn issues in women with Chediak-Higashi disease.

Background: Chediak-Higashi disease is an autosomal recessive condition with partial albinism, bleeding diathesis, immunodeficiency, and hemophagocytic lymphohistiocytosis. Without BMT mortality is high within the first decade of life. Little information is available about ob/gyn issues in CHD despite risks associated with immunodeficiency/bleeding.

Methods: 4 females with CHD were evaluated at the NIH Clinical Center. Testing included history, laboratory testing, review of records, physical examination and pelvic ultrasound.

Results: The patients ranged in age from 21-43 years. Half report monthly menses; half less frequent periods; 2 report heavy periods, 1 improved with ocp's. No patients had recurrent vaginal or pelvic infections. The only STD reported was HPV in 1 patient who also had cervical dysplasia. Gynecologic procedures included tubal ligation in 1 patient and LEEP in 1 patient with no abnormal bleeding. Two patients have been pregnant with a total of 3 pregnancies. One pregnancy was complicated by gestational hypertension and resulted in a vaginal delivery and retained placenta; the others were uncomplicated and resulted in vaginal deliveries without excessive bleeding.

Conclusions: There is a paucity of information in the literature about ob/gyn issues in CHD; patients and physicians often have questions about risk for vaginal/pelvic infections and menorrhagia given the immunodeficiency and bleeding diathesis. Questions also arise regarding fertility. Gaining a better understanding of the range of ob/gyn problems in CHD will expand the phenotype and is the first step toward developing ob/gyn therapeutic strategies for this patient population.

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Hyposialylation in glomerulopathies is mitigated by N-acetylmannosamine therapy

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Poster

Luhe Mian [1], May Christine Malicdan [1], Obiageri Okafor [1], Petcharat Leoyklang [1], T Yardeni [1,2], MF Starost [3], PM Zerfas [3], Y Anikster [2], A Volkov [4], B Dekel [5], JB Kopp [6], William A. Gahl [1], Marjan Huizing [1]

Biallelic mutations in murine Gne, coding for UDP-GlcNAc 2-epimerase/ManNAc kinase, the key enzyme in sialic acid biosynthesis, result in glomerular disease with podocyte effacement due to hyposialylation. We showed that oral supplementation with the sialic acid precursor N-acetylmannosamine (ManNAc) ameliorated the proteinuria and improved the podocyte foot process architecture and glomerular sialylation of mice. A panel of fluorescent-labeled lectins applied to kidney sections, indicated aberrant sialylation of predominantly O-linked glomerular glycans in mutant mice kidneys; this normalized after ManNAc treatment.

Since hyposialylation has sporadically been suggested in human glomerulopathies, we applied the lectin panel to renal tissue sections from patients with glomerulopathies. An unexpectedly high number of biopsies had glomerular hyposialylation similar to our mouse model, indicating that this condition may occur relatively frequently, and also that ManNAc may be a therapy.

To gather more preclinical data, we induced podocyte hyposialylation in mice by intraperitoneal injection of sialidase, removing sialic acids from glycans. Sialidase-injected mice developed proteinuria and renal failure in a dose-dependent manner. Their glomerular glycoproteins were hyposialylated and their podocytes were effaced, similar to our Gne knock-in mouse model. Importantly, oral prophylaxis and treatment with ManNAc significantly reduced their proteinuria and podocyte injury.

Although the exact mechanisms of glomerular hyposialylation requires further study, oral ManNAc therapy could benefit patients with glomerular hyposialylation. Moreover, ManNAc is currently being tested in a Phase 1 clinical trial for the treatment of the rare hyposialylation disorder GNE myopathy; it could be repurposed for trials in patients with glomerular hyposialylation.

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The NHGRI Microarray Core

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Poster

Weiwei Wu [1], Abdel Elkahloun [1]

The NHGRI Microarray Core (MAC) was established on 2000. 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 and epigenetics. Most investigator project requests are “Full Service”, in which the investigator will provide the chips, labeling kit and isolated RNA/DNA samples, and the core performs labeling, hybridization and data extraction. Upon project completion the investigator is then provided with a summary report including the data quality assessment, preliminary analysis and raw data output files. The core serves basic science, translational and clinical researchers and provides hands on training/education to investigators, postdoctoral fellows and students as well as consultation on experimental design.

The core support analysis on all commercial microarray platforms to offer a broad range of products and services, including whole genome gene expression, genotyping (SNP), epigenetics (DNA methylation, histone status), copy number variation (CNV, LOH and CGH), non-coding and microRNA analysis. The core also services protein array profiling.

Since its conception, the core has processed tens of thousands RNA/DNA samples thus building experience and expertise in bringing accuracy and consistency to our genomics services through rigorous standardization of protocols and multiple quality control checks.

1. NHGRI-CGB

Impaired autophagy leads to inflammasome activation and IL-1β secretion in gaucher macrophages

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Poster

Nima Moaven [1], Elma Aflaki [1], Barbara Stubblefield [1], Grisel Lopez [1], Ellen Sidransky [1]

Gaucher disease (GD) is caused by mutations in the gene GBA1, which codes for glucocerebrosidase (GCase). GCase breaks Glucosylceramide (GlcCer) to glucose and ceramide. GlcCer is essential for production of glycosphingolipids and regulation of ceramide in cellular level. While in GD GCase is deficient in all cell types, GlcCer accumulates within macrophages leading to "Gaucher cells," loaded with lipid-engorged lysosomes.

Autophagy and inflammasome are cellular processes with roles in innate immunity. While autophagosomes transport cytoplasmic components to lysosomes for degradation, inflammasomes are intracellular multiprotein complexes triggered by infection or stress, which regulate the activity of caspase-1 and maturation of IL-1β and IL-18. We investigated autophagy in macrophages derived from peripheral monocytes from ten type 1 GD patients (G-Macs) and from two iPSC-derived Gaucher macrophage lines.

Levels of both LC3-II and P62 were increased in G-Macs during erythrophagocytosis and inflammation. Increased P62 expression in these macrophages indicated impaired autophagy. Moreover, the expression of VPS34 and Rab7, proteins important in the fusion of early endosomes to late endosomes, was significantly lower in G-Macs as compared to control macrophages. Importantly, G-Macs show increased expression of IL-18 and IL-10, but decreased IL-1β expression.

Our data suggest that impaired secretion of some inflammatory cytokines results from the suppression of autophagy, leading to increased Atg16L expression in G-Macs. This inhibits the secretion and cleavage of IL-1β in these cells in the presence of LPS and ATP. We concluded that in G-Macs lysosomal dysfunction leads to the suppression of autophagy, followed by the inhibition of inflammasome activity.

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The Clinical Genome Resource (ClinGen): Annotating Sequence Variants for Use in Clinical Care

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Poster

Preetha Nandi [1], Yekaterina Vaydylevich [1], Lisa Brooks [2], Teri Manolio [1], Jeffery Schloss [2], Erin Ramos [1]

Medical and research centers are increasingly sequencing exomes or whole genomes of patients. However, identifying sequence variants relevant to disease is difficult. As a result, information on few genomic variants is used in clinical practice. One factor that limits the clinical use of variant information is the lack of an openly accessible knowledge base that captures genetic variants, their phenotypic and functional effects, and other clinical information. The Clinical Genome Resource (ClinGen) aims to collect phenotypic and clinical information on variants across the genome, develop a consensus approach to identify clinically relevant genetic variants, and disseminate information about the variants to researchers and clinicians.

ClinGen investigators are collecting sequence variants and related data from clinical laboratories and locus-specific databases and depositing these data into the central database ClinVar at NCBI. ClinGen working groups are curating these variants with additional clinical and functional data, and are developing methods to identify clinically relevant genetic variants that could be added to clinical care. The first working groups will focus on germline cancer, cardiovascular disease, and inborn errors of metabolism. As a central resource, ClinGen will provide comprehensive information about variants to reduce duplicative efforts of identifying clinically relevant variants. Additionally, collaboration with professional organizations will be critical to expanding the usefulness of this resource and for them to develop clinical practice guidelines. This resource is essential to advance the goals of implementing genomics in clinical care and will improve the understanding of phenotypic and functional effects of genetic variants and their clinical value.

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The NIH Intramural Sequencing Center (NISC)

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Poster

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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. NIH Intramural Sequencing Center/NHGRI/NIH

Genetic Analysis of Diamond-Blackfan Anemia

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Poster

Kelly O'Brien [1], Nancy Seidel [1], Jason Farrar [2], Adrianna Vlachos [3,4], NIH Intramural Sequencing Center [5], Abdel Elkahloun [1], Stacie Anderson [1], Xiuli An [6], Eva Atsidaftos [3], Harry Malech [7], Steven Ellis [8], Jeffrey Lipton [3,4], David Bodine [1]

Diamond-Blackfan anemia (DBA) is a rare, congenital bone marrow failure syndrome characterized by impaired erythropoiesis. Approximately 65% of DBA patients are heterozygous for mutations in ~ 13 ribosomal protein (RP) genes of either the large (RPL) or small (RPS) subunits. The genes responsible for the remaining ~35% of DBA patients are unidentified. To help identify these, we performed whole exome sequencing on DBA families who had no known mutations for DBA and no copy number variants and identified missense mutations in Mini Chromosome Maintenance Complex Component 2 (MCM2) and Polymerase RNA III beta subunit (POLR3B) in one family, Filamin B (FLNB) gene in one family, and SEMA7a in one family. Knockdown of MCM2 and FLNB resulted in significant reductions in the number of CD41-/CD235+ erythroid cells, indicating these genes play important roles in erythropoiesis. Furthermore, when MCM2 shRNA transduced CD34+ progenitor cells were plated in semi-solid medium, CFU-GM colony numbers were normal, while BFU-E colony formation was significantly reduced, suggesting an erythroid-specific role for MCM2. In conclusion, we have identified mutations in the non-ribosomal protein genes MCM2 and FLNB in patients with DBA and demonstrated an important role for these gene products in erythropoiesis. These findings would represent the first autosomal recessive mutations identified in DBA patients.

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Electronic Medical Records and Genomics Network - From GWAS to Implementation

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Poster

Jacqueline Odgis[1]

The eMERGE (Electronic Medical Records and Genomics) Network is a consortium funded by the National Human Genome Research Institute to investigate the use of electronic medical records (EMRs) and biorepositories in genomic research (Phase I, 2007-2011) and the incorporation of genomic variants into EMRs for use in clinical care (Phase II, 2011-2014). Phase I sites conducted genome-wide association studies (GWAs) for 13 phenotypes on ~19,000 genotyped participants. The Network published GWAs on LDL cholesterol, monocyte count, red blood cell traits, type 2 diabetes, white blood cell count, and hypothyroidism. These Network GWAs identified 12 novel loci and replicated 16 loci. In Phase II, the Network plans to conduct GWAs for 24 additional phenotypes on a total of ~87,000 genotyped participants. Goals in Phase II include defining standards for clinical validity and actionability, sharing executable phenotype definitions, creating standards for integrating CLIA certified results into EMRs, representing linked genotype-phenotype data in EMRs, and consulting with health care practitioners and patients about return of results. Implementation projects include genomic risk scores, measures of clinical utility and physician uptake of alerts, and a Network pilot study implementing specific pharmacogenomic variants in EMRs. eMERGE's collaborations within and outside the Network and its large, diverse group of genotyped participants linked to EMRs make it a suitable arena to test the feasibility of using genomic data in clinical care.

1. National Human Genome Research Institute, NIH

The altered landscape of the human skin microbiome in patients with primary immunodeficiencies

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Poster

Julia Oh [1], Alexandra Freeman [2], NISC [3], Morgan Park [3], Robert Sokolic [1], Steven Holland [2], Julia Segre [1], Heidi Kong [4]

While landmark studies have shown that microbiota activate and educate host immunity, how immune systems shape microbiomes and contribute to disease is incompletely characterized. Primary immunodeficiency (PID) patients suffer recurrent microbial infections, providing a unique opportunity to address this issue. To investigate the influence of host immunity on the skin microbiome, we examined skin microbiomes in patients with rare monogenic PIDs: hyper-IgE (STAT3-deficient), Wiskott-Aldrich, and dedicator of cytokinesis 8 syndromes. While specific immunologic defects differ, a shared hallmark is atopic dermatitis (AD)–like eczema. We compared bacterial and fungal skin microbiomes (41 PID, 13 AD, 49 healthy controls) at four clinically relevant sites representing the major skin microenvironments. PID skin displayed increased ecological permissiveness with altered population structures, decreased site specificity and temporal stability, and colonization with microbial species not observed in controls, including Clostridium species and Serratia marcescens. Elevated fungal diversity and increased representation of opportunistic fungi (Candida, Aspergillus) supported increased PID skin permissiveness, suggesting that skin may serve as a reservoir for the recurrent fungal infections observed in these patients. The overarching theme of increased ecological permissiveness in PID skin was counterbalanced by the maintenance of a phylum barrier; colonization remained restricted to typical human-associated phyla. Clinical parameters, including markers of disease severity, were positively correlated with prevalence of Staphylococcus, Corynebacterium, and other less abundant taxa. This study examines differences in microbial colonization and community stability in PID skin and informs our understanding of host–microbiome interactions, suggesting a bidirectional dialogue between skin commensals and the host organism.

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The NLRP3 Inflammasome is Regulated by Cofilin-1.

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Poster

Yong Hwan Park [1], Robert Kim [1], Daniel Kastner [1], Jae Jin Chae [1]

NLRP3 has a pivotal role in nucleating inflammasome, cytoplasmic multiprotein complexes that mediate the maturation of the proinflammatory cytokines interleukin-1β (IL-1b) by activating caspase-1. Mutations in the gene encoding NLRP3 cause a series of autoinflammatory disease, cryopyrin-associated periodic syndromes (CAPS). It has been reported that generation of reactive oxygen species (ROS) is one of the major NLRP3 inflammasome activating factor. However, the molecular mechanism of relationship between change of cellular redox state and NLRP3 inflammasome activation has not been elucidated. Here we show that cofilin-1, a redox sensitive actin binding protein, is involved in NLRP3 inflammasome activation. Cofilin-1 directly interacts to the nucleotide-binding domain (NBD) of NLRP3 protein in LPS-primed bone marrow derived macrophages (BMDMs). However, when the cells are stimulated with NLRP3 inflammasome activators, ATP or nigericin, cofilin-1 is dissociated from NLRP3 in a ROS-sensitive manner. Indeed, the interaction of cofilin-1 with NLRP3 is increased significantly when the oxidation sites of cofilin-1 are substituted from cysteine to alanine. These findings suggest that cofilin-1 is a key component in regulating the NLRP3 inflammasome in respond to ROS.

1. Inflammatory Disease Section, Medical Genetics Branch, NHGRI

Bisulfite conversion and sequencing of amplicons containing the ZNF154 promoter region from cell lines and tumor samples.

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Poster

Hanna Petrykowska [1], Francisco Sanchez-Vega [1], Gennady Margolin [1], Laura Elnitski [1]

Aberrant DNA methylation is a widespread phenomenon known to affect gene regulation andexpression. Next-generation sequencing of PCR amplicons from bisulfite treated DNA has emerged as a gold standard to assess the methylation status of target genomic regions. Technical considerations such as the choice of adequate primers need to be carefully evaluated to ensure reproducible results. We used bisulfite sequencing to analyze the methylation status of a 302 bp region at the promoter of gene ZNF154. This region was selected from a comprehensive analysis of TCGA, ENCODE and our own in-house samples. We developed a protocol for the amplification of target regions that includes PCR with sequencing adapters, bar coding and product cleanup. We applied this protocol to both cultured cells and solid tumor samples from cancer patients. Our amplicons extended the two CpG dinucleotides interrogated by probes in the Illumina 27K platform and included 18 additional, contiguous CpG locations. Methylation levels were higher in K562 cells than in GM12878 cells, consistent with results from Illumina arrays. We tested methylation kits to convert DNA into bis-DNA from several manufacturers and found important inconsistencies affecting some kits. Bisulfite sequencing reliably produced results that were consistent with those obtained with arrays while allowing analysis of methylation status over wider specific genomic regions. This can help to elucidate cis-regulatory mechanisms that play a role in locus specific methylation. Quantitative measurements of methylation at the level of methylated reads per sample can also lead to novel applications in clinical diagnosis.

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Genome sequence and initial analysis of malaria model Plasmodium coatneyi

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Poster

Arjun Prasad [1], Robert Morrison [2], NISC Comparative Sequencing Program [3], Patrick E. Duffy [4], James C. Mullikin [1,3]

Plasmodium falciparum malaria is the most deadly form of malaria, and infection leads to dangerous and often fatal complications such as cerebral malaria and severe anemia. P. falciparum is phenotypically distinct from other malarias that infect humans, in that it is highly sequestering, a process by which infected blood cells stick to the walls of small blood vessels. This sequestration may be responsible for the particularly severe complications of P. falciparum infection. Non-human models for sequestration are limited, and the most commonly used rodent models have some differences that have motivated the search for other models. A particularly promising model is the very strongly sequestering Plasmodium coatneyi infecting macaques. To help develop this model we sequenced, assembled, annotated, and analyzed the genome of P. coatneyi, demonstrating that it expresses a large and diverse repertoire of the host antigen switching SICAvar genes previously only known in P. knowlesi, and thought to be analogous to the PfEMP1 genes of P. falciparum involved in sequestration. The P. coatneyi genome also contains an expanded repertoire the Plasmodium interspersed repeat (PIR) genes that are also exported to the surface of infected cells. Phylogenomic analyses of 1,046 single-copy genes from all publicly available sequenced Plasmodium genomes and the related apicomplexan parasites Toxoplasma gondii and Babesia babis reveals very strong support for the sister relationship between Laverania (including P. falciparum) with the bird and rodent infecting malarias to the exclusion of the other primate infecting malarias (e.g., P. vivax and P. coatneyi).

1. Genome Technology Branch

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Imputation of Turkish Population Genotypes Using Immunochip Data and 1000 Genomes Reference Reveals Behçet's Disease Association of SNPs in the EGR2 Locus

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Poster

Elaine F Remmers [1], Masaki Takeuchi [1], Duran Ustek [2], Nobuhisa Mizuki [3], Ilknur Tugal-Tutkun [4], Emire Seyahi [5], Yilmaz Özyazgan [5], Ahmet Gül [4], Daniel L Kastner [1]

Behçet's disease is a genetically complex inflammatory disease with frequent occurrence of orogenital ulcers, uveitis, and skin lesions. The disease is common among modern-day populations who live along the ancient silk trade routes and is a leading cause of blindness in these countries. We recently identified disease-associated SNPs that exceeded genome-wide significance at genomic regions encompassing the major histocompatibility complex, IL10, IL23R, CCR1, STAT4, ERAP1, and the natural killer complex gene cluster on chromosome 12p13.2. We also found that low frequency and rare variants in TLR4 and MEFV contribute to disease susceptibility. In this study we genotyped 2014 cases and 1826 controls from Turkey using the Illumina Immunochip, which provides dense coverage for 186 genes found by GWAS in 12 autoimmune diseases and light coverage of additional immune disease candidate genes. We found a chromosome 10 region encompassing ZNF365, ADO, and EGR2 that contained many SNPs with near genome-wide significance. We therefore used IMPUTE2 to impute additional SNP genotypes with the 1000 Genomes reference haplotypes, revealing two disease-associated SNPs with $P < 5 \times 10\text{-}8$. These SNPs clustered 5′ of the EGR2 gene (early growth response 2), which encodes a transcription factor, E3 SUMO-protein ligase. Defects in this gene are responsible for several Charcot-Marie-Tooth disease types and are associated with increased risk of Ewing sarcoma. Recent work has shown roles of EGR2 and EGR3 in controlling inflammation and in promoting T and B cell antigen receptor signaling, suggesting a contribution of EGR2 variants to the hyperinflammatory state of Behçet's disease.

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Modeling Reticular Dysgenesis in Zebrafish

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Poster

Alberto Rissone [1], G. Jayashree Jagadeesh [1], Karen Simon [1], Kevin Bishop [2], Raman Sood [2], Fabio Candotti [1]

The members of adenylate kinase (AK) gene family are critical players in ensuring cellular energy homeostasis in all tissues. Mutations of the AK2 gene are responsible for a mitochondriopathy causing reticular dysgenesis (RD), an autosomal recessive form of severe combined immunodeficiency (SCID). RD is characterized by an early differentiation arrest in the granulocyte lineage and impaired lymphoid maturation and it represents less than 2% of total SCID. Affected children succumb to overwhelming infections early in life unless their immune system is successfully restored with allogeneic hematopoietic stem cells transplant (HSCT). The mechanisms underlying the pathophysiology of RD remain unclear. The phenotype of AK2 deficient animals has never been reported in the literature, we therefore used the zebrafish model to perform a comprehensive study of the effects of AK2 deficiency using MO injections and two different kinds of mutants (a knock-out mutant and an ENU-induced T371C/L124P missense mutant). We found that the loss of function of ak2 resulted in an impairment of hematopoietic stem cells development resulting in abnormalities distributed along all the hematopoietic lineages. Mechanistically, we observed in vivo that AK2 deficiency induced increased levels of reactive oxygen species triggering oxidative stress and consequent apoptosis in HSCs. Importantly, antioxidants treatment of the mutants induced the rescue of the hematopoietic phenotype indicating that it may represent a new approach to ameliorate the clinical conditions and more importantly the survival of RD patients.

1. Disorders of Immunity Section, GMBB, NHGRI

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Chediak-Higashi syndrome shows a strong genotype-phenotype correlation that predicts disease severity

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Poster

Joseph C. Roney [1], Wendy J. Introne [1], Camilo Toro [1], Marjan Huizing [1], William A. Gahl [1], Andrew R. Cullinane [1]

Chediak-Higashi syndrome (CHS) is a rare autosomal recessive disorder characterized by partial albinism, recurrent life-threatening infections, a mild bleeding tendency, and a later onset neurological dysfunction with features that include learning difficulties, cerebellar dysfunction, polyneuropathies, and L-dopa responsive Parkinsonism. CHS is caused by mutations in the lysosomal trafficking regulator gene (LYST), whose protein product is thought to function in the formation, regulation of size, and movement of lysosomes and lysosome-related organelles (LROs). Here, we emphasize a strong genotype-phenotype correlation for CHS, by which the nature of the LYST mutation predicts disease severity. Classic CHS patients, who require bone marrow transplantation in infancy, tend to have 2 nonsense alleles for LYST, whereas atypical patients (i.e., mild or late-presenting) tend to have at least 1 missense allele. However, recent case reports demonstrate exceptions to this association, and it is becoming apparent that the effect of the mutation on the level of LYST protein expression is a better prognostic marker for disease severity than genotype alone. Classic CHS patients do not express LYST protein, whereas atypical patients have residual expression. With less obvious clinical manifestations and later onset neurologic features that present in complex and nonspecific ways, atypical CHS patients are very challenging to diagnose. There are potentially hundreds of undiagnosed CHS patients within neurologic clinics who manifest the various neurological abnormalities of the disease. Identification of these individuals is not only essential for their close surveillance and clinical management, but will also provide more details about the complex neurological features of CHS.

1. Section of Human Biochemical Genetics, Medical Genetics Branch, NHGRI, NIH

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Poster

Communication is Key! Understanding How Families at Risk for Cancer Talk about Genetic Testing and Counseling Through Social Networks

Allie Rosen [1], Laura Koehly [1], Don Hadley [1], Chris Marcum [1]

People with Lynch Syndrome, (or Hereditary Nonpolyposis Colorectal Cancer) have higher rates of certain cancers which are caused by a genetic mutation. It is important that those at risk of inheriting the mutation discuss genetic counseling and testing with the members of their social network, especially blood-related family members. Approximately 5% of all colon cancer cases are due to this syndrome and children of those who are mutation-positive have a 50% chance of inheriting the mutation themselves. In this study, we use social network analysis to learn how social and biological relationships shape patterns of communication about genetic testing and counseling regarding Lynch Syndrome in families. In particular, we examine three important aspects of relationships within families that contribute to communication including: 1) being related by blood versus being socially related, 2) being close in age, and 3) having strong versus weak social bonds with each other. We find that having stronger social bonds, and being close in age, contributes to communication about genetic testing and counseling but communication between blood and non-blood relatives depends on the type of social relationship.

1. SBRB

Assaying interallelic complementation in vivo at the Mut locus with adeno-associated (AAV) viral gene delivery.

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Poster

Julien Senac [1], Charles Venditti [1]

Methylmalonic acidemia (MMA) is caused by mutations of the mitochondrial gene methylmalonyl-CoA mutase (MUT). Among the wide spectrum of partial activity or mut- mutations, the mechanism of enzymatic impairment has been defined only for selected mutations, such as p.G717V, which has been documented to be an adenosylcobalamin Km mutant. We generated transgenic mice that ubiquitously express the murine p.G717V homologue (p.G715V) as a stable transgene (*Mut-/-*;$Tg^{INS\text{-}CBA\text{-}G715V}$) and have established that this new model mimics the physiologic and phenotypic manifestations observed in *mut-* MMA patients, such as dietary induction of severe methylmalonic acidemia and the inducible formation of megamitochondria in the liver. Previous publications have demonstrated that cell lines harboring some MUT mutations, such as p.G717V mutation, could be `rescued' by alleles with distinct mutations, such as p.R93H. Using a yeast expression approach, we first established that the co-expression of the murine homologues of these mutations, Mut p.R91H and p.G715V, produced a recombinant Mut enzyme with improved kinetic parameters compared to either allele, supporting previous cell culture observations. We next hypothesized that we could recapitulate interallelic complementation in vivo using an rAAV vector to deliver the p.R91H mut transgene to *Mut-/-*;$Tg^{INS\text{-}CBA\text{-}G715V}$ mice.

A single retro-orbital injection of ($5e^{10}$ GC) of AAV2/9-CBA-Mut-pR91H was performed in adult mice. Following delivery, weight and metabolites were monitored. As early as one week post injection, *Mut-/-*;$Tg^{INS\text{-}CBA\text{-}G715V}$ mice demonstrated a significant weight increase ($p=0.04$) compared to treated heterozygote littermates, an effect similar in magnitude to that observed in MMA mice treated with a gene therapy vector configured to express wild type Mut. Our results suggest that the phenotypic improvement is secondary to increased Mut activity mediated by interallelic complementation of mutant alleles in vivo. We are conducting detailed physiologic and phenotypic characterization of the treated *Mut-/-*;$Tg^{INS\text{-}CBA\text{-}G715V}$ mice to further define the mechanism(s) and consequences of interallelic rescue. Using a *mut-* mouse model and rAAV mediated gene delivery, we establish a new platform to study the trans-effects of mutant MUT alleles and the foundation to examine small molecule activators of the MUT activity.

1. Organic Acid Research Section, GMBB/NHGRI/NIH

Meta-Analysis of Genome-Wide Association Studies in Myopia in Nine Populations

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Poster

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Myopia is a common refractive error which affects at least a third of most populations. Both genetic and environmental factors influence myopic development. It has a significant impact on the lives of affected individuals and carries high economic costs associated with treatment, loss of productivity and co-morbidity from vision impairment. Recent genome-wide association studies (GWAS) have identified many loci associated with myopia and refractive error. Here we report results of a large meta-analysis of myopia in nine cohorts, for a total of 17,787 individuals of European ancestry and replication in a further 8 cohorts for a total of 7953 individuals.
Genotypes in each population were imputed to HapMap2 and analyzed separately by each group. Cases defined as a spherical equivalent of -1 diopters (D) or worse; controls were defined as > 0D. Individuals between 0 and -1D coded as unknown. Analyses performed included age, sex and years of education and first 3 principal components to adjust for population structure. Meta-analysis performed in METAL with sample size schema. Due to large differences in numbers of cases and controls for some studies, effective sample sizes were calculated using the formula recommended by the authors of METAL. Residual population substructure was adjusted for with genomic control. SNPs with P < 1e-5 were identified and all SNPs within 500kb each side of that SNP selected for replication. The replication threshold was set by calculating the effective degrees of freedom using the Ramos method. SNPs were considered to replicate where the p value < 0.0026.

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Ontology based bed side phenotype capture enables phenologs aware exome analysis in rare diseases

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Poster

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Recent advances in DNA sequencing technologies made new tools available for rare disease research. Rare genetic diseases are mostly caused by mutations that occur in the exome (protein coding regions of the genome). Human exome sequencing has been used to identify the disease causing mutations in many recent studies with moderate success.
One of the main challenges to our ability in identifying the disease causing mutations is the fact that these diseases are so rare sometimes finding a second family with the same disease is very hard. Due to the fact that these cases are encountered infrequently in clinics around the world two natural consequences occur. First, it is hard to recognize these syndromes and second, it is very hard to meet the second case in the same clinic in temporal proximity. Therefore, there is a great need for centers around the world to have a standard way of describing the phenotypes of these cases so that a second case seen in another clinic can be found through automatic or semi-automatic searching.
One other critical challenge is the analysis of genome data in rare diseases. We still detect many thousands of new and private mutations in each sequenced individual or family. To tackle these problems we have improved and integrated a phenotype capturing tool (Phenotips) in to a new workflow based research information management system. Phenotips allows clinicians to record the patients' phenotypes using Human Phenotype Ontology.

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The expanding clinical spectrum of adenosine deaminase (ADA) deficiency

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Poster

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ADA deficiency manifests as profound immunodeficiency. Treatments that prolong survival allow other manifestations to become evident.

We described an association between ADA deficiency and multicentric dermatofibrosarcoma protuberans (DFSP). DFSP's in ADA deficiency present differently from typical DFSP's (i.e. multicentricity, pre-protuberant stage, reduced cellularity). Their natural history is unknown. We diagnosed DFSP in 9 of 17 patients, and followed the lesions for 2.5-7 years. Most enlarged, but none metastasized. Lesions that evolved to protuberant morphology or presented subcutaneous nodular/infiltrative pattern were resected. None recurred. This approach may limit morbidity without compromising oncologic outcomes.

We evaluated 19 ADA deficient patients with elevates transaminases for liver disease. Thirteen underwent imaging. Eight had changes consistent with fatty liver and/or hepatomegaly. Biopsy showed simple steatosis in three patients, non-alcoholic steatohepatitis and fibrosis in three, and cirrhosis in one. Liver stiffness, measured by ultrasonographic elastography, was elevated in five cases. Three children had increased BMI, abnormal lipids and insulin resistance consistent with metabolic syndrome. Liver abnormalities correlated with age and reduced ADA activity, but were observed regardless of adequate metabolic control (as defined by low levels of deoxynucleotides in RBC's) and immune function.

Lung pathologies in our cohort include hyperactive airways, obstructive lung disease reminiscent of bronchiolitis obliterans, granulomatous inflammation, fibrosis and subpleural cysts. These changes occurred in patients with otherwise adequate control of RBC deoxynucleotide levels and independent of immune function.

Non-immunologic manifestations of ADA deficiency may be life limiting and warrant assessment in all patients before and after interventions aimed at treating immunodeficiency.

1. National Human Genome Research Institute
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3. National Institute of Allergy and Infectious Disease
4. National Cancer Institute

The patterns of connectivity between subcortical and cortical structures and the adult outcome of Attention Deficit Hyperactivity Disorder

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Poster

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INTRODUCTION
Childhood ADHD persists into adulthood in around 30-50% of individuals, but little is known of either the genomic or neural factors determining the persistence of ADHD. Here we define the developmental changes in the relationship between brain regions as the brain's structural connectivity. We asked if children with ADHD who have symptoms persisting into adulthood show different patterns of age-related change in structural connectivity compared to those who recover. Such connectivity patterns, which are sensitive to the clinical course of ADHD, are a possible phenotype for future genomic studies.
METHODS
32 individuals whose childhood ADHD persisted into adulthood were contrasted against 32 with remitting ADHD and 64 age, sex matched typical individuals. All had one scan in childhood (mean 12.8 y.o.) and one in adulthood (mean 22.9 y.o.). Using neuroanatomical data (acquired using MRI) we estimated the connectivity between the surface area of the thalamus, globus pallidus, striatum, and cortex for each group in childhood and adulthood.
RESULTS
There were group differences in the age-related changes in structural connectivity. Specifically, the persistent ADHD group showed increasing connectivity with age when compared to the typical and remission groups ($p < .05$). The connections most robustly affected were between the left striatum and the posterior region of the thalamus, as well as between the right thalamus and the prefrontal cortex.
CONCLUSIONS
Individuals with persistent ADHD showed hyperconnectivity over time in regions involved in sensory integration and action planning. These coordinated anomalies could be promising candidates for phenotypes for future genomic studies.

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Clinical Sequencing Exploratory Research Program

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Poster

Kathie Sun [2,3], Joy Boyer [3], Charlisse Caga-Anan [4], Catherine Crawford [2], Adam Felsenfeld [1], Carolyn Hutter [2], Nicole Lockhart [3], Sheri Schully [4], Lucia Hindorff [2], Jean McEwen [3]

The 2011 National Human Genome Research Institute (NHGRI) Strategic Plan recognized the potential benefits to patients of comprehensive genomic data that soon will be available to clinicians with the rapid deployment of new DNA sequencing instruments and methods. NHGRI subsequently crafted the Clinical Sequencing Exploratory Research (CSER) initiative to: 1) leverage the Institute's long-standing experience in genomic sequencing and analysis to ease the adoption of these methods into clinical care, 2) guide the development and dissemination of best practices for the integration of clinical sequencing into clinical care, and 3) research the ethical, legal, and psychosocial implications of bringing broad genomic data into clinical decision-making. The CSER Consortium is currently composed of nine multi-disciplinary projects (six awarded in 2011 and three added in 2013), nine Ethical, Legal, and Social Implications (ELSI)-specific projects (which formerly comprised the Return of Results Consortium), and a Coordinating Center. Aims of the research include: generating genomic sequence data on patients in a variety of clinical contexts; outlining the principles and processes guiding the definition of an 'actionable' variant across the Consortium; and exploring standardized approaches to addressing the unique ELSI challenges relating to returning results in studies involving both adult and pediatric populations. The Consortium of grantees will cooperate to evaluate best practices in this rapidly advancing field and communicate these to the community. The organization of the Consortium across study sites and Working Groups, anticipated products and results, and the Consortium's role in broader efforts at NHGRI relating to genomic medicine will be described.

1. Division of Genome Sciences, National Human Genome Research Institute

2. Division of Genomic Medicine, National Human Genome Research Institute

3. Division of Genomics and Society, National Human Genome Research Institute

4. Epidemiology and Genomics Research Program, National Cancer Institute

Type I Error in Regression-based Genetic Model Building

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Poster

Heejong Sung [1], Alexa Sorant [1], Bhoom Suktitipat [1,2], Alexander Wilson [1]

The task of identifying genetic variants contributing to trait variation is increasingly challenging, given the large number and density of variant data being produced. Current methods of analyzing these data include regression-based variable selection methods which produce linear models incorporating the chosen variants. For example, the Tiled Regression method begins by examining relatively small segments of the genome called tiles. Selection of significant predictors, if any, is done first within individual tiles. However, type I error rates for such methods haven't been fully investigated, particularly considering correlation among variants. To investigate type I error in this situation, we simulated a mini-GWAS genome including 306,097 SNPs in 4,000 unrelated samples with 100 non-genetic traits. 53,060 tiles were defined by dividing the genome according to recombination hotspots. Stepwise regression and LASSO variable selection methods were performed within each tile. Type I error rates were calculated as the number of selected variants divided by the number considered, averaged over the 100 phenotypes. Overall rates for stepwise regression using fixed selection criteria of 0.05 and LASSO minimizing mean square error were 0.04 and 0.12, respectively. Considering separately each combination of tile size and multicollinearity (defined as 1 – the determinant of the genotype correlation matrix), observed type I error rates for stepwise regression tended to increase with the number of variants and decrease with increasing multicollinearity. With LASSO, the trends were in the opposite direction. Different ways of choosing selection criteria were investigated for both methods.

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Identification of a single nucleotide polymorphism variant in TYRO3 associated with coronary artery disease risk in the ClinSeq® Study

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Poster

Heejong Sung [1], Bhoom Suktitipat [1], Katie Lewis [2], Daivd Ng [2], Steve Gonsalves [2], NISC Comparative Sequencing Program [4], James Mullikin [3,4], Leslie Biesecker [2], Alexander Wilson [1]

ClinSeq® is a large-scale medical sequencing study designed to investigate associations of rare sequence variants with traits related to coronary artery disease (CAD). ClinSeq® currently includes 1092 non-smoking participants, ages 45-65, with normal to severe coronary artery calcification scores. About 200 CAD-related traits were measured at the NIH Clinical Research Center. Whole-exome sequencing was performed with the Agilent SureSelect 38Mb and 50Mb capture kits for 387 and 325 individuals, respectively, at NISC. After quality control screening, 439,807 sequence nucleotide variants (SNVs) common to both capture kits were retained for analysis. Of these, 74% and 46% had MAF < 0.01 and < 0.001, respectively. Individuals were classified by 10-year Framingham risk score for developing CAD: Bin1 (<5%), Bin2 (5-10%), Bin3 (>10%) and Bin4 (already-diagnosed CAD). All SNVs in 232 Bin1 individuals were compared with those in 89 Bin4 individuals using the Variant Annotation, Analysis, and Search Tool (VAAST), a probabilistic search tool in a likelihood framework based on amino acid substitution frequencies. This analysis identified the most likely disease-causing gene as TYRO3, with p-value 1.01e-81, due to a nonsynonymous variant (rs62001448, MAF=0.095). The variant allele (T) was found in heterozygous state in 16 (7%) times in Bin1 and 26 (29%) in Bin4, never in homozygous state. Mouse studies by Angelillo-Scherrer at al. (2005) and Cosemans et al. (2010) suggested a relationship between TYRO3 function and thrombus formation. This finding suggests the importance of the TYRO3 gene in CAD and provides insight into some of the underlying genetic mechanisms of CAD.

1. Genometrics Section, Inherited Disease Research Branch, NHGRI/NIH
2. Genetic Disease Research Branch, NHGRI/NIH
3. Comparative Genomics Unit, Genome Technology Branch, NHGRI/NIH
4. National Institutes of Health Intramural Sequencing Center

Generating Novel 'Social' Phenotypes for the Genomic Research of Childhood Emotional and Behavioral Problems.

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Poster

Eszter Szekely [1], Henning Tiemeier [2], Marina Verlinden [2], Philip Shaw [1]

Childhood emotional and behavioral problems are generally highly heritable. Likewise, there is evidence that the types of friendship a child forms within the peer network may have genetic contributors.

However, we have made limited progress in finding the specific genes that contribute to the development of childhood emotional and behavioral problems, underscoring the need for novel phenotypes. Here, we test two hypotheses:

1. The developmental course of early emotional and behavioral problems will uniquely predict the child's position within the peer network.
2. The child's position within the peer network is a potential 'social' phenotype for future genomic research.

We use data from a population-based prospective Dutch cohort (N=1590) to (1) model the developmental trajectories of early emotional and behavioral problems and (2) examine whether these trajectories uniquely predict children's peer status within the classroom.

Mothers rated their children's emotional and behavioral problems at ages 1.5, 3, and 5.5 years using the Child Behavior Checklist. Classroom peer relationships were assessed in grades 1-3 using the PEERS measure, an interactive, web-based computer program.

Significance. This work could help provide a novel phenotype, namely the child's position within the peer network for future genomic research. Understanding the structure of a child's peer network can also help identify points for treatment intervention.

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Hepatic mitochondrial adaptations during systemic immune activation

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Poster

Tatiana Tarasenko [1], Larry Singh [1], Julien Senac [1], Shingo Matsumoto [2], Keita Saito [2], Peter McGuire [1]

Viral infections play a significant role in precipitating life-threatening acute decompensations in inborn errors of metabolism (IEM). Acute decompensations are defined as a functional deterioration in metabolic status and may result in a range of hepatic metabolic perturbations including hypoglycemia, acidosis and hyperammonemia. We hypothesized that hepatic metabolic adaptations that normally occur during the immune response to infection will not be tolerated in IEM. To help define the hepatic metabolic response to immune activation, we employed mouse models of influenza infection and simulated viremia in C57Bl/6. After 5 days of infection with influenza, mice displayed hepatic sensitivity characterized by increased AST and ALT. mRNA microarray studies revealed antiviral response signatures suggestive of hepatic sensing of influenza. To simulate immune activation due to viremia, mice were injected with poly I:C. After 3 days of poly I:C, hepatic mRNA profiling showed activation of antiviral immune pathways, similar to natural infection. Metabolomic profiling showed a depletion of cofactors involved in energy metabolism. To address further perturbations in energy pathways, we profiled in vivo mitochondrial function by MR spectroscopy using hyperpolarized 13C-pyruvate. Following poly I:C treatment, mice displayed significant increases in 13C-lactate and 13C-alanine, consistent with decreased entry of 13C-pyruvate into the TCA cycle. In addition, a depletion of pyruvate dehydrogenase and components of respiratory chain were also seen by immunoblot. Overall, our findings suggest that immune activation may have direct effects on hepatic energy metabolism, which has implications for patients with mitochondrial disease and other IEM with significant mitochondrial dysfunction.

1. National Human Genome Research Institute

2. National Cancer Institute

Genome-wide comparison of an indigenous Ethiopian ethnic group with eleven HapMap populations reveals novel selection signals in HLA loci and energy metabolism genes

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Poster

Fasil Tekola-Ayele [1], Adebowale Adeyemo [1], Elena Hailu [2], Abraham Aseffa [2], Gail Davey [3], Melanie Newport [3], Charles Rotimi [1]

The recent feasibility of genome-wide studies of adaptation in human populations has provided novel insights into biological pathways that have been affected by adaptive pressures. However, only a few African populations have been investigated using the genome-wide approaches. Here, we performed a genome-wide analysis for evidence of recent positive selection in a sample of 120 individuals of Wolaita ethnicity belonging to Omotic speaking people that have inhabited the mid- and high-land areas of southern Ethiopia for millennia. Using the eleven HapMap populations as the comparison group, we found Wolaita-specific signals of recent positive selection in several HLA loci. Notably, the selected loci overlapped with HLA regions that we previously reported to be associated with podoconiosis – a geochemical lymphedema of the lower legs common in the Wolaita area. We found selection signals in PPAR-alpha, a gene involved in energy metabolism during prolonged food deficiency. This finding is consistent with the dietary use of enset, a crop with high carbohydrate and low fat and protein contents domesticated in Ethiopia subsequent to food deprivation 10,000 years ago, and with metabolic adaptation to high altitude hypoxia. We observed a novel selection signal in CDKAL1, a well-known diabetes susceptibility gene. Finally, the SLC24A5 gene locus known to be associated with skin pigmentation was in the top selection signals in the Wolaita, and the alleles of SNPs rs1426654 and rs1834640 (SLC24A5) associated with light skin pigmentation in Eurasian populations were of high frequency (47.9%) in this Omotic speaking indigenous Ethiopian population.

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NHGRI Embryonic Stem Cell and Transgenic Mouse Core

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Poster

Lisa Garrett [1], Jun Cheng [1], Gene Elliott [1], Kowser Hasneen [1], Karen Hazzard [1], Cecelia Rivas [1], Elsa Escobar [1]

The NHGRI Transgenic Core specializes in generating genetically altered mouse lines for basic studies of gene function and regulation and for the creation of mouse models of human genetic diseases. Several technologies are utilized by the Core to generate genetically altered mice. First is to create conventional transgenics by microinjection of DNA into fertilized embryos (pronuclear microinjection) to generate germline mice. Secondly, targeted transgenics are generated by microinjecting genetically altered embryonic stem cells (ES cells). The ES cells are modified via homologous recombination of targeted genes in the Core or, are imported ES cell lines from other institutions.

The Core archives, in multiple locations, mutant strains by cryopreservation of sperm and embryos and reconstitutes the lines by in vitro fertilization. The Core rederives animals into our facility by in vitro fertilization and embryo transfer of fertilized eggs.

The Core utilizes inhibitors, GSK3b and MAPK (2i media) to generate new ES cell lines from mutant mice. These factors enhance cell pluripotency/ cell quality and chimera production. The Core establishes mouse induced Pluripotent Stem (miPS) cells from mutant mice fibroblasts and can generate germline transmitting chimeras.

The Core is presently developing protocols for gene editing with the CRISPR/Cas system. The Core has plasmids available for in vitro transcription of gRNA and Cas9 for direct microinjection as well as plasmids for transfection into ES cells. This technology will allow for simulataneous targeting of ES cells for multiple genes, as well as homolgous directed recombination of multiple genes via direct microinjection of fertilized eggs.

1. NHGRI/GDRB/Transgenic Mouse Core

EGFR-mediated activation of STAT-1 can augment production of PD-L1

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Poster

Arun Unni [1], Shih-Queen Lee-Lin [1], Harold Varmus [1]

The epidermal growth factor receptor (EGFR) is frequently altered in cancers of diverse origin. These alterations include mutations within the kinase domain, deletions in the extracellular domain, and gene amplification. EGFR activation is typically associated with cellular growth and survival. Several signaling modules are implicated in these outcomes, in particular the RAS-MAPK-ERK pathway. In this context, the activation of Stat-1 following EGFR activation is a puzzling but frequently reported finding. However, the full transcriptional program that defines Stat-1 activation downstream of the interferon pathway is not observed. Here, we show that this program can be fully deployed when high levels of EGFR are present or when kinase domain mutant alleles, common in lung cancer, are expressed. One of the transcriptional targets of this program is the PD-L1 gene, encoding an inhibitory cell surface protein that suppresses the immune response. The enhanced expression of PD-L1 observed in diverse tumors may in part be the consequence of strong activation of EGFR or other receptor tyrosine kinase pathways.

1. NIH, National Human Genome Research Institute, Cancer Genetics Branch

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Poster

Spatial structure of the human skin microbiome

Alex Valm [1], Sean Conlan [1], Cynthia Ng [1], Heidi Kong [2], Julie Segre [1]

The human skin comprises an ecosystem; composed of host epithelial and immune cells and commensal bacteria and fungi. Because the function of any biological system is implicit in its structure, the physical architecture of the commensal microbial community associated with the human skin correlates with its physiology and plays an integral role in maintaining human health and underlying disease. The 30 most abundant genera of microbes present on the human skin were identified from previous molecular-based surveys of the human skin microbiota. The unique 16S sequences from the 20 body sites surveyed were pooled to create a comprehensive database of skin microbiome sequences. FISH probes were designed to target microbial organisms at the genus level. Representative, cultivable species of skin microbes from these genera were cultured in the lab or otherwise procured from the Microbiology Laboratory at the NIH Clinical Center or from the American Type Culture Collection (ATCC) and were used to empirically test probe specificity. Additionally, a non-invasive protocol for removing skin specimens and associated microbial cells was developed. Dermatologic tape strips were applied serially to human volunteers removing layers of epidermis. Tape strip specimens were fixed and labeled in FISH experiments with the EUB338 probe which targets a highly conserved region of the bacterial 16S rRNA revealing the spatial distribution of bacteria with relation to host epidermal cells and hairs.

1. NHGRI

2. NCI

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Poster

A High-throughput Strategy for Targeted Mutagenesis using CRISPR/Cas9 in Zebrafish

Gaurav Varshney [1], Wuhong Pei [1], Matthew LaFave [1], Ashrifia Adomoko-Ankomah [1], Viviana Gallardo [1], Lisha Xu [1], Shannon DeMaria [1], Jennifer Idol [1], Donna Wang [1], Johan Ledin [1], Shawn Burgess [1]

The zebrafish genome is now completed and is the only third vertebrate to have a fully annotated reference genome, which facilitates systemic large-scale functional genomic studies. The number of large-scale mutagenesis projects has increased in last few years, further enhancing the utility of zebrafish as a model organism. Most of these projects are using random mutagenesis approaches thus limiting the number of genes that can be mutagenize with this approach. However, the development of targeted mutagenesis approaches such as TALENs and CRISPR/Cas9 have opened up new avenues to mutagenize genome in a systematic fashion. We developed an inexpensive high-throughput method of multi-allelic targeted mutagenesis using CRISPR/cas9 system. We also generated a highly fecund lab strain NHGRI-1.0 and mapped all the polymorphisms in this strain. By having all polymorphisms identified, it allows us to computationally design CRISPR targets without additional target validation. As a proof-of principle, we are targeting all zebrafish genes known or believed to cause deafness in humans, and all genes encoding kinases. All these mutants will also be released to the community through ZIRC.

1. Genome Technology Branch

Completion of 1000 Genomes: A Deep Catalog of Human Genetic Variation

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Poster

Yekaterina Vaydylevich [1], Lisa Brooks [1], 1000 Genomes Consortium [2]

The 1000 Genomes Project has expanded the scope of known human genomic variation through the detection of over 70 million SNPs, indels, and larger structural variants. More than 95% of variants with an allele frequency of at least 1% in the human population, will have been characterized by the project, with a false detection rate of less than 5%. In the final phase of the project, data were collected from an additional 12 populations, with a focus on African and South Asian ancestry. In total, genotype data and low coverage (~7.5X) whole genome sequence data were combined with high coverage (~80X) exome data for 2535 individuals from 26 global populations. Additionally, deep sequencing was done on a subset of the samples, and two trios.

The project has been working towards identifying more complex types of variation across a larger portion of the genome with novel analytic methods. By integrating multiple detection approaches that leverage information from read mapping, locally reassembly, and full-scale de novo assembly, we are able to generate a more complete picture of human genetic variation. In order to place all discovered variants on phased haplotypes, we are also extending current statistical phasing approaches to allow for the simultaneous integration of multiallelic small variants and larger structural variants.

The variants, haplotypes, and cell lines the project provides have been used for better mapping of disease-associated variants in GWAS studies, studies of function, expression, methylation, and drug response, and studies of population genetics and admixture.

1. NHGRI

2. International

Identification of disease causing mutations in a new congenital neutrophil defect syndrome

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Poster

Thierry Vilboux [1], Atar Lev [2], May Christine Malicdan [1], Simon Amos [2], Raman Sood [3], Yair Anikster [4], Christoph Klein [5], William Gahl [1], Raz Somech [6]

Neutrophils are the predominant phagocytes that protect the host against bacterial and fungal infections. The important role of neutrophils in innate immunity is underscored by the multiple congenital defects that lead to severe infections. These disorders are characterized by genetic abnormalities that alter the neutrophil number and/or their function.

To identify the causative gene, we combined the data of single-nucleotide polymorphism arrays of multiple families and a single exome. Functional studies were performed on patient neutrophils and fibroblasts, and on a zebrafish model.

We describe seven patients from five families, who displayed a unique constellation of severe neutrophil dysfunction, bone marrow fibrosis and nephromegaly. From our analysis, this new immunodeficiency is associated with biallelic mutations in the VPS45 gene, encoding a protein that regulates membrane traffic through the endosomal system. Two distinct homozygous VPS45 mutations were identified in patients from different ethnic origins. Functional studies showed that VPS45 mutations affected protein recycling, altered cell migration and neutrophil maturation, and induced cell apoptosis. Both the migration defect and the enhanced apoptosis were rescued by expressing the wild-type VPS45 in patient cells. Neutrophil defect was recapitulated in a zebrafish model.

Mutations in VPS45 underlie a new immunodeficiency syndrome with bone marrow fibrosis and severe neutropenia. This can pave the way into understanding and finding more immunodeficiencies that are associated with endocytosis or phagosome defect.

1. Medical Genetics Branch, NHGRI, NIH, Bethesda MD
2. Sheba Medical Center, Sheba Cancer Research Center, Pediatric Immunology, Tel Aviv, Israel
3. Zebrafish Core, Genetics and Molecular Branch, NHGRI, NIH, Bethesda MD
4. Edmond and Lily Safra Children's Hospital Tel Aviv University, Israel
5. Ludwig Maximilians University Munich Pediatrics, Munich, Germany
6. Edmond and Lily Safra Children's Hospital Sackler Faculty of Medicine, Tel Aviv, Israel

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Poster

Expanding the Spectrum of Phenotypes with Polydactyly – Mutation Discovery in Individuals with Overlapping Features of Pallister-Hall and Oral-Facial Digital Syndromes

Ingrid Wentzensen [1,2], Jennifer Johnston [1], Julie Sapp [1], Leslie Biesecker [1]

Background: Mutations in GLI3, a zinc-finger transcription factor encoding gene on 7p14.1, have been associated with Pallister-Hall syndrome (PHS) and Greig Cephalopolysyndactyly syndrome (GCPS). Oral-facial digital syndrome (OFDS) comprises the key features of oral hamartomas/frenula, cleft lip/palate, polydactyly, or cerebellar vermis hypoplasia. Molecular etiology for OFDS is still largely unknown. Johnston et al., 2010, described mutations in GLI3 in 29% of cases with overlapping features of OFDS, PHS, and GCPS. GLI2 encodes for a related transcription factor which, when mutated leads to defective anterior pituitary formation, with forebrain cleavage or midface abnormalities, and polydactyly.

Methods: We use exome sequencing to discover causative molecular lesions in patients with overlapping features of described GLI3 phenotypes and OFDS previously negative for GLI3 mutations. We hypothesized that mutations in GLI2 could be involved and performed candidate gene sequencing in 6 patients with polydactyly and midline defects prior to exome analysis. Twenty-one individuals were sent for exome sequencing, including 6 trios. Varsifter software is used to identify relevant variants. Causal variants are CLIA-validated and returned to the families. Validated secondary variants are returned as well.

Results: No mutations in GLI2 were identified in 6 selected individuals through Sanger sequencing. Homozygous mutations in C5orf42 were identified in a patient with polydactyly, imperforate anus, cystic kidneys, and absent cerebellar vermis.

Summary: With this ongoing project, we anticipate identifying different genes in this heterogeneous group and aim to add new insights to expanding knowledge about the spectrum of polydactyly phenotypes.

1. Genetic Disease Research Branch, NHGRI, NIH

2. Institute of Genetic Medicine, Johns Hopkins School of Medicine

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Poster

Germline variation modulates susceptibility to aggressive disease development in a mouse model of prostate tumorigenesis

Kendra Williams [1], Sujata Bupp [1], Shashank Patel [1], Jonathan Andreas [1], Suiyuan Zhang [2], Alfredo Molinolo [3], Silvio Gutkind [3], Nigel Crawford [1]

Although prostate cancer is common, with over 238,000 new cases being diagnosed in the US in 2012, it typically runs an indolent course with most men succumbing to unrelated disease processes. It is critical to identify modifiers that increase susceptibility to aggressive disease to accurately identify men at risk of fatal disease forms. The goal of this work is to map prostate tumor progression and metastasis modifier loci mapping using the C57BL/6-Tg(TRAMP)8247Ng/J (TRAMP) mouse model of prostate carcinoma. We hypothesize that germline variation influences tumor progression and metastasis in prostate cancer.

The effect of germline variation in TRAMP mice was investigated by crossing it to Collaborative Cross progenitor strains and quantifying tumor progression and metastasis in transgene1 males. Strains with the greatest phenotypic variation from the wildtype TRAMP C57BL6/J mice were chosen for modifier mapping using an F2 intercross. F2 mice were genotyped using a linkage panel consisting of 1,449 SNPs and modifier loci analyzed using j/qtl. The greatest number of loci achieving genome-wide significance were observed in the TRAMPxNOD/ShiLtJ F2 cross (n=232). Modifier loci associated with primary tumor growth were observed on chromosomes 4, 7 and 8. Additionally, loci associated with metastasis were observed on chromosomes 1, 11, 13 and 17.

We identified multiple loci associated with aggressive disease development in a mouse model of prostate cancer. Candidate gene identification is ongoing, and focuses upon characterizing cis-eQTLs in TRAMPxNOD/ShiLtJ F2 primary tumors. Our eventual aim is to confirm candidate genes identified in the TRAMP mouse in human association cohorts.

1. Genetics and Molecular Biology Branch, NHGRI

2. Genome Technology Branch, NHGRI

3. National Institute of Dental and Craniofacial Research, NIH, Bethesda MD.

BY CHiP, PYRIN BINDS THE IRF2 PROMOTER

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Poster

Geryl Wood [1], Hong-Wei Sun [2], James Balow Jr [1], Yuka Kanno [3], Ivona Aksentijevich [1], D.L. Kastner [1]

The gene causing familial Mediterranean fever (FMF), MEFV, encodes a protein, pyrin, which is expressed at high levels in granulocytes, monocytes, dendritic cells and in some human myeloid leukemia cell lines, such as THP.1. Studies of pyrin localization show a cell-type dependency. Endogenous pyrin is predominantly nuclear in granulocytes, dendritic cells, and synovial fibroblasts, but it is cytoplasmic in monocytes. Previously, we evaluated changes in global gene expression in THP.1 cells between endogenous pyrin (scrambled control, SC) and knockdown (siMEFV) using cDNA microarray and identified 10 down-regulated genes (CD36, LY96, S100A8, CCR1, CD53, TIRAP, DEDD, SGK, MyD88, CD14) for further study. 7 of 10 genes showed a comparable mRNA and protein alteration consistent with the microarray analysis. Promoter analyses of the genes suggest an enrichment of binding sites for interferon regulatory factors (IRFs). We identified Interferon regulatory factor 2, IRF2 as the most significantly enriched IRF family member with a p value of 0.008. siMEFV treated cells showed diminution in IRF2 mRNA and protein compared to SC. Using CHiP-qPCR, we demonstrated binding of pyrin within the promoter of IRF2. Our findings suggest that in THP1 cells pyrin might regulate expression of innate immune genes by binding to the promoter of the transcription factor IRF2. Further research is needed to examine the possibility that pyrin may bind to other DNA using CHiP coupled with next generation sequencing.

1. IDS/MGB/NHGRI, Bethesda, USA

2. Biodata Mining and Discovery Section/OST/NIAMS, Bethesda, USA

3. LCBS/MIIB/NIAMS, Bethesda, USA

NHGRI Study "Clinical and Molecular Investigations into Ciliopathies": Findings on 103 Patients with Joubert Syndrome and Related Disorders (JSRD)

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Poster

Deniz Yildirimli [1,4], Courtney Sinclair [1,4], Danny Chang [1], Thierry Vilboux [1], Andrew R. Cullinane [1], Joy Bryant Bryant [2], Roxanne Fischer [1], Meghana Vemulapalli [3], Baishali Maskeri [3], Alice Young [3], Jim Mullikin [3], NISC Comparative Sequencing Program [3], Marjan Huizing [1], William A. Gahl [1], Meral Gunay-Aygun [1]

JSRD is a clinically and genetically heterogenous group of ciliopathies defined based on a distinctive brain malformation (molar tooth sign) on brain imaging. Most JSRD patients display hypotonia, developmental delay, abnormal eye movements, and an abnormal respiratory pattern in infancy. Variable features include fibrocystic kidney disease, congenital hepatic fibrosis, retinal degeneration, retinal colobomas, and polydactyly. Twenty one genes identified to date account for approximately 50% of JSRD. The exact frequency and nature of kidney, liver, retina, brain and other organ system involvement in JSRD is not well defined. Under the NHGRI ciliopathy study, we evaluated a total of 275 patients including 103 JSRD patients. The enrollment criterion for JSRD was the presence of the "molar tooth sign" on brain MRI. Our cohort of 103 JSRD patients included 89 families; 10 families had 2 affected siblings and two families had 3. Molecular Inversion Probe sequencing identified mutations in known JSRD genes in 45% of families. Exome sequencing analysis of the remaining families is ongoing; to date, we have identified 5 novel JSRD genes, reducing the number of unknown families to 16%. Among JSRD patients, kidney disease was diagnosed in 34% (34/101), liver disease in 30% (30/101), retinal degeneration in 34% (34/101) and uveal colobomas in 30% (30/101). Neurocognitive functioning ranged from average to severely impaired; the distribution suggested that genotyping may explain the variability. Identification of JSRD genes and further description of the related phenotypes will provide the groundwork for more focused studies and future therapeutic interventions.

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2. Office of the Clinical Director, NHGRI, NIH

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4. These authors contributed equally

GSVseq - Detection of Genomic Structural Variation Using Multiple Features of NGS Data

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Poster

Kai Ying [1], Zhengdao Wang [2], Nancy Hansen [1], Jim Mullikin [1]

Genomic structural variation(SV) and especially copy number variation (CNV) play an important role in many complex diseases such as autism, Alzheimer's disease and cancer. Recent advances in high-throughput DNA sequencing technologies have enabled us to sequence the whole genomes or Exomes of many samples at reasonable costs.

However, current analytical methods for CNV detection are still unsatisfactory in both sensitivity and accuracy. Current CNV detection methods usually utilize only one kind of sequence feature information, e.g. read depth (RD), allele frequency aberration (AF), paired end distributions (PE) and split reads (SR) to call CNVs. Combination methods just find the overlap regions of different programs' predictions, which does not maximize the detection power.

Here we propose an integrated model that combines different feature information in a unified statistical model for CNV detection. First, read depth and allele frequency of each base pair were fed to a single base pair Bayes model to estimate the likelihood of different copy numbers on a single base level. Then, a Hidden Markov Model (HMM) was applied to combine neighboring information to identify contiguous genomic regions that show an abnormal CNV signal. Finally, smoothing and statistical testing were performed to control the false discovery rate (FDR).In addition to the observed read information, prior information, such as population allele frequencies or known mutation hot spots, can also be integrated into our model.

1. National Human Genome Research Institute, National Institutes of Health

2. Department of Electrical and Computer Engineering, Iowa State University

Studies of regulatory B cell function in Wiskott-Aldrich syndrome gene knockout mice

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Poster

Tadafumi Yokoyama [1], Karen Simon [1], Fabio Candotti [1]

The Wiskott-Aldrich syndrome (WAS) is a rare X-linked primary immunodeficiency characterized by recurrent infections, thrombocytopenia, eczema, and high incidence of malignancy and autoimmunity. The cellular mechanisms underlying autoimmune complications in WAS patients remain poorly defined. Interleukin (IL)-10-producing regulatory B cells (B10 cells) are emerging as important mediator cells with immunosuppressive activity in different autoimmunity models. However, there are no reports that describe the function of Breg cells in WAS patients. In this study, we investigated the characteristics of B10 cells in the Was gene knockout (WKO) mouse model.

Splenocytes from WKO and wild type 129S6/SvEvTac (WT) mice were stained for the CD19, CD1d, and CD5 B10 cell surface markers and analyzed by flow cytometry. WKO mice are known to have lower total numbers of B cells. However, we also found that the percentage of CD19+ CD1dhigh CD5+, B10 cells was lower in WKO mice compared to WT controls. We also analyzed the proportion of IL-10 producing B cells and found them to be lower in WKO mice than in WT animals. Finally, quantitation of IL-10 production in the supernatant of LPS-stimulated B cells showed reduced concentrations in WKO cultures compared to WT samples.

In summary, our results show decreased number and impaired IL-10 production of WKO B10 regulatory cells. This defect may contribute to the susceptibility to autoimmunity in patients with WAS.

1. NHGRI, GMBB

Genetics Researchers' Attitudes about Obligations to Return Genetic Incidental Findings and Individual Research Results to Participants

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Poster

Caroline Young [1,2], Barbara Biesecker [1], David Kaufman [3], Lori Erby [2]

Researchers in genetics are increasingly facing decisions about returning incidental findings (IFs) and individual research results (IRRs) to participants in their research. Studies have shown that participants are interested in receiving such results, and there has been vigorous debate in the bioethics community about the extent of researcher obligation to return both IRRs and IFs. However, little research has focused on whether the researchers themselves feel such an obligation, and whether feelings of obligation actually translate into the return of results. This study (a secondary data analysis) will seek to describe the extent of researcher feelings of obligation to return results, whether feelings differ for IRRs and IFs, and describe predictors of such feelings. It will also examine variables related to the practice of return of results, including demographic factors, research characteristics, and feelings of obligation. Lastly, for those who do not return results, this study will describe what went into their decision. This will involve a secondary data analysis of a larger survey of genetics researchers (who are members of the American Society of Human Genetics) and biobank managers about to be conducted by the Genetics and Public Policy Center that seeks to describe perspectives about current issues in genetics including consent, privacy protections, data sharing, and the return of individual research results.

1. National Human Genome Research Institute

2. Johns Hopkins School of Public Health

3. Genetics and Public Policy Center

NHGRI Zebrafish Core Facility

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Poster

Raman Sood [1], Kevin Bishop [1], Blake Carrington [1]

The external development and optical clarity of zebrafish embryos make them an ideal model system for functional genomics. They are also beneficial for generating disease models to understand the pathophysiology of disease and to develop therapeutic approaches. NHGRI established a Zebrafish Core in 2005 to facilitate zebrafish research without the need to dedicate space and resources of multiple laboratories. The Core provides the following resources and technical services:

• Generations of genetic mutants using Zinc Finger (ZFNs), Tal effector Nucleases (ZFNs), and Clustered Regularly Interspaced Short Palindromic Repeat (CRISPERs)

• Microinjections to transiently knockdown gene expression using morpholinos, induce over expression using mRNA and or generate transgenic lines using reporter constructs.

• Whole mount in situ hybridizations using embryos fixed at a series developmental stages to determine the spatial and temporal expression of genes.

• Maintenance of commonly used wildtype strains, mutant lines and transgenic lines

1. NHGRI

Identification of a potential chemotherapeutic agent to treat lynch syndrome tumors

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Poster

Yongliang Zhang [1], Jennifer Fox [1,2], Suhyeon Yoon [1], Kyungjae Myung [1]

Impairing the cell division of rapidly dividing cancer cells by small molecules has been a primary goal in the development of chemotherapeutic agents. One way to impair cell division is by the administration of genotoxic compounds that increase DNA damage to the point of inducing cancer cell death. Cancer cells often exhibit deficiencies in one of the major DNA repair or damage response pathways. Thus, the identification of chemotherapeutic agents acting on compromised DNA repair pathways in cancer cells would result in more efficient and personalized treatment to kill cancer cells. Lynch Syndrome tumors have proven to be resistant to most conventional chemotherapeutic agents, making surgery the best treatment option available. We screened DNA damaging agents for their ability to selectively kill cells with mutations in the MSH2 gene, and identified baicalein as a small molecule that could target MMR-deficient cancer cells. Baicalein is a flavone, and we found that it can intercalate DNA, triggering a DNA damage response in MSH2-proficeint cells. We also found that in MSH2-deficient cells, continued replication in the presence of baicalein leads to the formation of DNA double-strand breaks and ultimately results in apoptosis in the G2/M phase of the cell cycle. These data, combined with our previous finding that baicalein is not mutagenic, suggest that baicalein may offer an improved treatment option for patients with tumors characterized mutations in the MMR pathway.

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Early-Onset Stroke and Vasculopathy Associated with Mutations in ADA2

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Poster

Qing Zhou [1], Dan Yang [2], Amanda Ombrello [1], Camilo Toro [3], Raman Sood [4], Shawn Burgess [5], Manfred Boehm [2], Daniel Kastner [1], Ivona Aksentijevich [1]

Background: We observed a syndrome of intermittent fevers and early-onset lacunar strokes, livedoid rash, hepatosplenomegaly, and systemic vasculopathy in three unrelated patients. We suspected a genetic cause because the disorder presented in early childhood.
Methods: We performed whole-exome sequencing on the initial three patients and their unaffected parents, and candidate gene sequencing in three patients with a similar phenotype, two children with polyarteritis nodosum (PAN), and one patient with small vessel vasculitis. Enzyme assays, immunoblotting, immunohistochemistry, flow cytometry, and cytokine profiling were also performed on patient samples. We used morpholino knockdowns in zebrafish and shRNA knockdowns in U937 cells cultured with human dermal endothelial cells to study gene function.
Results: All nine patients carried recessively inherited predicted-deleterious mutations in CECR1, encoding adenosine deaminase 2 (ADA2), that were rare or absent in healthy controls. Six patients were compound heterozygous for eight different ADA2 mutations, while the three patients with PAN or small vessel vasculitis were homozygous for the p.Gly47Arg ADA2 mutation. Patients had a marked reduction in blood ADA2, and dramatically reduced ADA2-specific enzyme activity. Skin, liver, and brain biopsies demonstrated vasculopathic changes characterized by compromised endothelial integrity, endothelial cellular activation, and inflammation. Knockdown of a zebrafish ADA2 homolog caused intracranial hemorrhages and neutropenia, phenotypes that were rescued by wild type but not mutant human CECR1. Patient monocytes induced damage in cocultured endothelial cell layers.
Conclusion: Loss-of-function mutations in CECR1 are associated with a spectrum of vascular and inflammatory phenotypes ranging from early-onset recurrent stroke to systemic vasculopathy and/or vasculitis.

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Using the Encyclopedia of DNA Elements (ENCODE) to Better Understand Human Disease

Xiao-Qiao Zhou [1], Preetha Nandi [1], Elise Feingold [1], Mike Pazin [1], Peter Good [1], On behalf of the ENCODE Consortium

After completing the sequence of the human genome, biologists faced the challenges of interpreting this sequence and understanding its role in health and disease. The purpose of the Encyclopedia of DNA Elements (ENCODE) Project is to create a public resource of high-quality data that identifies all functional elements of the human genome. This poster will demonstrate how to access and download ENCODE data through the public portal, as well as provide examples of how ENCODE data can be used to study human disease.

The ENCODE Consortium consists of data production, analysis, and coordination centers that apply high-throughput biochemical assays to a variety of human and mouse cell types. All ENCODE data are rapidly released to the public through the ENCODE portal (www.encodeproject.org). ENCODE data have been used in over 290 publications by researchers outside of the project in a range of applications. For example, ENCODE has commonly been used to interpret genome-wide associations that lie in non-protein coding regions of the genome. A package of 30 papers published in 2012 by the ENCODE Consortium provided integrative analysis of ENCODE data and illustrated ways of using and accessing the data.

ENCODE data can be used to help identify functional variants, target genes, or target cell types in order to refine hypotheses from genetic studies. ENCODE hopes to enable the scientific and medical communities to interpret the human genome sequence in order to better understand human biology and to improve health.

1. Division of Genome Sciences, Extramural Research Programs

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