Burroughs Wellcome Fund
Maryland Genetics, Epidemiology and Medicine (MD-GEM) Training Grant

Abstract Book
Genetics Research Day
February 27, 2015

Contents

Dear Participants,

On behalf of the Maryland-Genetics, Epidemiology, Medicine Training Program (MD-GEM) it is our pleasure to welcome you to the second annual Genetics Research Day at Johns Hopkins University. MD-GEM includes faculty spanning the Mckusick-Nathans Institute of Genetic Medicine, the Johns Hopkins Bloomberg School of Public Health, the Johns Hopkins School of Medicine and the National Human Genome Research Institute, who join together to train doctoral students in population and laboratory sciences focused on genetics.

This Genetics Research Day provides the greater JHU community an opportunity to promote discussion and collaboration across JHU/NHGRI and to integrate students from different disciplines into the wide breadth of genetics research. We welcome all faculty, post-doctoral fellows and students, especially those new to the field of genetics, We look forward to continued partnerships and new relationships across the fields of Epidemiology, Biostatistics, Human Genetics, Biology, Computer Science, Mathematics and more. The posters represent the Departments of Biochemistry and Molecular Biology Epidemiology, Biostatistics, and Mental Health in the Bloomberg School of Public Health; the Departments of Human Genetics, Oncology, Pathology, and Pediatrics in the School of Medicine; The Whiting School of Engineering, Johns Hopkins University; The Berman Institute for Bioethics, The Greenberg Center for Skeletal Dysplasias, The Lieber Institute for Brain Development, The McKusick-Nathans Institute for Genetic Medicine,The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University; and the Computational and Statistical Genomics Branch and Medical Genetics Branch of the National Human Genome Research Institute.

A very special thank you to Dr. Nancy Cox, Vanderbilt University, for joining us as our plenary speaker. Thank you to all of the faculty judges who have generously lent us their expertise and time and to whom we are indebted. We extend our sincere thanks to Sandy Muscelli, Jon Eichberger and Tracie Wyman for all of their help in organizing and promoting this event. We are especially grateful for the tireless efforts of Jennifer Deal who graciously attended to every detail to bring this day together.

Thank you for participating.

Sincerely,

Priya Duggal, PhD, MPH
Director, MD-GEM
Johns Hopkins Bloomberg School of Public Health

David Valle, MD, PHD
Director, MD-GEM
Mckusick-Nathans Institute of Genetics Medicine

Dani Fallin, PhD
Associate Director, MD-GEM
Johns Hopkins Bloomberg School of Public Health

Nancy J. Cox is a quantitative human geneticist focused on identifying and characterizing the genetic component to human common diseases and related complex traits. She was educated at the University of Notre Dame 1974-1978 (B.S. in Biology) and Yale University 1978-1982 (PhD in Human Genetics), and did post-doctoral research at Washington University and the University of Pennsylvania before settling at the University of Chicago as a faculty member for 28 years. She moved in December of 2014 to Vanderbilt University as the Mary Phillips Edmonds Gray Professor of Genetics and Director of the Vanderbilt Genetics Institute and the Division of Genetic Medicine. A major current focus of Dr. Cox's research is integrating information on genome function into studies of common and rare variants in common disease. In particular, she is now focused on conducting these activities within BioVU, the Vanderbilt University biobank with more than 195,000 subjects, to enable further discovery and translational genomic science for common human disease.

Burroughs Wellcome Fund

The *Burroughs Wellcome Fund* is an independent private foundation dedicated to advancing the biomedical sciences by supporting research and other scientific and educational activities. Within this broad mission, BWF has two primary goals:

- To help scientists early in their careers develop as independent investigators
- To advance fields in the basic biomedical sciences that are undervalued or in need of particular encouragement

BWF's financial support is channeled primarily through competitive peer-reviewed award programs. A Board of Directors comprising distinguished scientists and business leaders governs BWF. BWF was founded in 1955 as the corporate foundation of the pharmaceutical firm Burroughs Wellcome Co. In 1993, a generous gift from the Wellcome Trust in the United Kingdom, enabled BWF to become fully independent from the company, which was acquired by Glaxo in 1995. BWF has no affiliation with any corporation.

http://www.bwfund.org/

Maryland Genetics, Epidemiology and Medicine (MD-GEM) Training Program

The *Maryland Genetics, Epidemiology and Medicine (MD-GEM)* is a pre-doctoral training program that comprehensively integrates Genetics, Epidemiology, and Medicine (GEM). Funded by the Burroughs-Wellcome Fund, the MD-GEM training grant brings together the expertise and training infrastructure of the Johns Hopkins Schools of Public Health and Medicine and the National Human Genome Research Institute. Together, these three institutions can provide laboratory, methodological and clinical expertise and coursework to train the next generation of scientists who can forge new avenues of research and address the rapidly changing field of human genetics. This program trains pre-doctoral students through integration of these important areas by partnering with established mentors and offering integrated learning. We envision a training program that will prepare scientists for the next generation of genetics research.

http://www.hopkinsgenetics.org/

MD-GEM Faculty

Priya Duggal, Co-Director
David Valle, Co-Director
M. Daniele Fallin, Associate Director
Dan Arking
Dimitrios Avramopoulos
Joan E. Bailey-Wilson
Terri Beaty
Aravinda Chakravarti
Debra Mathews
Ingo Ruczinski
Steven Salzberg
Kathleen Barnes
Diane M. Becker
Lewis Becker
Larry Brody
Josef Coresh
Derek Cummings
Gary Cutting
Jennifer Deal
Hal Dietz
Andrew Feinberg
Gail Geller
Ada Hamosh
Kasper Hansen
Julie Hoover-Fong
William Isaacs
Lisa Jacobson
Corrine Keet
Alison Klein
Christine Ladd-Acosta
Jeffrey Leek
Justin Lessler
Brion Maher
Rasika Mathias
Shruti Mehta
Ana Navas-Acien
Elaine A. Ostrander
Ann Pulver
Stuart Ray
Debra Roter
Robert Scharpf
Alan Scott
Margaret Taub
David Thomas
Kala Visvanathan
Jeremy Walston
Xiaobin Wang
Alexander Wilson
Robert Wojciechowski
Peter Zandi

20	Bracha Erlanger	Metaplastic Breast Cancer: Tracking Changes	22
21	Rachel Dvoskin	Ethical, Legal and Social Implications of Genomics in Infectious Disease Management	20
22	Dylan McLaughlin	Does SUMO Wrestle Thymine DNA Glycosylase Off DNA?	34
23	Xuan Pham	A polymorphic di-nucleotide repeat (DNR) variant in the 5'UTR of *DPYSL2* gene affects its regulation via mTOR signaling	37
24	Nicole Eckart	Regulatory Function of Schizophrenia-Associated Variants in CACNA1C	21
25	Dolly Singh	Comparison of GDNF mRNA Expression in Testes of Fertile and Infertile Men and Fertile Mice	39
26	Danyelle Winchester	Genes Involved in the Immune Response and Intraprostatic Inflammation in the Placebo Arm of the Prostate Cancer Prevention Trial	42
27	Kipper Fletez-Brant	Histone Mark Signal Correlates with Gene Expression for Fixed Regions Across Individuals	25
28	Margaret M Parker	Exome Array Analysis of Pulmonary Function (FEV1/FVC) in the COPDgene Study	36
29	Samuel F. Gilbert	Analysis of a Histiocytic Sarcoma Locus – Canine's Utility for Fetching Candidates	26
30	Fei Chen	Exome Array Analysis Identified Novel Susceptibility Loci for Refractive Error	18
31	Rebecca L Beer	Adult zebrafish centroacinar cells contribute to β-cell regeneration and are similar to larval endocrine pancreas progenitors	13
32	William Wu	Exploration of Functional Consequences of Alu Insertion Polymorphisms in ACE and ARID5B	43
33	Poojitha Balakrishnan	PPAR-γ is Associated with HOMA-B among American Indians: the Strong Heart Family Study	10
34	Melissa Russo	Prenatal Diagnosis of Campomelic Dysplasia via 3D Ultrasound of the Scapula	38
35	Bashira A. Charles	Metabolic Products of Purine Metabolism Play a Role in Development of Proliferative Diabetic	17

Blood-brain DNA Methylation Concordance in Autism Spectrum Disorders

Shan Andrews[1], Lisa Croen[2], Laura Schieve[3], Kasper D. Hansen[1], Brian K. Lee[4], Craig Newschaffer[4], Andrew P. Feinberg[1], Christine Ladd-Acosta[1], and M. Daniele Fallin[1]

[1]Johns Hopkins University
[2]Kaiser Permanente
[3]Centers for Disease Control and Prevention
[4]Drexel University School of Public Health

Presented by Shan Andrews

Background: Epigenetic processes in the brain have been investigated for their potential implications in many neurological disorders, because of the important role of epigenetics in brain function and development. While many useful epigenetic signatures have been discovered in the brain for numerous disorders, such as Alzheimer and Parkinson diseases, and autism spectrum disorders (ASD), an obvious limitation is the inability to test for them pre-mortem. Objectives: The purpose of this work is to identify the extent to which a more easily-accessible tissue, such as blood, can be used as an indicator of epigenetic signatures for ASD that have been discovered in the brain. Methods: Ladd-Acosta et al. found four regions of the genome to be differentially methylated between ASD cases and controls in a cohort of 41 post-mortem brain tissue samples. We will attempt to replicate these regions using methylation measurements in whole-blood derived DNA from 609 individuals, including 292 ASD cases and 317 controls, enrolled in the Study to Explore Early Development (SEED), a multisite population-based case-control study of children aged 2-5 years with ASD and a control group drawn from the general population. Results: We will present results detailing the extent to which differentially methylated regions discovered in the brain samples are replicated in the SEED cohort. Conclusion: The utility of replication in this context would be to provide additional evidence for the methylation differences identified in these regions, and to inform the development of non-invasive biomarkers for both ASD and ASD-related exposures.

Content Area: Computational Genetics, Genetic Epidemiology
Keywords: DNA methylation, Autism Spectrum Disorder

PPAR-γ is Associated with HOMA-B among American Indians: the Strong Heart Family Study

Poojitha Balakrishnan[1-2], Dhananjay Vaidya[1,3], Nora Franceschini[4], V. Saroja Voruganti[5], Kari E. North[4], Karin Haack[6], Sandra Laston[6], Barbara V. Howard[7,8], Jason Umans[7,8], Elisa T. Lee[9], Lyle G. Best[10], Jean W. MacCluer[6], Shelley A. Cole[6], Ana Navas-Acien[1-2,11-12]

[1] Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland;
[2] The Welch Center for Prevention, Epidemiology and Clinical Research, Johns Hopkins Medical Institutions, Baltimore, Maryland
[3] Clinical and Translational Research, Johns Hopkins School of Medicine, Baltimore, Maryland
[4] Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina
[5] Department of Nutrition and UNC Nutrition Research Institute, University of North Carolina at Chapel Hill, Kannapolis, North Carolina
[6] Department of Genetics, Texas Biomedical Research Institute, San Antonio, Texas
[7] MedStar Health Research Institute, Hyattsville, Maryland
[8] Georgetown and Howard Universities Center for Clinical and Translational Science, Washington, DC
[9] Center for American Indian Health Research, College of Public Health, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma
[10] Missouri Breaks Industries Research, Inc., Timber Lake, South Dakota
[11] Department of Environmental Health Sciences, Johns Hopkins University Bloomberg School of Public Health, Baltimore, Maryland
[12] Department of Oncology, Johns Hopkins School of Medicine, Baltimore, Maryland

Presented by Poojitha Balakrishnan

Background: According to the American Diabetes Association (ADA), 9.3% or around 29.1 million U.S. adults have type 2 diabetes (T2D). American Indians are disproportionately affected by T2D, with a U.S. prevalence of 15.9% and around 50% at baseline in the Strong Heart Study (SHS). Previous studies have been successful in identifying loci for metabolic traits in European Americans; PPAR-γ has been highlighted for a range of disorders, including T2D and dyslipidemia, and regulates adipocyte development and glucose metabolism. Our objective is to examine whether PPAR-γ is associated with metabolic traits in American Indians, including T2D, Homeostasis Model Assessment – Insulin Resistance (HOMA-IR), and Homeostasis Model Assessment – β cell dysfunction (HOMA-B).

Methods and Materials: The Strong Heart Family Study (SHFS), an expansion of the original SHS into a multigenerational family study includes 3,800 American Indian participants, 2,415 of whom were free of diabetes at baseline with complete data, and recruited from Arizona, Oklahoma and North and South Dakota. T2D was defined using ADA criteria: fasting plasma glucose ≥126 mg/dL, or use of insulin or oral hypoglycemic medications. The HOMA-IR and HOMA-B were calculated from fasting plasma glucose and insulin measurements at baseline. The single nucleotide polymorphisms (SNPs) were genotyped with Illumina MetaboChip and were imputed accounting for family structure with Merlin using all reference populations in 1000 Genomes. SNP quality control included Hardy-Weinberg equilibrium, Mendelian error (>5%), genotyping inconsistencies, excess identity by state (IBS) sharing, mismatch for gender, and outliers in either IBS or principal components analysis. Association analyses were performed under an additive model using logistic regression for T2D and linear regression for baseline log-transformed HOMA-IR and HOMA-B. These models were adjusted for age, sex and body mass index. Population stratification was assessed with Linkage Disequilibrium Analyses for Quantitative and Discrete Traits

(QTDT) using pedigree structures for top SNPs. All p-values were corrected for multiple testing (p <5.9x10-4).

Results: Over a median follow-up of 5.9 years (range 3-12.25), 367 out of 2415 individuals developed T2D. At baseline, the mean log-transformed HOMA-IR was 1.12 and the mean log-transformed HOMA-B was 0.14. After imputation and quality control, there were a total of 242 SNPs on PPAR-γ located on chromosome 3. All 242 SNPs were statistically significant for baseline log-transformed HOMA-B. The index SNP rs73027216; C>A) was associated with better beta-cell function (p 1.86x10-30). No SNPs passed multiple testing correction for other T2D-realted traits. The top SNP for T2D (rs9814788; C>A) had nominal p 0.17 and the top SNP for baseline log-transformed HOMA-IR (rs115004741; A>G) had nominal p 1.99x10-3. QTDT analysis showed little evidence of population stratification.\

Conclusion: Common PPAR-γ variants affect beta cell function, implicating that PPAR-γ based drugs may be more effective based on genotype. Given the difference in allele frequencies, PPAR-γ variants may partially explain the difference in prevalence of T2D in American Indians. The absence of statistically significant associations for the other traits could be due to power, differences in linkage disequilibrium or genetic architecture in American Indians. Further investigation of common variants among American Indians is warranted.

Content Area: Genetic Epidemiology
Keywords: American Indians, Diabetes, Association Analysis

Undiagnosed Stickler Syndrome: The Value of Genetics in Cleft Clinic

Natalie Beck[1], Carrie Blout[1], Kim Seifert[2], Richard Redett[2], and Julie Hoover-Fong[1]

[1] Greenberg Center for Skeletal Dysplasias, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University
[2] Department of Plastic and Reconstructive Surgery, Johns Hopkins Hospital

Presented by Natalie Beck

BACKGROUND: Autosomal dominant Stickler syndrome refers to a group of connective tissue disorders due to abnormalities in the COL2A1, COL11A1, COL11A2 genes. Mutations in these genes lead to dysfunctional assembly of collagens II and/or XI that affect the ocular, auditory, craniofacial and musculoskeletal systems. Patients with Stickler syndrome often have hearing loss, precocious arthritis, and palatal anomalies with ocular manifestations (e.g. high myopia) found only in patients with COL2A1 and COL11A1 mutations. Due to the multisystem involvement, patients with Stickler syndrome are followed by multiple providers who may raise the suspicion for this diagnosis that includes multidisciplinary cleft clinic (including plastic surgery, ENT, audiology, nutrition/airway management), genetics, genetic counseling, ophthalmology, and orthopedics as needed. As active care providers in a large multidisciplinary cleft clinic, our team of genetics professionals has diagnosed a cohort of previously undiagnosed patients with Stickler syndrome; allowing for expansion of access to genetics for patients and improved recognition of the variability of Stickler syndrome among the cleft clinic care team.

METHODS: A retrospective review of the clinical database maintained for patients evaluated by the genetics professionals in the multidisciplinary cleft clinic for 11 consecutive months beginning in August 2012 was done.

RESULTS: In the cleft clinic, 167 unique patients were evaluated by Genetics. Of those, we clinically suspected 10 (5.99%) to have Stickler syndrome; 7/10 had previously been evaluated by a genetics professional previously and a diagnosis of Stickler syndrome had been suggested in 2. Following our evaluations, 1 patient was determined to have multiple epiphyseal dysplasia by molecular testing (i.e. mutation in DTDST), with 6 of the remaining 9 patients (66.67%) completing molecular testing for Stickler syndrome, 5 having pathogenic mutations and 1 with negative results.

DISCUSSION: Genetic professionals within the multidisciplinary cleft clinic provided expanded services to previously suspected and new patients with Stickler syndrome, with significant phenotypic variation observed. Our evaluations led to confirmed diagnoses of Stickler syndrome and clarification of a patient with another genetic syndrome. A confirmed diagnosis allows for targeted genetic counseling for patient medical management, confirmatory parental testing, and accurate recurrence risks. Patients with pleiotropic disorders like Stickler syndrome may present to a variety of subspecialists for care; we have demonstrated that the addition of genetics professionals to these groups allows for consolidation and coordination of patient management through accurate diagnoses and counseling.

Adult zebrafish centroacinar cells contribute to β-cell regeneration and are similar to larval endocrine pancreas progenitors

Rebecca L Beer[1], Fabien Delaspre[1], Meritxell Rovira[2], Wei Huang[1], Guangliang Wang[1], Stephen Gee[1], Maria del Carmen Vitery[1], Sarah J Wheelan[1], and Michael J Parsons[1]

[1] Institute of Genetic Medicine, Johns Hopkins School of Medicine
[2] Genomic Programming of Beta-Cells Laboratory, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS)

Presented by Rebecca L Beer

Diabetes is associated with a paucity of insulin-producing β cells. With the goal of finding therapeutic routes to treat diabetes, we aim to find molecular and cellular mechanisms involved in β-cell neogenesis and regeneration. To facilitate discovery of such mechanisms we utilize a vertebrate organism where pancreatic cells readily regenerate. The larval zebrafish pancreas contains Notch-responsive progenitors that during development give rise to adult ductal, endocrine, and centroacinar cells (CACs). Adult CACs are also Notch-responsive and are morphologically similar to their larval predecessors. To test our hypothesis that adult CACs are also progenitors we took two complementary approaches: 1) we established the transcriptome for adult CACs. Using gene ontology and in situ hybridization we found that the CAC transcriptome is enriched for progenitor markers. 2) Using lineage tracing we demonstrated that CACs do form new endocrine cells following β-cell ablation or partial pancreatectomy. We concluded that CACs and their larval predecessors are the same cell type and represent an opportune model by which to study both β-cell neogenesis and β-cell regeneration.

Content Area: Molecular Genetics
Keywords: diabetes, regeneration, pancreas, beta cells

A Method to Identify Causal Rare Variants Using Targeted Sequence Data

Ferdouse Begum[1], Ingo Ruczinski[2], Terri H. Beaty[1], and Margaret Taub[2]

1 Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Maryland, USA
2 Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Maryland, USA

Presented by Ferdouse Begum

High-throughput sequencing data holds the promise of identifying specific mutations directly involved in disease etiology by sifting through all DNA variants. Several statistical methods for identifying rare causal variants from sequencing data using case-control study designs are available. Methods for identifying causal variants using sequencing data from family-based studies are less well studied. Available collapsing methods do not attempt to isolate the truly causal variants. We are developing methods for targeted sequence data from case-parent trios (an affected child with both parents). The focus of this study is to identify sets of functional rare variant sets in or near regions of established association/linkage with common variants. Using different experimental thresholds, we focused on only sets of rare variant predicted to damage the gene product (MAF<1%, CADD>20) and searched for sets of rare variants which are transmitted with the risk allele at a SNP showing evidence of association and the corresponding set of rare variants not transmitted with the 'risk' allele but transmitted with the 'protective' allele. We test for a minimal set of rare variants, which can separate the maximum number of people. Our data set came from a targeted sequencing study of ~1500 case-parent trios (both parents and affected child with oral clefts) from three ethnic groups (of Chinese, Filipino and European ancestry). Among 13 targeted regions, one 700kb long region encompassed the MAFB gene on chromosome 20. We separately analyzed different ethnic groups and here discuss the Chinese. Analysis of common variants showed significant signal for linkage and association at SNP rs2865509 ($p<1x10^{-6}$ for T allele) among Chinese families. We identified a cluster of 33 rare variants with transmitted haplotypes carrying risk allele T at rs2865509 and a cluster of 33 rare variants on untransmitted haplotypes carrying protective allele C at rs2865509. The total of these 66 rare SNVs could distinguish 80 individuals with T or C alleles at SNP rs2865509. We plan to develop permutation-based tests to assess evidence that these rare variant sets are associated with the deleterious or protective allele at the common variant beyond what is expected by chance. We will expand this approach to take into account the linkage disequilibrium structure and further characterize putative harmful effects of the sets of rare variants. We are exploring patterns across all thirteen targeted regions on different chromosomes to integrate information.

Content Area: Statistical Genetics
Keywords: Trio, Rare variant, Targeted sequence

ABCA4 Variants in Stargardt Disease: Preliminary Results from the ProgStar Study

Samantha Bomotti[1], Rupert W. Strauss[2,3], Hendrick P. Scholl[4], Robert Wojciechowski[1,3]

[1] Department of Epidemiology, Bloomberg School of Public Health, The Johns Hopkins University, Baltimore, MD, USA
[2] Department of Ophthalmology, Medical University Graz, Graz, Austria
[3] Johns Hopkins Wilmer Eye Institute, The Johns Hopkins University, Baltimore, MD, USA

Presented by Samantha Bomotti

Purpose: Stargardt disease type 1 (OMIM 248200) is the most common juvenile macular dystrophy. It follows an autosomal recessive mode of inheritance. There is currently no cure and Stargardt remains difficult to diagnose due to its high allelic and phenotypic heterogeneity. We are compiling phenotypic data and genetic variant information at the ABCA4 gene in Stargardt patients at nine clinical centers across the United States and Europe. The data will be used to establish genotype-phenotype relationships for ABCA4 mutations, and to identify the effects of variants of unknown clinical significance.

Methods: To date, genetic and clinical data have been collected from 244 Stargardt patients. A variety of biological assays have been employed to identify the ABCA4 variants, depending on the technologies in use at the time of diagnosis (from 2008 or earlier to present). We report on the distribution of ABCA4 variants in this selected Stargardt population. These preliminary analyses are being conducted as the ProgStar study nears enrollment completion, at which point complete data will be available for in-depth analysis.

Results: Among the 244 Stargardt individuals for whom we have genotype information thus far, the majority (86.9%) were heterozygous for ABCA4 mutations. In 26 cases (10.7%), only one ABCA4 mutation was identified, suggesting incomplete coverage of the functional regions of ABCA4 or locus heterogeneity. The most common variant was c.5882G>A, found in 67 (27.5%) participants. Three (4.5%) of these individuals were homozygous for this variant. The second most common mutation, c.2588G>C, was observed in one copy in 28 (11.5%) individuals. These variants are among the most commonly found in previously published data. Thirty-three individuals (13.5%) had three putatively pathogenic ABCA4 variants and three (1.2%) had four variants.

Conclusions: Initial mutation analyses in the ProgStar study confirm the high allelic heterogeneity of Stargardt disease. These data will shed new light on variant effects on the progression of Stargardt disease. It should be noted that testing protocols varied considerably across testing laboratories that contributed data to ProgStar. Most assays were based on mutations known at the time of testing, and favored screening of more common mutations. High-throughput sequencing technologies will allow for an unbiased ascertainment of pathogenic ABCA4 mutations in Stargardt disease.

Content Area: Genetic Epidemiology
Keywords: Stargardt, ABCA4, Macular dystrophy

Kabuki Syndrome, a Disorder of Histone Methylation, Demonstrates Characteristic DNA Methylation Abnormalities Illustrating Potential Interplay between Histone and DNA Methylation Machineries

Brucato M[1], Sobreira N[2], Zhang L[2], Ladd-Acosta C[1], Ongaco C[3], Romm J[3], Baker M[2], Doheny K[3], Doheny K[3,4], Chong K[4], Perez ABA[5], Melaragno M[5], Meloni V[5], Valle D[2], and Bjornsson HT[2,6]

[1] Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
[2] Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD
[3] Center for Inherited Disease Research (CIDR), Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD
[4] Unidade de Genética, Instituto da Criança, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
[5] Genetics Division, Department of Morphology and Genetics, Universidade Federal de São Paulo, Brazil
[6] Department of Pediatrics at the Johns Hopkins University School of Medicine, Baltimore, MD

Presented by Martha Brucato

Kabuki Syndrome (KS) is a genetic cause of intellectual disability which also leads to a characteristic facial dysmorphology. About 80% of individuals with KS have a mutation in KMT2D, a histone methyltransferase gene, or KDM6A, a histone demethylase gene; this suggests that KS is primarily a disorder of dysregulated histone methylation. However, in 20% of clinically defined cases of KS, no genetic cause can yet be identified. We aim to further elucidate KS etiology so that additional patients can receive a definitive molecular diagnosis. We have collected a cohort of clinically diagnosed KS patients (n=29) and age-and-sex-matched controls (n=9) from Brazil. Here we report detection of de novo mutations in an additional histone methyltransferase gene, KMT2A, in two KS patients who have no other probable causative mutation. This gene has not been previously associated with KS. Notably, there is phenotypic overlap between KS and ICF (Immunodeficiency, Centromeric region instability, Facial anomalies syndrome), a Mendelian genetic disease that is caused by mutations in DNA methylation machinery. To further explore the mechanisms for disease development and pathogenesis in KS, given that mutations in histone or DNA methylation can lead to a similar disease phenotype, we conducted a genome-scale investigation of DNA methylation in our KS cohort using the Illumina Infinium HumanMethylation450 BeadChip platform. The cohort was divided into patients with identified mutations in histone machinery (KMT2D and KMT2A mutations, n = 13) and those without an identified genetic cause or a non-histone machinery mutation (ZBTB24, DNMT3B, HCFC1; n = 15). The first grouping of patients represents the canonical mechanism of KS, and may have different underlying DNA methylation alterations. The canonical KS patients were compared to normal controls using two complementary approaches to detect differential methylation. First, we searched for single differentially methylated positions (DMPs). Second, we used a region-finding approach that can take advantage of probe clustering on the 450k platform to detect larger areas of consistent methylation differences between cases and controls (differentially methylated regions, DMRs). Two DMPs met the strict Bonferroni cut-off for significance ($p < 1.0e-7$) and an additional 55 DMPs had a false discovery rate q-value of < 0.05; no significant DMRs were found with a family-wise error rate of < 0.05. Similarly, KS patients without an observed mutation in the histone machinery genes were compared to controls using single site and region finding approaches. No significant DMPs were identified; one significant DMR was detected near the ZFP57 gene. Suspecting a lack of power due to small sample size, we went on to test some non-significant but promising findings using bisulphite pyrosequencing, a quantitative and independent measurement technology. Significant differences ($p < 0.01$) were found in DNA methylation in both groups of KS patients compared to controls at regions near the genes DEGS2, MYO1F and LAMB2. These results demonstrate that a disorder of histone methylation leads to characteristic DNA methylation abnormalities, suggesting interplay between histone methylation and DNA methylation machinery.

Content Area: Genetic Epidemiology
Keywords: Kabuki syndrome, epigenetics, DNA methylation

Metabolic Products of Purine Metabolism Play a Role in Development of Proliferative Diabetic Retinopathy

Bashira A. Charles[1], Yvette P. Conley[2], Susan M. Sereika[2], Rachel G. Miller[2], Charles N. Rotimi[3], Joan Bailey-Wilson[1], Michael B. Gorin[4], Robert E. Ferrell[2], and Trevor J. Orchard[2]

1 Computational and Statistical Genomics Branch, National Human Genome Research Institute
2 University of Pittsburgh
3 Center for Research on Genomics and Global Health, National Human Genome Research Institute
4 Jules Stein Eye Institute, University of California Los Angeles

Presented by Bashira A. Charles

Uric acid is a catabolic end product of purine metabolism in man. Most mammals possess a functioning urate oxidase (UOX) gene, which produces the protein urate ozidase. This enzyme binds with copper to oxidize uric acid to allantoin. Allantoin is then filtered by the kidneys and excreted in the urine. Humans developed non-sense mutations for the UOX gene greater than 8 million years ago, making it impossible for humans to catabolize uric acid to allantoin resulting in an accumulation of uric acid in the blood which is then filtered by the kidneys and excreted in the urine. Excessive consumption of alcohol, excessive intake of foods high in purine content, and or ineffective renal excretion of uric acid due to kidney disease may result in development of elevated serum uric acid levels (hyperuricemia). Genome-wide association studies in European and African-ancestry samples have identified the Solute Carrier Family 2 Member 9 (SLC2A9) gene to be associated with serum uric acid levels in human samples.

Hyperuricemia is associated with hypertension, endothelial dysfunction, and diabetes. Diabetes affects 8.3% of the global population and 9.3% United States population, placing affected individuals at an increased risk for developing diabetic retinopathy, a microvascular complication of diabetes. Approximately 5% of the individuals diagnosed with diabetes have type 1 diabetes, placing them at an increased risk of developing proliferative diabetic retinopathy (PDR), the most vision threatening form of the disease.

Adenosine and its receptors are in the same metabolic pathway as uric acid. Adenosine acting via its receptors ameliorates the hypoxia, oxidative stress, cellular damage and tissue injury caused by diabetes. It also plays a role in glucose transport and promotes angiogenesis. Hyperuricemia is pro-inflammatory and plays a role in tissue damage. Many individuals with diabetes are hyperuricemic and uric acid has been identified in the vitreous of individuals with DR. Most individuals with Type 1 Diabetes (T1D) develop some form of DR, however not all progress to PDR, the most vision threatening form of the disease. Prior to the Diabetes Control and Complications Trial (DCCT) 50% and 60% of individuals developed PDR after 15 years and 20 years with T1D respectively. Post DCCT 39% and 50% developed PDR after 20 and 25 years with T1D respectively.

To provide insight into the genetic basis of Adenosine in the development of PDR we conducted a candidate gene association study in 496 participants of the Epidemiology of Diabetes Complications prospective (25 years) cohort of individuals with Type 1 Diabetes (T1D). Variants of the Adenosine A2A receptor (ADORA2A) gene were investigated for association with baseline, incident, and any PDR during the course of the study.

Data were analyzed with logistic regression and Cox proportional hazards regression models using PLINK and SAS statistical software packages. Recessive, dominant and additive genetic models were explored.

Variant rs2236624 (odds ratio=0.18; p-value=0.004) was associated with protection from development of PDR.

Our results demonstrate that homozygosity for the “T” allele for variants of ADORA2A, are associated with protection from development of PDR in this European Ancestry cohort.

Content Area: Genetic Epidemiology
Keywords: Uric, Diabetes, Retinopathy, Adenosine, Receptor

Exome Array Analysis Identified Novel Susceptibility Loci for Refractive Error

Fei Chen[1], Priya Duggal[1], Barbara E.K. Klein[2], Kristine E. Lee[2], Barbara Truitt[3], Ronald Klein[2], Sudha K. Iyengar[3], Alison P. Klein[1,4,5]

[1] Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA
2 Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin, USA
[3] Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio, USA
[4] Department of Oncology, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland, USA
[5] Department of Pathology, Johns Hopkins School of Medicine, Baltimore, Maryland, USA

Presented by Fei Chen

Purpose: Ocular spherical equivalent, the measurement of refraction, is the power of external lens required to focus images on the retina. The pathologic conditions associate with errors in refraction, myopia (nearsightedness) and hyperopia (farsightedness), are the most common eye disorders and the leading causes of visual impairment and blindness worldwide. The goal of this study is to identify rare and less common variants that influence refractive error.

Methods: After genotyping 1,632 individuals from a population-based cohort, the Beaver Dam Eye Study (BDES) using the Illumina exome array, we conducted both variant level and gene-level quantitative trait association analysis for mean spherical equivalent to 35,195 single nucleotide variants and 6,943 autosomal genes across the genome.

Results: Spherical equivalent was associated with two novel variants in TCTE1 gene region at 6p21.1 (rs2297336, MAF = 14.2%, β = -0.67, P = 3.5 × 10-7; rs324146, MAF = 17.2%, β = -0.59, P = 1.7 × 10-6), and with the FSCB gene at 14q21.1 (P = 3.0 × 10-6). We successfully replicated the association on rs634990 near GJD2 at 15q14 (MAF = 46.8%, β = -0.34, P = 2.1 × 10-4), and discovered a novel association with spherical equivalent on rs1550094 (MAF = 30.5%, β = -0.27, P = 8.0 × 10-3), a locus in PRSS56 at 2q37.1 that was previously reported for myopia.

Conclusions: Novel genetic variants and genes with multiple rare and less common variants may play a role in the control of spherical equivalent. Our results contribute to the increasing evidence of GJD2 gene and PRSS56 gene in the development of refractive errors. The implication of these two genes, as the common genetic factor for both spherical equivalent and myopia may provide new insights to the underlying mechanism leading to myopia and consequent vision loss.

Content Area: Genetic Epidemiology
Keywords: refractive error; susceptibility; exom

LINE-1 Expression and Retrotransposition in Barrett's Esophagus and Esophageal Carcinoma

Tara Doucet[1,2], Nemanja Rodić[3], Reema Sharma[3], Isha Darbari[1], Gabriela Abril[1], Jungbin A. Choi[1], Ji Young Ahn[1], Yulan Cheng[4], Kathleen H. Burns[1,3], Stephen J. Meltzer[4], and Haig H. Kazazian Jr.[1]

[1] McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA
[2] Pre-doctoral training program in Human Genetics, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205
[3] Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States of America
[4] Division of Gastroenterology and Hepatology, Department of Medicine and Department of Oncology, Johns Hopkins University, Baltimore, Maryland

Presented by Tara Doucet

Barrett's esophagus (BE) is a common disease in which the lining of the esophagus transitions from stratified squamous epithelium to metaplastic columnar epithelium that can predispose individuals to developing esophageal adenocarcinoma (EAC). We hypothesized that BE provides a unique environment for increased L1 retrotransposition. To test this hypothesis, we evaluated 5 patients with benign BE, 5 patients with BE and concomitant esophageal adenocarcinoma (EAC), and 10 additional patients with EAC to determine L1 activity in this progressive disease. Using L1-seq, we confirmed 118 somatic insertions by PCR in 10 of 20 individuals. We observed clonal amplification of several insertions which appeared to originate in normal esophagus (NE) or BE and were later clonally expanded in BE or in EAC. Additionally, we observed evidence of clonality within the EAC cases: specifically, 22 of 25 EAC-only insertions were present identically in distinct regions available from the same tumor (20 in 6/6 regions tested and 2 insertions in 2/2 available sections tested), suggesting that these insertions occurred in the founding tumor cell of these lesions. We detected ORF1p, one of two proteins encoded by L1, in 8/9 tumors evaluated with immunohistochemistry. In addition, Western blots revealed ORF1p expression in normal squamous esophagus, BE, and EAC suggesting that L1 expression is permitted in these tissues in BE patients. In summary, our data show that somatic retrotransposition occurs early in many patients with BE and EAC, and indicate that early events occurring even in histologically normal esophageal cells may be clonally expanded in esophageal adenocarcinogenesis.

Content Area: Human Genetics
Keywords: Retrotransposons, LINE-1, Cancer, Genomic Instability

Ethical, Legal and Social Implications of Genomics in Infectious Disease Management

Rachel Dvoskin[1], Theodore Bailey[1], Priya Duggal[2], Ruth A. Karron[2], Michelle H. Lewis[1], Daniel Salmon[2], Andrea Sutherland[2], Chloe L. Thio[3], Jeffrey P. Kahn[1] and Gail Geller[1,2,3]

[1] Berman Institute of Bioethics, Johns Hopkins University
[2] Bloomberg School of Public Health, Johns Hopkins University
[3] School of Medicine, Johns Hopkins University

Presented by Rachel Dvoskin

Advances in genomics are contributing to the development of more effective, personalized approaches to the prevention and treatment of infectious diseases. Genetic sequencing technologies are furthering our understanding of how genomic signatures in human hosts, pathogens and even vectors—and their interactions—contribute to individual differences in immunologic responses to infections, vaccines, and drug therapies. With the potential for tailored interventions for particular individuals, populations or subpopulations, ethical, legal and social implications (ELSIs) may arise for public health and clinical practice. Potential ELSI considerations include balancing health-related benefits and harms between individuals and the larger community, minimizing threats to individual privacy and autonomy, and ensuring just distribution of scarce resources.

The specific ELSI challenges that arise will depend not only on the type of genomic information that is available but also, importantly, on the characteristics of the disease in question, including disease severity, chronicity, ease (and mode) of transmission, preventability, and treatability. We will present specific examples of recent genomic advances and their current or potential implications for infectious disease management along with examples of the ELSI issues that could arise as a result. For example, there are genetic markers associated with increased susceptibility to influenza infection, with severity of illness from infection, and with response to vaccination. In the case of a flu pandemic, where infection can be spread casually and widely, one could imagine that genetic information could be used to make decisions about workforce restrictions on healthcare personnel. Consider an example of an infectious disease that is spread by more intimate contact: in the case of hepatitis B, genetic variants have been found to be associated with vaccine non-response. Could decisions about prioritizing access to therapy for vaccine non-responders be based on genotype, particularly in resource limited settings? Could people with a genetic predisposition to have no (or lower) protection from the hepatitis B vaccine be exempted from job-dependent mandatory vaccination? In light of the current Ebola crisis, it is still unknown whether human genetic variation contributes to individual differences in disease susceptibility and severity; however, might discoveries of this nature lead to genotype-based quarantine policy or travel restrictions or triage decisions in resource-limited settings?

We will address these types of questions, highlighting the need for addressing this current gap in scholarship, and the importance of anticipating these ELSI issues in advance of new scientific discoveries.

Content Area: Human Genetics; Other
Keywords: genomics, infectious disease, ethics, ELSI

Regulatory Function of Schizophrenia-Associated Variants in CACNA1C

Nicole Eckart[1], Ruihua Wang[1], Mariela Zeledon[1], Megan Szymanski-Pierce[1], David Valle[1,2], Dimitri Avramopoulos[1]

[1] McKusick-Nathans Institute of Genetic Medicine at Johns Hopkins University School of Medicine
[2] Department of Pediatrics at Johns Hopkins University School of Medicine

Presented by Nicole Eckart

Schizophrenia (SZ) and bipolar disorder (BP) are complex psychiatric disorders, together affecting over 3.5% of the US population. They have overlapping clinical presentations, and onset in the second or third decade of life. Association and family studies indicate a shared genetic risk. One variant that has been independently and repeatedly associated with both disorders is rs1006737, a single nucleotide polymorphism (SNP) in the third intron of the CACNA1C gene.

Variants identified by association studies are often in non-coding regions of the genome and enriched in expression quantitative trait loci (eQTLs). We previously reported that the risk allele of rs1006737 is correlated with decreased expression of CACNA1C ($p=0.001$) in a study of 195 post-mortem tissue samples from the superior temporal gyrus (STG). In 100 post-mortem tissue samples from the dorsolateral prefrontal cortex (DLPFC) we found that the risk allele trends toward increased expression of CACNA1C, .in agreement with published data from Bigos et al. in the DLPFC ($p=0.002$).

The variant rs1006737 tags a haplotype with several other SNPs, all located in the third intron. We are testing these for allele-specific regulatory potential using dual luciferase reporter assays. Following up on previous results, now using 4 biological replicates for each construct, we report that for two constructs, one containing rs2159100 and a second containing both rs1077306 and rs10744560, the risk alleles show statistically significant increases in luciferase expression compared to the common alleles when transiently transfected in HEK293 cells ($p=4.8*10\text{-}4$ and $p=2.1*10\text{-}3$, respectively).

In addition to differences in regulatory potential, we also explore allele-specific protein binding, using electrophoretic mobility shift assays (EMSAs). We show that 4 of 7 the SNPs tested have allelic differences in protein binding profiles in HEK293 and SK-N-SH cells. In a protein microarray, we find that the risk allele for rs11062170 binds 7 proteins that do not show strong binding with the common allele. These proteins include calcium dependent proteins (ANXA11), transcription factors (MAPK8, MAX, MLX), and splicing factors (ROD1).

Our data indicate that the haplotype tagged by rs1006737 plays a role in regulating transcription of CACNA1C. It appears there may be multiple functional variants, as we see allelic differences in enhancer activity as well as protein binding profiles for multiple SNPs.

Content Area: Human Genetics
Keywords: Schizophrenia, Regulation of Transcription

Metaplastic Breast Cancer: Tracking Changes

Bracha Erlanger[1], Ashley Cimino-Mathews[2], Roisin Connolly[1], Sarah J. Wheelan[1], and Ben Ho Park[1]

[1] The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University School of Medicine, Baltimore, MD 21287
[2] Department of Pathology, The Johns Hopkins Hospital, Baltimore, MD 21231

Presented by Bracha Erlanger

Metaplastic Breast Cancer is a rare subtype of breast cancer prevalent in less than 1% of all Breast Cancer cases, with an increasing annual incidence. MBC presents as neoplasms that differentiate into mesenchymal and/or Squamous Epithelial lineages with differing histologies, often displaying triple negative (ER-/PR-/HER2-) receptor status with poor prognosis, large tumor size and rapid growth rate.

This study aims to determine whether tumors with different histologies arise from the same clone, and further if MC stems from more common ductual tumors or if they arise independently. The hypothesis is that independently arising lesions would share some driver mutations and few passenger mutations, since those occur randomly during tumorigenesis. However, tumors that evolved from each other would share driver mutations and many more passenger mutations.

To that end, whole exome sequencing was performed on Formalin Fixed Paraffin Embedded (FFPE) Invasive Ductal Carcinoma (IDC) and Metaplastic Carcinoma (MC) tumor pairs from 8 patients. Variant calling and CNV analysis is used to answer these questions by way of comparing variants between samples and tracking the rate of mutation between and within IDC-MC tumor pairs.

Content Area: Human Genetics, Molecular Genetics, Computational Genetics
Keywords: Metaplastic Breast Cancer, Variants, WES

Growth Standards for Children and Adolescents with Smith-Magenis Syndrome

L.R. Fleming[1], F. Duncan[2], W.Introne[2], J. McGready[3,5], K. Schulze[4,5], A. Alade[5,6], J. Hoover-Fong[5,6], A.C.M. Smith[2]

[1] Medical Genetics Branch, NHGRI/NIH, Bethesda, MD
[2] Office of the Clinical Director, NHGRI/NIH, Bethesda, MD
[3] Dept of Biostatistics, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD
[4] Center for Human Nutrition, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD
[5] Greenberg Center for Skeletal Dysplasia, Johns Hopkins University, Baltimore, MD
[6] McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD

Presented by L. R. Fleming

Background: Smith-Magenis syndrome (SMS) is a complex genetic disorder, with an incidence of 1/15,000, characterized by distinctive craniofacial features, cognitive impairment, sleep disturbances, and neurobehavioral abnormalities. Poor linear growth and weight gain are common in infancy and short stature has been reported in 50-78% of individuals. However SMS-specific growth curves have not been available previously to allow clinicians toassess growth.

Methods: Anthropometric data (height, weight and head circumference) were collected by clinicianmeasurement, review of medical records and parent submission for 143 individuals (82 Females, 61 Males) with SMS in Europe, the USA and Australia. Of these, 138 (96.5%) had deletions and 5 (3.5%) had point mutations in *RAI1*. Penalized cubic smoothing splines were used to estimate gender specific SMS curves for height and weight for periods of 0-36 months and 2-18 years. These curves were then compared to WHO (0-36 month) and CDC (2-18 year) norms.
Results: At birth, the majority of term infants with SMS are within the clinically normal range for weight and length. Infants with SMS then exhibit a decline in height velocity, with the median height tracking < 5th centile of average stature by 3 years of age. Females with SMS show a more gradual decline in height velocity than males. Relative short stature persists through early adolescence, with median height for individuals with SMS through age 15 near the 5th centile for sex specific CDC age norms. By 6 months of age there is substantial attenuation of weight velocity in SMS males. A more gradual attenuation in weight velocity over the first year of life is seen in SMS females. Weight curves for 2-18 years of age show accelerated weight gain beginning in late childhood (~7 yrs). In comparison to CDC female norms, the median weight in SMS females falls well above the CDC 50th centile through 15 years of age. In comparison to CDC male norms, median weight gradually decreases after age 14 years and is concurrent with the CDC 50th centile by 18 years of age.

Discussion: This study represents the first set of standardized growth curves created for individuals with SMS. Syndrome specific growth curves can be used to set expectations for growth and to manage syndrome related symptoms. These curves can also be used in future research to better understand the natural history of SMS and the genotype-phenotype relationships in these patients

Osteopathia Striata with Cranial Sclerosis Complicated by Carotid Artery Stenosis: A Case Report and Review of the Literature

Leah R Fleming[1], MD, Aida Bytyci Telegrafi[2], MS, and Julie E Hoover-Fong[3], MD, PhD, FACMG

[1] National Institutes of Health, Bethesda, MD
[2] GeneDx, Gaithersburg, MD
[3] Johns Hopkins University, Baltimore , MD

Presented by Leah R Fleming

Description: Osteopathia striata with cranial sclerosis (OSCS) is an X-linked disorder of endochondral bone formation that results in a sclerosing bone dysplasia characterized by progressive development of pathognomonic linear striations of the long bones and pelvis, as well as sclerosis of the cranial base and calvarium. OSCS arises from inherited or *de novo* deletions or truncating mutations within *WTX* (*AMER1*, *FAM123B*). It has an incidence of 0.1/million. While somatic mutations in *WTX* are linked to increased risk of Wilms tumor, there is no evidence of increased cancer risk in individuals with OSCS.

We report a 6.5 year old girl with a history of choanal atresia, cleft palate, micrognathia and obstructive sleep apnea treated with tracheostomy, PDA, ASD, multiple muscular VSDs, T4-5 butterfly vertebra, recurrent otitis media, periodic limb movement disorder, mild developmental delay particularly of expressive language and fine motor skills, and a history of severe transient headaches. One year prior to presentation at our clinic, the patient suffered a transient ischemic attack and was found to have hypoplastic mastoid bones, diffuse thickening of the skull base, occlusion of the right terminal ICA segment, and high-grade stenosis of the left ICA segment.

On physical exam the patient had characteristic features of OSCS including a broad forehead, wide spaced eyes, broad flat nasal bridge, small nose, widely spaced teeth with dental anomalies (in this case absent central lower incisors and cone shaped lateral incisors), macrocephaly (>97th centile) and short stature (below 3rd centile), weight 35th centile for age. Her skull had a trapezoidal appearance due to bi-temporal narrowing rather than the more characteristic square shape. Linear striations of the long bones were present on skeletal survey. Molecular testing of *WTX* (Baylor College of Medicine) revealed a previously published point mutation [c.1072 C>T] resulting in a premature stop codon.[(1)] Neurosurgical consultation with imaging of the carotid stenosis showed development of sufficient collaterals to defer surgery.

The phenotype of osteopathia striate with cranial sclerosis varies widely from sub-clinical cases found upon family evaluation to lethality in some affected males.[(1)] Previously reported complications in OSCS include cranial nerve palsies, chronic otitis media, and vertebral abnormalities, however we are not aware of other descriptions of transient ischemic attack secondary to compression of the carotid artery. This case expands the phenotype for OSCS. We would recommend considering evaluation for ICA stenosis in patients with OSCS who develop new neurologic symptoms.

Histone Mark Signal Correlates with Gene Expression for Fixed Regions Across Individuals

Kipper Fletez-Brant[1,2] and Kasper Daniel Hansen[1,2]

1 Institute of Genetic Medicine, Johns Hopkins School of Medicine
2 Department of Biostatistics, Johns Hopkins School of Public Health

Presented by Kipper Fletez-Brant

DNA is organized in the nucleus of a cell in part by wrapping around histones, which are composed of the proteins H2A, H2B, H# and H4. Histone marks are chemical modification of the tails of histones, and are known to have functional properties. There are two broad categories of histone mark: those that indicate activity of nearby DNA and those that indicate inacivity of nearby DNA. Examples of the former cateagory are H3K4me1, H3k4me3 and H3K27ac, while an example of the latter is H3K27me3. In the present study we have analyzed ChIP-Seq data from H3K4me1, H3K4me3 and H3K27ac, as well as RNA-Seq data, from 15 HapMap cell lines. Specifically, we examine the relationship between histone mark signal and gene expression on a per-region basis, correlating the two across individuals. We find that in aggregrate, the distribution of correlations between chip signal and gene expression is strongly shifted in the positive direction. This positive shift indicates that an individual's strength of histone mark signal is predictive of their gene expression.

Content Area: Human Genetics, Computational Genetics, Statistical Genetics
Keywords: genomics, statistics

Analysis of a Histiocytic Sarcoma Locus – Canine's Utility for Fetching Candidates

Samuel F. Gilbert MPH[1,2], Heidi G. Parker PhD[2], Abigail L. Shearin PhD, DMV[2], and Elaine A. Ostrander PhD[2]

[1] The Johns Hopkins University Department of Human Genetics
[2] Cancer Genetics and Comparative Genomics Branch, NHGRI

Presented by Samuel F. Gilbert

Histiocytic Sarcoma (HS) is a rare human hematopoietic neoplasm that accounts for less than 1% of hematolymphoid cancers and occurs either concurrently with or subsequently to B- or T-lymphoblastic lymphoma and other B-cell cancers. As such, its genetic etiology has been difficult to establish. HS in dogs, by comparison, shows strong breed specificity with 20% of flat-coated retrievers and 25% of Bernese mountain dogs (BMD) estimated to develop the disease in their lifetime. Therefore, the domestic dog represents a unique system in which to investigate the genetic predispositions of HS. Of particular note are the differential clinical features of HS, which can present disseminated across multiple organs or localized to the skin and joints. Both however are essentially uniformly fatal. Our lab has previously undertaken genome wide association studies (GWAS) on both American and European BMD in order to more clearly understand the susceptibility genetics. Both American and European BMD demonstrated evidence for a significant locus on canine chromosome (CFA) CFA11; fine mapping revealed a single haplotype spanning the MTAP (methylthioadenosine phosphorylase) gene and part of the cyclin-dependent kinase inhibitor 2A (CDKN2A) gene, which was present in 96% of BMD with HS. GWAS in European BMD, however, also showed a significant locus on CFA14. I have since undertaken targeted resequencing of the CFA14 locus in a subset of European BMD and identified 34,000 variants. Promising candidate genes in this region include GRM8, which inhibits the cyclic AMP cascade in the central nervous system and has shown to be involved in several cancers; POT1, which is involved in telomere length maintenance and protection; and HYAL4, a hyaluronidase potentially involved in extracellular matrix degradation. I am performing PCR-based resequencing of a larger subset of 20 European BMD HS cases and 20 controls (which is now underway), to elucidate a risk haplotype for HS for this population. In addition, the laboratory has generated a 30x sequence from one HS case. I am working to include that detailed information in my analysis, interpolating my haplotypes as necessary to find a disease associated haplotype that I can test in our larger collection of 572 BMD cases and 615 controls that may point out factors involved in HS as well as the different clinical presentation of the disease.

Content Area: Molecular Genetics
Keywords: Histiocytic Sarcoma, Canine Genetics, Cancer

r2VIM: Variable Selection Method for Identifying Interaction Effects

Emily Holzinger[1], Silke Szymczak[2], James Malley[3], Abhijit Dasgupta[4], Qing Li[1], and Joan Bailey-Wilson[1]

[1] Computational and Statistical Genomics Branch (NHGRI/NIH)
[2] Institute of Clinical and Molecular Biology, Christian-Albrechts University of Kiel
[3] Division of Computational Bioscience (CIT/NIH)
[4] Clinical Trials and Outcomes Branch (NIAMS/NIH)

Presented by Emily Holzinger

Standard analysis methods for genome wide association studies (GWAS) are not robust to complex disease models, such as interactions between variables with small main effects. These types of effects likely contribute to the heritability of complex human traits. Machine learning methods that are capable of identifying interactions, such as Random Forests (RF), are an alternative analysis approach. One caveat to RF is that there is no standardized method of selecting variables so that false positives are reduced while retaining adequate power. To this end, we have developed a novel variable selection method called r2VIM. This method incorporates recurrency and variance estimation to assist in optimal threshold selection. For this study, we specifically address how this method performs in simulated data with close to completely epistatic effects (i.e. no marginal effects).

Our initial findings indicate that the optimal selection threshold can often identify both interacting loci while reducing the number of false positives in the selected variables. However, the optimal threshold is highly dependent on the underlying simulated genetic model, which is unknown in biological data. To address this, we also test a permutation procedure to generate null VIM distributions based on the actual genotype data to guide threshold selection. We permute the phenotype and re-run r2VIM to get a new estimate of the null variance. This is then used to choose a selection threshold for the non-permuted analysis based on the rate of false positive identification in the permuted data. We tested the method on a subset of the simulated data used in the initial analysis. The results suggest that the permutation procedure can guide optimal threshold selection in data with strong interaction effects in a manner that retains locus detection power and reduces the false positive selection rate.

Content Area: Statistical Genetics
Keywords: machine Learning, variable selection, GWAS

A Human-Specific Isoform of AS3MT Regulated by a Human-Unique Genetic Variation Explains Susceptibility to Psychiatric Illness

Ming Li[1], Ran Tao[1], Andrew E. Jaffe[1], Joo Heon Shin[1], Yanhong Wang[1], Qiang Chen[1], Chao Li[1], Yankai Jia[1], Kazutaka Ohi[1], Brady J. Maher[1, 2], Richard E. Straub[1], Joshua Chenoweth[1], Huijun Wei[1], James C. Barrow[1], Ronald McKay[1], Thomas M. Hyde[1, 2], Joel E. Kleinman[1], and Joel E. Kleinman[1, 2]

[1] Lieber Institute for Brain Development, Johns Hopkins University, Baltimore, Maryland, USA
[2] Departments of Psychiatry, Neurology, Neuroscience and the McKusick-Nathans Institute of Genetic , Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Presented by Ming Li

Genome-wide association studies have reported numerous single nucleotide polymorphisms (SNPs) associated with predisposition to psychiatric disorders. However, our knowledge remains very tentative regarding the causative genomic sequences and the specific molecular mechanisms. Here, we identify a human unique AS3MT exonic variable number tandem repeat (VNTR) in high linkage disequilibrium (LD) with SNPs in the 10q24.32 locus showing genome-wide statistical association with several psychiatric disorders. The VNTR risk allele is significantly associated with increased mRNA expression in brain of a human specific AS3MTd2d3 isoform (N=604, p=1.99×10-30), which is directionally consistent with the diagnostic association of higher expression in brains of patients with schizophrenia than in healthy subjects (p=0.003). AS3MTd2d3 mRNA, though expressed at relatively low levels in peripheral tissues, is most abundant in brain, particularly in hippocampus. In vitro luciferase assays demonstrate that the number of VNTR repeats predicts AS3MT promoter activity. The disease associated AS3MTd2d3 isoform encodes a truncated protein lacking 102 amino acids from the methyltransferase domain, but shows comparable abundance in brain tissues to the full length. This isoform is expressed very early during human stem cell differentiation and lacks arsenic methyltransferase activity, implicating a novel and evolutionarily recent role in early brain development. These findings identify a previously undescribed AS3MT isoform involved in risk of psychiatric illness, providing a potential new therapeutic drug target.

Content Area: Human Genetics, Molecular Genetics
Keywords: Psychiatric disorders, AS3MT, RNA-seq, VNTR

Modified Random Forest Algorithm to Identify GxG Interaction Using Case-Parent Trios of Non-syndromic Cleft Lip With or Without Cleft Palate

Qing Li[1], Emily Holzinger[1], Jacqueline B. Hetmanski[2], Mary L Marazita[3], Terri H. Beaty[2], and Joan E. Bailey-Wilson[1]

[1] Computational and Statistical Genomics Branch, National Human Genome Research Institute, National Institutes of Health, Baltimore, MD, USA
[2] Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA
[3] Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh PA, USA

Presented by Qing Li

The case-parent trio design has the advantage of controlling for population stratification and facilitates error checking. However, methods designed to test for gene-gene interaction using trio data are very limited. In this project, we have modified the Random Forest (RF) algorithm to identify gene-gene (GxG) interactions in matched case-control and trio data. Our method accounts for the randomness in the generation of pseudo-controls to match with the cases, as well as the inherent randomness of machine learning procedures. We first sample from the set of all possible genotypes of the pseudo-controls to match with those of the cases and then employ recurrency measures. To evaluate our RF method, we simulated matched case-control and case-parent trio data, and applied our method to select the most important predictors. The results are compared with Logic Regression and trio Logic Regression. We show our method for variable selection maintains correct genome-wide false positive rates.

Genome-wide association studies (GWAS) for non-syndromic cleft lip with or without cleft palate (CL/P) have identified multiple genes as important in the etiology of this common birth defect. Using these genome-wide data, we performed a candidate gene/pathway analysis explicitly considering G×G interactions to further explore the etiology of CL/P. Animal models have shown the WNT signaling pathway plays an important role in mid-facial development, and various genes in this pathway have been associated with non-syndromic CL/P in previous studies. We used our modified RF algorithm to identify possible genetic interactions between genes in the WNT family, and between WNT family genes and other genes identified by GWAS in case-parent trios. Results are compared with results from other methods, including trio Logic regression, RF++ and case-only analysis.

Content Area: Genetic Epidemiology
Keywords: gene-gene interaction, oral cleft

Strategy for Generating and Characterizing a Zebrafish Knockout Model of Spondylometaphyseal Dysplasia with Cone-rod Dystrophy

J. Jurgens[1], C. Woods[1], P. Hook[1], N. Sobreira[1], A. McCallion[1],and D. Valle[1]

[1] Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD

Presented by J. Jurgens

Spondylometaphyseal dysplasia with cone-rod dystrophy (SMD-CRD) is a rare, autosomal recessive condition characterized by short stature, bowing and metaphyseal flaring of the long bones, rhizomelic shortening, platyspondyly, scoliosis, and early-onset progressive degeneration of cone and rod photoreceptors. By whole exome sequencing and Sanger sequencing, Hoover-Fong et al. (2014) identified novel homozygous or compound heterozygous variants in *PCYT1A* in 6 unrelated probands. Independently, Yamamoto et al. (2014) described two novel homozygous *PCYT1A* variants, p.Glu129Lys and p.Ser323Argfs*38, in two unrelated SMD-CRD probands. *PCYT1A* encodes CTP:phosphocholine cytidydyltransferase α (CCTα), an enzyme which catalyzes the rate-limiting step in de novo phosphatidylcholine biosynthesis by the Kennedy pathway. Little is known about the pathophysiological mechanism of SMD-CRD due, in part, to the lack of an existing model organism for the disorder. Here we describe a strategy for generating and characterizing a zebrafish knockout model of the two zebrafish orthologs of *PCYT1A*, *pcyt1aa* and *pcyt1ab*, using the CRISPR/Cas9 system. By targeting each of these loci for double-stranded DNA breakage by the Cas9 protein followed by an endogenous error-prone mechanism for repair, we generated F_0 mosaic *pcyt1aa* and *pcyt1ab* knockout fish. We then screened these mosaics for germline transmission of the *pcyt1aa* and *pcyt1ab* knockout alleles and established F_1 heterozygous knockout fish. By breeding these heterozygous knockout fish, we plan to make homozygous knockouts for the *pcyt1aa* and *pcyt1ab* alleles, both individually and in combination. We will then characterize these knockout fish using a variety of methods including staining of the bone and retina; testing retinal function by measuring the optokinetic response and electroretinograms; and obtaining measurements of fin and body length to characterize skeletal features. We anticipate that these models will provide a resource for screening potential small molecule and/or nutritional therapies. We suggest that creating and characterizing a zebrafish model of SMD-CRD will facilitate greater understanding of this disorder and perhaps other bone or retinal dysplasias.

An Analysis of Anthropometrics in the SEED Study: Exploring Phenotypic Expression in Autism Spectrum Disorders as an Insight into Possible Mechanisms

Norazlin Kamal Nor[1], M. Dani Fallin[2], Julie Hoover-Fong [3,4], and Terri Beaty[1]

[1] Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health
[2] Department of Mental Health, Johns Hopkins Bloomberg School of Public Health
[3] McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University
[4] Department of Pediatrics, Johns Hopkins University

Presented by Norazlin Kamal Nor

Introduction: Autism spectrum disorders (ASD) are a group of complex neurodevelopmental disorders characterized by impairments of social interaction, communication difficulties and repetitive or stereotypic behaviors. The specific etiology of ASD is unknown, although it is widely recognized that genetic, environmental and gene-environment interactions are risk factors. ASD presents as a spectrum, with variability in manifestations and severity, leading to the hypothesis of ASDs as having differing phenotypes. The various 'autisms' in this spectrum include, but are not limited to, sleep disturbance, gastrointestinal disturbance and dysmorphology, as well as the more recognized 'autisms' such as social-communication impairments and behavioral peculiarities. Understanding the phenotype classification in ASD, for example ASD dysmorphology, may allow greater insight into the mechanisms leading to ASD. With this in mind, the aim of this study is to consider dysmorphological findings, specifically anthropometric measurements, in children with ASD as a tool to investigate ASD etiology. Anthropometry describes the science defining physical measurements of height, weight and head circumference in the human individual.

Methodology: The study population were children enrolled in the Study to Explore Early Development (SEED) 1, a multisite population-based case-control study of children aged 2-5 years with ASD (145 children) as well as two control groups, one with non-ASD developmental difficulties (99 children) and another obtained from the general population (129 children), in six diverse sites in the United States. A large number of bio-specimens as well as clinical examinations were performed on these children. Children who were detected to have physical abnormalities indicative of dysmophology were referred to Clinical Geneticists and underwent a comprehensive dysmorphology review. This dysmorphology review was standardized in the Dysmorphology Review Form (DRF), which utilized both objective and subjective measures. Scores were given for each measure on a Likert scale between 0 to 4, with '0' designated as not having abnormality, and '4' designated as having the highest level of abnormality. Specific measurements were also undertaken for anthropometrics, including weight, height and head circumference. In this preliminary analysis, the primary objective is to compare the head circumference, weight and height of children with ASD to those with non-ASD developmental difficulties and those from the normal population. The secondary objective is to compare the head circumference, weight and height of children with ASD who have dysmorphology compared to those who don't. Previous research have demonstrated a link between differential growth in head circumference in the first five years of life in children with ASD compared to those in the general population. This study aims to build on this to investigate differences in anthropometry comparing the diagnostic neurodevelopment groups (ASD and non-ASD developmental impairments) and the normal population, as well as the differences between the group of children diagnosed with ASD who are dysmorphic and those who aren't.

Content Area: Epidemiology
Keywords: Autism Spectrum Disorders, Anthropometry, SEED

Prenatal Exposure to Selective Serotonin Reuptake Inhibitors and DNA Methylation Changes in Childhood

Weiyan Li[1], Brian K Lee[2], Nicole Gidaya[2], Craig Newschaffer[2], Laura A. Schieve[3], Diana E. Schendel[4], Nicole Jones[5], Julie L. Daniels[6], Gayle C. Windham[7], Lisa A. Croen[8], Andrew P. Feinberg[9], M. Daniele Fallin[10], and Christine Ladd-Acosta[1]

[1] Department of Epidemiology, Johns Hopkins School of Public Health, Baltimore, MD
[2] Department of Epidemiology and Biostatistics and the A.J. Autism Institute, Drexel University School of Public Health, Philadelphia, PA
[3] National Center on Birth Defects and Developmental Disabilities, Centers for Disease Control and Prevention, Atlanta, GA
[4] Department of Public Health, Institute of Epidemiology and Social Medicine, Aarhus University, Aarhus, Denmark, Department of Economics and Business, National Centre for Register-based Research, Aarhus University, Aarhus, Denmark, and Lundbeck Foundation Initiative for Integrative Psychiatric Research (iPSYCH), Denmark
[5] Biomedical Research Informatics Core, Michigan State University, East Lansing, MI
[6] Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC
[7] California Department of Public Health, Richmond, CA
[8] Kaiser Permanente Division of Research, Oakland, CA
[9] Center for Epigenetics, Johns Hopkins School of Medicine, Baltimore, MD
[10] Department of Mental Health, Johns Hopkins School of Public Health, Baltimore, MD

Presented by Weiyan Li

About 1 in 68 children in the United States is affected by Autism Spectrum Disorders (ASD). Etiology of autism is believed to be an interplay of genetics and environmental influences, and recent studies showed that the environment plays a larger role than previously expected. Epigenetics is an important regulatory mechanism of gene expression and is responsive to environmental stimuli, thus a promising candidate to study environmental influences on complex diseases. Selective serotonin reuptake inhibitors (SSRIs) have been commonly prescribed for treatment of maternal depression during pregnancy, and recent reports showed that prenatal exposure to SSRI is associated with an increased risk for ASD. To investigate the role of epigenetics in the association of prenatal SSRI use and ASD in offspring, a first step would be examining the relationship between prenatal SSRI use and changes in DNAm signatures. To our knowledge, no such studies have been conducted. Here we plan to utilize an unique data source with individual level exposure data and DNAm profile from the Study to Explore

Early Development (SEED) population and perform an epigenome wide scan for DNA methylation signatures related to prenatal SSRI exposure. Our goal is to identify (1) potential biomarkers for prenatal exposure to SSRI, and (2) potential mechanisms mediating the effect of prenatal SSRI use on ASD risk in the offspring. After implementation of rigorous quality control measures, DNAm profiles were available for 570 children. Prenatal SSRI exposure data were collected via telephone interviews with the mothers. Preliminary analysis showed the prevalence of prenatal SSRI use at any time during pregnancy in this population is 7%. Also, suggestive shift of DNAm were observed at several loci, but results were not significant after adjusting for multiple testing, blood cell composition estimates, race and other potential confounders. Approximately 370 additional samples are expected in the near future, and this will greatly increase our statistical power in further analysis to detect DNAm signatures related to prenatal exposure to SSRI.

Content Area: Genetic Epidemiology
Keywords: DNA methylation, EWAS, ASD, SSRIs

Population Screening Methods in the Precision Medicine Era: Cystic Fibrosis as a Case Example

Allison McCague[1], Debra J.H. Mathews[1,2], and Garry R. Cutting[1]

[1]McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University
[2]Berman Institute of Bioethics, Johns Hopkins University

Presented by Allison McCague

With President Obama's recent announcement of a new initiative in precision medicine, the ongoing discussion of personalized medicine – both its implementation and potential impact on public health – has shifted into high gear. Within medical genetics, this includes a debate about when it is appropriate to use population-wide screening to identify carriers of genetic disease. For some diseases, such as Huntington's Disease, mutation carriers are identified primarily through "cascade testing," whereby once one member of a family has been diagnosed, subsequent testing is sought for family members who are now at increased risk. For other diseases, such as cystic fibrosis, carrier screening on a population-wide basis is becoming standard of care. There have recently been calls for population-wide screening as standard of care for other genetic mutations, such as the cancer-predisposing mutations in BRCA1 and BRCA2. As we move toward precision medicine as the frame for the integration of genetics into medical care, it will be important to assess what we know about the different models of testing. Here we use cystic fibrosis as a case example to evaluate the benefits and drawbacks of cascade testing versus population-wide screening, in order to better inform the field moving forward in the precision medicine era.

Content Area: Genetic Epidemiology
Keywords: population screening, cystic fibrosis

Does SUMO Wrestle Thymine DNA Glycosylase Off DNA?

Dylan McLaughlin[1], Wei-Chih Yang[1], Christopher Coey[2], Alexander Drohat[2], and Michael Matunis[1]

[1] Department of Biochemistry and Molecular Biology, Johns Hopkins School of Public Health
[2] Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine

Presented by Dylan McLaughlin

Thymine DNA glycosylase (TDG) is an enzyme that recognizes and repairs G/T and G/U mismatches in the base excision repair (BER) pathway. Its BER function is important in maintaining genome integrity and in regulating DNA gene expression through DNA demethylation of 5-methylcystosine (5mC). Small ubiquitin-related modifiers (SUMOs) are post-translationally conjugated to other proteins to regulate a wide range of cellular processes, including DNA repair. Based on in vitro studies, SUMOylation of TDG has been proposed as one mechanism for alleviating product inhibition at the abasic site after enzyme processing and allowing for dissociation of TDG from DNA. Subsequent desumoylation of TDG would be required for complete enzymatic turnover. The effect of sumoylation on TDG activity in vivo, however, has not been studied. Here we have devised an in vivo assay to study the role of sumoylation in regulating TDG activity. Ten-eleven translocation (TET) enzyme iteratively oxidizes 5mC of genomic DNA to a final product of 5-carboxylcytosine (5caC). Because TDG is the only enzyme capable of recognizing and repairing 5caC, we co-expressed TDG wild type or SUMOylation mutant constructs with TET in HEK293T cells and measured 5caC levels of genomic DNA to monitor TDG activity. Our findings demonstrate that SUMOylation of TDG is not critical for its base excision repair activity in vivo. This suggests that SUMOylation may instead regulate other functions of TDG, including control of gene regulation, development, and cellular differentiation.

Content Area: Molecular Genetics
Keywords: Base Excision Repair, SUMOylation

Differential Transcriptome Profiling of African Americans with Uncontrolled Hypertension and Chronic Kidney Disease (CKD) versus Controlled Hypertension and without CKD: Study Design

Priyanka Nandakumar[1] and Adrienne Tin[2]

[1] Johns Hopkins School of Medicine, Institute of Genetic Medicine
[2] Johns Hopkins Bloomberg School of Public Health, Department of Epidemiology

Presented by Priyanka Nandakumar and Adrienne Tin

Background: Hypertension-attributed chronic kidney disease (CKD) is highly resistant to treatment in African-Americans, and contributes to racial disparity in end-stage renal disease (ESRD). In older adults (aged 70-74), African-Americans have 4-fold higher risk of developing hypertension-attributed ESRD than European-Americans, and incidence of hypertension-attributed ESRD increases with age. A hypothesized mechanism linking hypertension and progressive CKD is the innate immune response and inflammation. Inflammation biomarkers have been associated with kidney function decline and incident hypertension. Gene expression in peripheral blood can provide a view of the innate immune activation profile, and prior gene expression association studies in hypertensive subjects have demonstrated success. Additionally, APOL1, a gene that is involved in the innate immune response, is known to harbor renal risk variants that is common in African Americans and rare in other ethnicities. However, the mechanisms by which the APOL1 renal risk variants influence CKD progression are still unknown,
Aims: Thus, we proposed a pilot study of transcriptome profiling in African-Americans with treated high blood pressure and CKD as cases, and controlled blood pressure and no CKD as controls to (1) identify differentially expressed genes and pathways, (2) investigate the relative expression of APOL1 and genes within its co-expression network.
Study Design: The cases and controls (N=2x15) were selected from those without diabetes and matched by age, gender, body mass index, and medication use to reduce heterogeneity. Hypertension under treatment is defined as on hypertension medication and with systolic blood pressure (SBP) â‰¥ 145 mm Hg. CKD is defined as estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73m2. The cases were selected from those with both hypertension under treatment and CKD. The controls were selected from those with blood pressure controlled by hypertensive medications (SBP <135 mm Hg and diastolic blood pressure < 90 mm Hg) and without CKD (eGFR between 90 and 120 mL/min/1.73m2 and urine albumin-to-creatinine ratio < 30mg/g).
The cases were selected from the extremes of the phenotype distributions to maximize the differences between the cases and controls. The case-control pairs with APOL1 high-risk genotype were over sampled to study the APOL1-associated renal risk.
RNA Sequencing: Our study is quantifying the transcriptome using RNA sequencing, which has enhanced reproducibility and sensitivity down to the splice variant level. Paired-end sequencing will generate roughly 100 million reads for mRNA and 15 million reads for miRNA per sample. Each case-control pair will be processed in the same run to minimize the confounding of batch effects.
Analyses: Differential expression analysis will use the HTSeq-count/DESeq2 pipeline. HTSeq-count estimates the gene expression levels. DESeq2 normalizes the read counts and assumes a negative binomial distribution for read counts to evaluate the association between gene expression and case status. DESeq2 was found to have a lower false positive rate than similar software.
Study Significance This study brings together the population-based method of matched case-control design and the novel laboratory method of RNA sequencing to investigate the systemic biological pathways in hypertension-attributed CKD. Our findings may yield novel insights on potential intervention targets for CKD prevention or treatment.

Content Area: Human Genetics, Genetic Epidemiology
Keywords: RNA-seq, Hypertension, CKD, African-Americans

Exome Array Analysis of Pulmonary Function (FEV1/FVC) in the COPDgene Study

Margaret M Parker[1], Brian Hobbs[2], Michael Cho[2], Ingo Ruczinski[3], Rasika Mathias[4], Terri Beaty[1] on behalf of the COPDgene Investigators

[1] Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore MD, USA 21202
[2] Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA
[3] Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore MD, USA 21202
[4] Division of Allergy and Clinical Immunology, Department of Medicine, Johns Hopkins University, Baltimore MD, USA 21224

Presented by Margaret M Parker

Chronic obstructive pulmonary disease (COPD) is a progressive respiratory disease characterized by airflow restriction and decreased lung function. It is the 4th leading cause of death in the world, and is expected to be the 3rd leading cause by 2030. It is diagnosed using the ratio of forced expiratory volume in one second to forced vital capacity (FEV1/FVC). This measure reflects the severity of airway obstruction and predicts population morbidity and mortality. The primary environmental cause of COPD is cigarette smoking, but genetics also play a role in individual susceptibility and disease progression. Genome-wide association studies (GWAS) have identified over 30 loci associated with FEV1/FVC, but together the identified variants explain only a small proportion of the variation in FEV1/FVC. Recently, major advances in next-generation sequencing and exome array technologies have allowed for the identification of rare functional variants hypothesized to influence disease susceptibility. This study aims to identify functional genetic variation associated with pulmonary function (FEV1/FVC) using exome array data from 10,000 smoking subjects enrolled in the COPDgene study. We present the results of single variant, burden and SKAT tests of the association between genetic markers and spirometry measures in 6,581 NHW and 3,321 AA COPDgene subjects.

Content Area: Genetic Epidemiology
Keywords: exome array, COPD, rare variants

A Polymorphic Di-nucleotide Repeat (DNR) Variant in the 5'UTR of *DPYSL2* Gene Affects its Regulation via mTOR Signaling

Xuan Pham[1], Yaping Liu[1], Ruihua Wang[1], Ann Pulver[1], David Valle[1], Dimitrios Avramopoulos[1]

[1]JHU School of Medicine, Human Genetics Department

Presented by Xuan Pham

Schizophrenia (SZ) is a common, disabling neuropsychiatric disorder with a complex etiology. It is estimated that as much as 80% can be attributed to genetic factors. Previous linkage and association studies have implicated DPYSL2 on chr8p21 as a candidate gene for SZ. *DPYSL2* encodes CRMP2 which is important in axonal growth, and its dysfunction may result in neurodevelopmental abnormalities. We previously identified a polymorphic dinucleotide repeat (DNR) located in the 5'-untranslated region of DPYSL2, with a characteristic 5'-terminal oligopyrimidine (5'-TOP) tract, a target for mTOR mediated regulation pathway. The 13 CT repeat allele (risk) of the DNR was found to be associated with SZ compared to the 11 CT repeat common allele (WT). We performed dual luciferase assays in HEK293 cells and mouse primary cortical neurons and the risk allele showed ~3-fold decrease in luciferase activity as compared to the WT allele in both cell types. Further, polysome profiling of the constructs showed the fraction of luciferase mRNA in polysomes associated with the risk allele was reduced by ~3-fold compared to the WT allele. Here we show that increasing concentrations of Rapamycin, an allosteric mTOR inhibitor, reduced luciferase expression in constructs from both alleles, the risk allele remaining lower at 0-30 nM. At concentrations higher than 30 nM, both alleles reached a plateau at the same levels. The same trend was recapitulated in both HEK293 cells and mouse primary cortical neurons. Our results suggest that the difference we observe between the two DNR alleles is mediated by mTOR signaling. Using arrays of > 4,000 human transcription factors and proteins, we screened for those that bind differentially to the two alleles within the 5'-TOP of *DPYSL2* and might produce the decreased gene expression observed in the risk DNR allele. We identified a number of such proteins, one of which is a ribosomal binding protein (RBP) *HuD/ELAVL4* that is also involved in mTOR signaling. Further *HuD/ELAVL4* has been shown to play a crucial role in neuronal differentiation. How this RBP interacts with *DPYSL2* in the WT and risk form to mediate gene expression in neurons is the subject of our current studies. In conclusion, we show that *DPYSL2* is regulated by the mTOR signaling pathway and a SZ associated DNR variant in the 5'-UTR of *DPYSL2* affects this regulation.

Keywords: Schizophrenia, DPYSL2, mTOR, HuD, ELAVL4, dinucleotide repeat, Rapamycin

Prenatal Diagnosis of Campomelic Dysplasia via 3D Ultrasound of the Scapula

Melissa Russo[1], Karin Blakemore[1], Jude Crino[1], Erin Oehler[1], Shannon Trebes[1], and Julie Hoover-Fong[2]

[1]Johns Hopkins Hospital, Baltimore, MD
[2]Johns Hopkins University, Baltimore , MD

Presented by Melissa Russo

BACKGROUND: Prenatal ultrasound allows detection of a bent bone dysplasia in the second trimester of gestation. Once detected, the differential is extensive, including campomelic dysplasia, osteogenesis imperfecta, thanatophoric dysplasia, short rib polydactyly dysplasias and others. The bent bone dysplasias span the severity spectrum from lethal e.g. thanatophoric dysplasia to benign e.g. femoral hypoplasia unusual facies. Detection of phenotypic features that direct molecular testing to optimize genetic counseling in the prenatal period is of tremendous utility. Although there are radiographic and gross physical features which help differentiate these bent bone dysplasias postnatally, these characteristics are sometimes difficult to appreciate in the fetal period by routine ultrasound. Here, we present use of 3D ultrasound to examine the shape of the scapula and direct molecular testing in a case of campomelic dysplasia.

CASE: A 29 year old G1P0 female presented at 18-4/7 weeks for suspicion of fetal skeletal dysplasia by outside prenatal ultrasound. She had an abnormal first trimester ultrasound with an increased nuchal translucency (11.9 mm). Karyotype by CVS was normal [46, XX] and molecular testing for thanatophoric dysplasia was negative. At the 18 week ultrasound, the femurs and humeri were bowed and two weeks behind in growth; tibia/fibula were 4 weeks behind. Chest circumference, head and abdomen were normal. The differential diagnosis included diastrophic dysplasia, kyphomelic dysplasia, metatropic dysplasia, campomelic dysplasia and atelosteogenesis type II. At 21-4/7 weeks a second ultrasound showed progressive shortening of the long bones and bowed femurs and humeri. Facial profile was flat with hypoplastic nasal bone. At 25 weeks, fetal head and abdominal circumference were appropriate but long bones remained short and bowed. There were 11 ribs and the scapulae were hypoplastic in 2D coronal and sagittal views. 3D imaging clearly delineated hypoplastic, rectangular scapulae with absent inferior wings, suggestive of campomelic dysplasia. Based on the scapula's appearance, DNA from cultured CVS cells was tested for SOX9 mutation. There was a nonsense mutation (c.126C>T), changing glutamine to a termination codon in exon 3. The patient chose to terminate the pregnancy. Post-mortem x-rays confirmed hypoplastic scapulae, shortened long bones and bowed femurs and humeri. Full autopsy was declined by the patient.

DISCUSSION: Mortier et al. reported radiographic differences of the scapula among several bent bone dysplasias and other diagnoses. The characteristic appearance of the scapula in campomelic dysplasia is a hypoplastic and rectangular shape, both of which we were able to appreciate on a 25 week 3D ultrasound of the fetus. This narrowed the differential in the prenatal period; genetic testing was performed in a timely fashion with molecular confirmation of campomelic dysplasia. We conclude that 3D ultrasound of the scapula may be a valuable tool in the prenatal differentiation of bent bone dysplasias.

Mortier et al. The scapula as a window to the diagnosis of skeletal dysplasia. Pediatr Radiol.1997

Comparison of GDNF mRNA Expression in Testes of Fertile and Infertile Men and Fertile Mice

Dolly Singh[1], Kyle Orwig[2], Darius A. Paduch[3], Peter N. Schlegel[3], and William Wright[1]

[1] Department of Biochemistry and Molecular Biology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
[2] McGee Womans Research Institute, University of Pittsburgh, Pittsburgh PA
[3] Department of Urology, Weill Cornell Medical College, New York, NY

Presented by Dolly Singh

Normal spermatogonial stem cells (SSCs) renewal and differentiation is essential for maintaining spermatogenesis in the adult testis. SSCs are a subset of undifferentiated spermatogonia and have the ability to self-renew or give rise to progenitor spermatogonia. Glial cell line-derived neurotropic factor (GDNF), secreted by Sertoli cells, has been shown to be one of the growth factors required for SSCs regulation in mice (1, PMID: 22232066; 2, PMID: 25165119). Importantly, GDNF is also expressed in human testes, raising the possibility that this growth factor plays a role in male infertility (3, PMID: 11732574). The objective of this study was to compare testicular GDNF mRNA in testes of men with normal spermatogenesis, and infertile men with either maturation arrest (MA) or Sertoli Cell Only syndrome (SCO), which results from loss of SSCs. Additionally, we compared GDNF mRNA expression in testes of fertile mice and men to gain insight into whether the mouse was a good model for studying GDNF expression in humans. Clusterin mRNA was measured as a control and data were normalized to 18S rRNA and subjected to one-way ANOVA. Human GDNF mRNA was measured by digital PCR. Human and mouse clusterin mRNA's, mouse GDNF mRNA &18S mRNA were measured using Taqman assays. From each sample of RNA, cDNA was synthesized and was used across all assays. GDNF mRNA levels varied significantly among the normal, MA and NOA patients (n=3 per group; $p<0.05$). Expression of GDNF in human testes decreased by 57% in SCO men and increased 32% in MA testes when compared with normal testes. This increase in GDNF mRNA levels in MA patients can be due to a decrease in testicular area causing a higher ratio of Sertoli cells /gram tissue vs. normal men. Clusterin mRNA levels were 37% and 75% higher in MA and SCO testes vs. the normal population, respectively ($p=0.06$). GDNF mRNA levels in testes of fertile men and mice were identical, while clusterin mRNA levels in normal human testes were 3% of mouse levels. The low levels of GDNF expression in SCO men may reflect an intrinsic testicular defect that could have contributed to limiting SSCs. If these levels can be raised, the testicular environment may be able to support spermatogenesis in the presence of limited SSCs, potentially restoring fertility in a subset of infertile men. Supported by 5R01HD074542-04.

Content Area: Human Genetics
Keywords: Spermatogonia, Male infertility

Impaired Cognition as a Mediator of the Genetic Risk for Schizophrenia

Richard E. Straub[1], Dwight Dickinson[2], and Daniel R. Weinberger[1,3]

[1] Lieber Institute for Brain Development, Johns Hopkins University, Baltimore, Maryland, USA
[2] Clinical Brain Disorders Branch, Intramural Research Program, National Institute of Mental Health, NIH, Bethesda, MD, USA
[3] Departments of Psychiatry, Neurology, Neuroscience and the McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Presented by Richard E. Straub

People with schizophrenia show broad cognitive impairment, which is seen in attenuated form in their unaffected relatives, including co-twins, siblings and parents, suggesting that relatively impaired cognition is an intermediate phenotype related to genetic risk for schizophrenia. The strength of the genetic relationship between current cognitive performance and illness is high, with extensive overlap in the genes that contribute to cognition and schizophrenia.

A genetic risk profile score (RPS), also called the polygene score, can be constructed for each subject as the weighted sum of its trait-associated alleles. Based on the schizophrenia association results from the Psychiatric Genetics Consortium, we calculated RPS (24694 SNPs, p value cutoff 0.05) and examined the relationship between RPS, cognition, and clinical diagnosis.

Our sample consisted of 594 controls and 339 people with schizophrenia, a subset of participants in the CBDB/NIMH Study of Schizophrenia Genetics (D.R. Weinberger, P.I.), who were self-identified as Caucasian of European descent. Participants were administered a battery of cognitive tests and 25 variables were used in exploratory and confirmatory factor analyses, reducing these data to composites representing domains of verbal memory, visual memory, N-back, processing speed, card sorting, and working memory span. A composite of all 25 variables was calculated to represent “g”, commonly referred to as general cognitive ability.

The Pearson correlation coefficient between RPS and g in schizophrenics is highly significant (-0.213, p=1e-8), whereas in controls it not significant (-0.041, p=0.43). RPS predicted 14.6% of the variance in the binary diagnosis, and g predicted 65.9%. Regressing g out of RPS left a residual that predicted only 2.2% which is a decrease of 85%. Conversely, regressing RPS out of g left a residual that predicted 54.2%, a decrease of only 18%. These results are consistent with an interpretation that perhaps the bulk of the genetic risk being indexed by this particular RPS is being mediated by g.

We pursued this further using formal mediation analysis with the INDIRECT program by Hayes and Preacher, as implemented in SPSS. Consistent with the results above, we found evidence for a strong indirect effect of RPS on diagnosis via g. In addition, we found the reciprocal, ie. an indirect effect of RPS on g via diagnosis. The latter is intriguing. It is known for example that for a subset of patients, cognition declines rapidly upon the onset of illness, and that therapeutic medications can cause impairment – these susceptibilities may also be in part genetic. This preliminary study yielded a predictably complicated set of relationships that we will explore further with structural equation modeling including latent phenotypes.

Content Area: Human Genetics; Molecular Genetics; Statistical Genetics
Keywords: cognition, schizophrenia, RPS, polygene score

GWAS derived Risk Profile Score is Associated with Schizophrenia Only in Individuals Exposed to Obstetric Complications

Gianluca Ursini[1], Stefano Marenco[2], Qiang Chen[1], Richard E. Straub[1], Giovanna Punzi[1], and Daniel R. Weinberger[1]

[1] Lieber Institute for Brain Development, Johns Hopkins Medical Campus, Baltimore, MD
[2] Clinical Brain Disorders Branch, Intramural Research Program, National Institute of Mental Health, National Institutes of Health, Bethesda, MD

Presented by Gianluca Ursini

BACKGROUND: Schizophrenia GWASs suggest that genetic risk is conferred by many small effect alleles (1). To date, GWAS have not provided well-defined mechanistic hypotheses for follow-up experiments (1). Environmental factors also have a role in the pathophysiology of schizophrenia, and obstetric complications and intrauterine adversity (OCs) slightly but significantly increase risk for adult emergence of this disorder (2). Here, we test whether risk profile scores (RPSs) constructed from alleles showing association with schizophrenia (1) interact with OCs in predicting case-control status.
METHODS: 272 healthy subjects and 228 patients with schizophrenia were assessed for OC exposure, using the McNeil-Sjostrom Scale (2). RPSs were generated using odds ratios derived from the PGC2 datasets (1). Regression analyses were performed in 'R', with case-control status as dependent variable, and i)RPS, ii)OCs, iii)RPS, OCs and their interaction as predictors.
RESULTS: All the RPSs generated using different threshold for selecting risk alleles predict case-control status without taking into account OCs ($p<3.65e-06$); OCs exposure alone does not predict case-control status. Strikingly, analysis of the interaction between OCs and the RPS obtained with the set of SNPs showing GWAS significant association with schizophrenia ($p<5E-08$, RPS1) show that OC exposure predicts case-control status ($p=0.04$), while RPS1 does not ($p>0.34$); moreover OCs and RPS1 significantly interact to predict case-control status ($p<0.01$), so that only in presence of OCs is the RPS1 associated with schizophrenia. No significant interaction ($p>0.08$) was found between OCs and RPSs generated using less restrictive thresholds.
CONCLUSIONS: Our data suggest that the RPS obtained from SNPs showing GWAS significant association with schizophrenia interact with OCs exposure in affecting risk for schizophrenia. More specifically, the RPS obtained from these SNPs predicts case-control status in our sample only in the presence of serious OCs exposure. Our data raise the possibility that the weak effect sizes of the GWAS SNPs is because they only increase risk in the context of developmental risk factors, which are not universal. Our results require replication in other samples.

Ref.:
1.Psychiatric Genomics Consortium. Nature. 2014 Jul 24; 511 (7510):421-7.
2.Nicodemus KK. Mol Psychiatry 2008;13:873-7.

Content Area: Other
Keywords: schizophrenia, obstetric complications, GWAS

Genes Involved in the Immune Response and Intraprostatic Inflammation in the Placebo Arm of the Prostate Cancer Prevention Trial*

Danyelle Winchester[1], Bora Gurel[2, 3], Cathee Till[4], Phyllis J. Goodman[4], Catherine M. Tangen[4], Regina M. Santella[5], Teresa L. Johnson-Pais[6], Robin J. Leach[6], Ian M. Thompson[6], Jianfeng Xu[7], S. Lilly Zheng[8], M. Scott Lucia[9], Scott M. Lippmann[10], Howard L. Parnes[11], William B. Isaacs[12,13], Charles G. Drake[12, 13, 14], Angelo M. De Marzo[2,12,13], and Elizabeth A. Platz[1,12, 13]

[1] Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
[2] Department of Pathology, Johns Hopkins School of Medicine, Baltimore, MD, USA
[3] Department of Pathology, Kocaeli University School of Medicine, Kocaeli, Turkey
[4] SWOG Statistical Center, Fred Hutchinson Cancer Research Center, Seattle, WA
[5] Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY
[6] Department of Urology, University of Texas Health Science Center San Antonio, San Antonio, TX
[7] Program for Personalized Cancer Care and Department of Surgery, NorthShore University Health System, Evanston, IL
[8] Center for Cancer Genomics, Wake Forest University School of Medicine, Winston-Salem, NC
[9] Department of Pathology, University of Colorado Denver School of Medicine, Aurora, CO
[10] Moores Cancer Center, University of California San Diego, La Jolla, CA
[11] Division of Cancer Prevention, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD
[12] James Buchanan Brady Urological Institute and Department of Urology, Johns Hopkins University School of Medicine, Baltimore, MD
[13] Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD
[14] Department of Immunology, Johns Hopkins University School of Medicine, Baltimore, MD

Presented by Danyelle Winchester

Background: We previously reported that both intraprostatic inflammation and SNPs in genes involved in the immune response are associated with prostate cancer risk and disease grade. In the present study, we evaluated the association between these SNPs and intraprostatic inflammation in men without a prostate cancer diagnosis. **Methods:** Included in this cross-sectional study were 205 white controls from a case-control study nested in the placebo arm of the Prostate Cancer Prevention Trial. We analyzed inflammation data from the review of H&E stained prostate tissue sections from biopsies performed per protocol at the end of the trial irrespective of clinical indication, and data for 16 SNPs in genes involved in the immune response (IL1β, IL2, IL4, IL6, IL8, IL10, IL12(p40), IFNG, MSR1, RNASEL, TLR4, TNFA; 7 tagSNPs in IL10). Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association between carrying at least one minor allele and having at least one biopsy core (of a mean of 3 reviewed) with inflammation. **Results:** None of the SNPs was statistically significantly associated with having at least one core with inflammation. However, possible inverse associations were present for carrying the minor allele of rs2069762 (G) in IL2 (OR=0.51, 95% CI 0.25-1.02); carrying two copies of the minor allele of rs1800871 (T) of IL10 (OR=0.29, 95% CI 0.08-1.00); and carrying the minor allele of rs486907 (A) in RNASEL (OR=0.52, 95% CI 0.26-1.06), especially in leaner men (BMI<25 kg/m2; OR=0.33, 95% CI 0.11-1.00). **Conclusion:** Overall, our findings do not support a strong cross-sectional link between genes involved in the immune response and intraprostatic inflammation. Funding: P01 CA108964, U10 CA37429, UM1 CA182883, T32 CA009314.
*A SWOG-Coordinated Study S9217

Content Area: Genetic Epidemiology
Keywords: Prostate Cancer, Intraprostatic inflammation, SNPs

Exploration of Functional Consequences of Alu Insertion Polymorphisms in ACE and ARID5B

Kathleen Burns[1,2], Chunhong Liu[3], Peilin Shen[1], William Wu[1,3], and Ally Moyer[1,4]

[1] Johns Hopkins University School of Medicine, Dept. of Pathology
[2] McKusick-Nathans Institute of Genetic Medicine
[3] Johns Hopkins University, Zanvyl Krieger School of Arts and Sciences
[4] Johns Hopkins University, Whiting School of Engineering

Presented by William Wu

Genome wide association studies (GWAS) have associated a predisposition for the development of many common disease phenotypes with the inheritance of specific common genetic variants, in particular trait associated SNPs (TASs). Alone however, these TASs, which are frequently intronic or intergenic, are of dubious functional significance. Previous work found that retrotransposon insertion polymorphisms (RIPs) abound in the vicinity of TASs. Furthermore, many RIPs themselves have been shown to associate with TASs, tagging haplotypes. We hypothesize that these RIPs may serve as distinct functional variants, holding roles in the regulation of surrounding genetic elements, and therefore be responsible for phenotypic effects. We have selected 2 RIPs in high linkage disequilibrium with nearby TASs, each situated within a gene directly relevant to the associated disease phenotype: An intronic Alu in ACE strongly associated with serum immunoreactive ACE concentrations and an intronic Alu in ARID5B linked to TAS associated with increased predisposition for the development of childhood precursor B-cell ALL. To study the functional implications of these RIPs, we first have used the pRED/ET rpsL-neo counterselection recombination system to construct modified BACs by replacing the region immediately surrounding each Alu insertion with a synthesized “Alu pop in/pop out” DNA fragment. Utilizing different recombinase schemes, we can create transgenic mice containing modified human transgenes either with or without the Alu element for in vivo phenotypic characterization. In addition to mouse models, the CRISPR/Cas9 system will be used to artificially target insertion or deletion of Alu in existing cell lines, and appropriate qPCR, western blot, and RT-PCR assays will be employed to elucidate aspects of gene expression, protein expression, and transcript splicing affected by Alu presence. Our current work pioneers a process to isolate, manipulate and measure the effects of naturally occurring RIPs in allelogenic transgenic mouse and in vitro models to discover mechanisms by which they might act on medically significant phenotypes. We hope these efforts will serve as a paragon for future studies of additional loci.

Content Area: Human Genetics
Keywords: RIP, GWAS, recombineering, CRISPR/Cas9, transgenic

Detecting Gene-Gene Interactions for Cleft Lip With or Without Cleft Palate by Targeted Sequencing of GWAS Loci

Yanzi Xiao[1] and Terri Beaty[1]

[1] Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health

Presented by Yanzi Xiao

Genome-wide association studies (GWAS) for non-syndromic cleft lip with or without cleft palate (CL/P) have identified multiple single-nucleotide polymorphisms (SNPs) that individually predispose to this common birth defect. However, many genetic risk factors such as epistasis remain unaccounted for. To following up on previous GWAS, we selected thirteen strongly associated regions, performed targeted sequencing in 1,409 Asian and European trios. We propose an exhaustive search for epistatic effects using regression-based methods and found robust evidence of GXG interaction between markers in ARHGAP29 and IRF6 (p-values= 6.29E-07 in European trios).

Content Area: Genetic Epidemiology
Keywords: complex disease, gene-gene interaction