7TH ANNUAL HUMAN GENOMICS SYMPOSIUM

UNIVERSITY OF CALIFORNIA DAVIS MEDICAL CENTER

NOVEMBER 6, 2020 » Virtual Symposium

PROGRAM

8:00 AM	**POSTER VIEWING OPENS**
8:50	**LOG INTO ZOOM**
9:00	**WELCOME** Katherine Rauen, MD, PhD, Chief, Division of Genomic Medicine, Department of Pediatrics, Symposium Chair
9:05	**KEYNOTE ADDRESS**: **The Mouse Model as Patient Avatar: Its Role Informing Disease Diagnosis, Patient Care, and Therapeutic Strategies** Kristin Grimsrud, DVM, PhD, Assistant Clinical Professor, Department of Pathology and Laboratory Medicine, Associate Director of Vivaria and Veterinary Care, Mouse Biology Program, University of California, Davis
9:45	» **PLATFORM PRESENTATIONS** **1. Genetic polymorphisms in ACE2, ARDS-related endothelial damage markers, inflammatory factors genes and 3p21.31 loci are associated with case-fatality differences in the COVID-19 pandemic** *Graciela Molina et al* **2. DNA methylation editing of genes on the X-chromosome via adeno-associated viruses** *Julian Halmai et al* **3. CGG allele instability and mosaicism in FMR1 premutation carriers** *Ye Hyun (Jenny) Hwang et al* **4. An automated system for early diagnosis, severity and progression identification in Duchenne Muscular Dystrophy: A machine learning and deep learning approach** *Albara Ramli et al*
10:45	**BREAK**
11:00	» **PLATFORM PRESENTATIONS** **5. Diverse molecular mechanisms contribute to differential expression of human duplicated genes** *Colin Shaw et al* **6. Assessment of allele frequency of a Latin American-specific, breast cancer-protective variant across breast tumor subtypes** *Valentina Zavala et al* **7. Sexually dimorphic gene expression molecular correlates of improvement in human ischemic stroke** *Hajar Amini et al* **8. Case report of oldest described adult with SETD2 variant: an expansion of phenotype that highlights the intersection of SETD2 gene neurodevelopmental and tumor suppression roles** *Alena Egense et al*
12:00 PM	**KEYNOTE ADDRESS: Ocular gene therapy for a retinal synaptic disease** Paul A. Sieving, MD, PhD, Professor of Ophthalmology, Director, Center for Ocular Regenerative Therapy, UC Davis School of Medicine
12:45	**CLOSING REMARKS AND ADJOURN**
2:00	**POSTER VIEWING CLOSES**

SYMPOSIUM CHAIR

KATHERINE (KATE) RAUEN, MD, PHD is a Professor in the Department of Pediatrics, Division of Genomic Medicine at UC Davis where she currently serves as the Chief of Genomic Medicine. Dr. Rauen is internationally known for her pioneering work in the early application of microarray technology in clinical genetics and as a leader and major contributor to the understanding of the RASopathies, the Ras/MAPK pathway genetics syndromes. Her research program involves the clinical and basic science study of cancer syndromes with effort to identify underlying genetic abnormalities affecting common developmental and cancer pathways.

ABSTRACT AND PROGRAM COMMITTEE

MAIJA KIURU, MD, PHD is an Assistant Professor in the Department of Clinical Dermatology and Pathology. She received her doctorate degree in cancer genetics. Her research identified an inherited genetic defect causing a syndrome with skin tumors, uterine fibroids, and kidney cancer. She continued to pursue her research interests in genetic medicine as a post-doctoral research fellow at Weill Cornell Medical College and at Columbia University studying hereditary skin blistering and hair and nail disorders. Her research interests include genetic alterations in skin tumors and familial skin diseases.

JOHN MCPHERSON, PHD is a Professor in the Department of Biochemistry and Molecular Medicine. He has deep expertise in DNA sequencing and cancer genomics through his involvement in the Human Genome Project and large-scale tumor sequencing as a founding member of the International Cancer Genome Consortium. His current interests lie in understanding the mechanisms underlying structural rearrangements in tumors, in bringing advanced genomic technologies to clinical application in personalized diagnosis and targeted therapeutics through maximizing the data yield from small biopsies and circulating cell free DNA, and in reducing chemotherapy- induced side effects during cancer treatment.

DAVID (DAVE) SEGAL, PHD is Professor of Biochemistry and Molecular Medicine in the UC Davis Genome Center, with joint appointments in the Department of Pharmacology, and the MIND Institute. He received his PhD from the University of Utah and post-doc'ed at The Scripps Research Institute. He joined the faculty of the University of Arizona in 2002 and UC Davis in 2005. Dr. Segal's research focus is on genetic and epigenetic editing for the study and treatment of neurologic disease.

SUMA SHANKAR, MD, PHD is an Associate Professor at the University of California, Davis in the Department of Pediatrics and Ophthalmology. She is a fellow of the Royal College of Surgeons, Edinburgh, UK and is board certified in Medical Genetics from the American College of Medical Genetics. She holds a PhD in Molecular Biology from University of Iowa. As director of precision genomic program her goal is to provide personalized health care and facilitate precision medicine practice using state-of-the-art whole genome sequencing to determine the underlying genetic etiology in patients with complex medical conditions.

WILLIAM (BILL) TIDYMAN, PHD is a Specialist in the Department of Pediatrics, Division of Behavioral and Developmental Pediatrics at the UC Davis Medical Center. He received his Master's in Physiology at San Francisco State University and went on to obtain his PhD in Physiology from University of California, Davis. Dr. Tidyman has extensive research experience in the study of both cardiac and skeletal muscle gene regulation and development. His current research examines the mechanism of dysregulated Ras/MAPK signaling in Costello and CFC syndrome models and how this disrupts early myogenesis.

KEYNOTE PRESENTERS

THE MOUSE MODEL AS PATIENT AVATAR: ITS ROLE INFORMING DISEASE DIAGNOSIS, PATIENT CARE, AND THERAPEUTIC STRATEGIES

KRISTIN GRIMSRUD, DVM, PHD
Assistant Clinical Professor, Department of Pathology and Laboratory Medicine
Associate Director of Vivaria and Veterinary Care, Mouse Biology Program
University of California, Davis

Dr. Kristin Grimsrud is an Assistant Clinical Professor in the Department of Pathology and Laboratory Medicine in the School of Medicine at the University of California Davis. Additionally, she is the Associate Director of Vivaria and Veterinary Care at the UC Davis Mouse Biology Program. Prior to this role she obtained a Doctor of Veterinary Medicine and her PhD in Pharmacology and Toxicology. She completed a residency in Laboratory Animal Medicine and did a fellowship in Clinical Pharmacology and a postdoc in Cardiothoracic Surgery with an emphasis in regenerative medicine in large animal models at UC Davis. Her current role at the Mouse Biology Program includes overseeing the rodent vivariums, providing clinical care for the animals, consulting on research and serving on a number of NIH consortium grants including the Knockout Mouse Project, the Mutant Mouse Resource and Research Center and the Mouse Metabolic Phenotyping Program. She has additional research focus of developing and analyzing precision animal models based on human patient variants, with a specific interest in the Champ1 gene. Her other research interests focus on characterizing individual variation in drug pharmacokinetics in special populations, specifically burn and pediatric patients. She has a currently funded K01 and Shriner's Hospital for Children grant to investigate the impact of pharmacogenetics on opioid metabolism and efficacy.

7TH ANNUAL HUMAN GENOMICS SYMPOSIUM

UNIVERSITY OF CALIFORNIA DAVIS MEDICAL CENTER

NOVEMBER 6, 2020 » Virtual Symposium

OCULAR GENE THERAPY FOR A RETINAL SYNAPTIC DISEASE

PAUL A. SIEVING, MD, PHD
Professor of Ophthalmology
Director, Center for Ocular Regenerative Therapy
School of Medicine
University of California, Davis

Dr. Sieving is Professor of Ophthalmology and Founding Director of the Center for Ocular Regenerative Therapy at the University of California at Davis. He recently joined the faculty in 2019 after serving as Director of the National Eye Institute, National Institutes of Health from 2001-2019. Dr. Sieving is known internationally for studies of human retinal neurodegenerative diseases. After graduate work in nuclear physics at Yale University, he attended Yale Law School (1973-74), and then attended the University of Illinois and received his MD degree (1978) and a PhD in Bioengineering (1981). He was an ophthalmology resident under Morton F. Goldberg, MD, at the University of Illinois Eye and Ear Infirmary, and he studied retinal physiology with Roy H. Steinberg MD, PhD, at UCSF as a post-doctoral fellow (1982-84). His clinical fellowship was with Eliot L. Berson, MD, in inherited retinal degenerative diseases, at Harvard (1984-85). Dr. Sieving was on the faculty of the University of Michigan (1985-2001) and held the Paul R. Lichter Chair in Ophthalmic Genetics. As Director of the National Eye Institute, he originated the "NEI Audacious Goals Initiative in Regenerative Medicine." Dr. Sieving identified a novel mechanism to protect photoreceptors by modulating the retinoid cycle in the eye using 13-cis retinoic acid (PNAS 2001), which has led to several therapy efforts for Stargardt macular degeneration. He conducted the first human clinical trial with CNTF neurotrophic factor to rescue rod and cone photoreceptors from slowly progressive death from RP (PNAS 2006). Dr. Sieving has worked for many years on the pathophysiologic basis of X-linked retinoschisis. He created a transgenic XLRS mouse model (IOVS 2004) and demonstrated that XLRS is a synaptic disease with direct involvement of the rod-to-bipolar synapse (JCI 2015). He used gene therapy to deliver a normal RS1 gene into eyes of XLRS mice, and this reverses the synaptic pathology and closes the retinal schisis cavities. These pre-clinical studies culminated in initiating a human AAV8-RS1 gene therapy trial for XLRS subjects in 2015 at the NEI. He has published over 300 peer reviewed papers in ocular genetics and the pathophysiology of retinal neurodegenerative diseases and was elected to membership in the US National Academy of Medicine in 2006 and the German National Academy of Sciences in 2013.

7TH ANNUAL HUMAN GENOMICS SYMPOSIUM

UNIVERSITY OF CALIFORNIA DAVIS MEDICAL CENTER

NOVEMBER 6, 2020 » Virtual Symposium

SEMINAR SERIES

UC DAVIS GENOMIC MEDICINE, GENOME CENTER AND COMPREHENSIVE CANCER CENTER PRESENT:

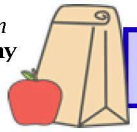
2020-2021 HUMAN GENOMICS SEMINAR SERIES

Web Connect *https://ucdhs.webex.com* or *https://www.webex.com*
Meeting Number: 820 659 459 Password: HGSS [All Caps]

Oct 7, 2020	**Joseph Shen, M.D., Ph.D.**	
12:00 PM	*UC Davis Department of Pediatrics*	
WebEx*	**A Proposal to Add Criteria That Incorporates Clinical Response to Treatment into the 2015 ACMG Genomic Variant Classification Guidelines**	
Nov 4, 2020	**Michelle Andrea La Merrill, Ph.D.**	
12:00 PM	*UC Davis Department of Environmental Toxicology*	
WebEx	**A Role of Thermogenesis in Human Genetics and Environmental Exposures Underlying Obesity**	
Dec 2, 2020	**Leonard Abbeduto, Ph.D.**	
12:00 PM	*UC Davis Department of Psychiatry and Behavioral Sciences*	
WebEx	**Down Syndrome: Research Perspectives on the Phenotype and Treatment**	
Jan 6, 2021	**K. C. Kent Lloyd, D.V.M., Ph.D.**	
12:00 PM	*UC Davis Department of Surgery*	
CHT 1341	**In Vivo Modeling of Functional Genomics**	
Feb 3, 2021	**Madelena Martin, M.D.**	
12:00 PM	*UC Davis Department of Biochemical Genetics*	
CHT 1341	**Newborn Screening: The Past, Present and Future**	
Mar 3, 2021	**John Albeck, Ph.D.**	
12:00 PM	*UC Davis Department of Molecular and Cellular Biology*	
CHT 1341	**Using Live-Cell Imaging to Quantify Cell Signaling Phenotypes**	
Apr 7, 2021	**Krishna Singh, M.D.**	
12:00 PM	*UC Davis Department of Obstetrics and Gynecology*	
CHT 1341	**Prenatal Genetics: Screening, Diagnostic Tests and Applications**	
May 5, 2021	**Craig McDonald, M.D.**	
12:00 PM	*UC Davis Department of Physical Medicine and Rehabilitation*	
CHT 1341	**Genetic-Based Therapies for Duchenne Muscular Dystrophy**	
Jun 2, 2021	**Alena Egense, M.S., L.C.G.C.**	
12:00 PM	*UC Davis Department of Pediatrics*	
CHT 1341	**Holding Things Together: A Review and Update on Cohesinopathies**	

ACCME ACCREDITED WITH COMMENDATION

Approved for 1.0 AMA PRA Category One Credit



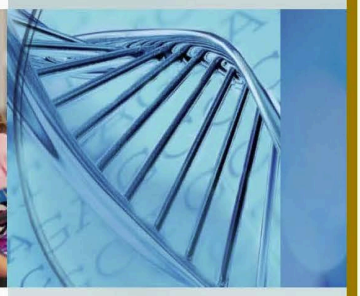
Lunch will be provided!**

**Location subject to change **Lunch provided for in-person sessions only*

UCDAVIS HEALTH | CHILDREN'S HOSPITAL | UCDAVIS GENOME CENTER | UCDAVIS HEALTH | COMPREHENSIVE CANCER CENTER

Sponsored by: UC Davis Health Office of Continuing Medical Education.

CLINICAL ROUNDS



GENOMIC MEDICINE CLINICAL ROUNDS

1.5 CME credits available

GOAL

This meeting is designed to enhance provider practice and knowledge of Genomic Medicine. Participants will be kept abreast of topics in the rapidly changing field of clinical genomics through discussion of known and novel genetic/genomic conditions, clinical cases, next generation diagnostic genomic testing, treatment, therapies, and research.

WHAT IS GENOMIC MEDICINE?

Genomic Medicine integrates the understanding of gene interactions and environmental factors as they contribute to human diseases. The Division of Genomic Medicine in the Department of Pediatrics at UC Davis applies this information to improve health care outcomes for both adults and children with genetic/genomic disorders through diagnostic testing, clinical care, education, and research.

This activity is approved - *AMA PRA Category 1.5 Credit(s)*TM

WHEN

Every Friday

12:00 PM—1:30 PM

LOCATION

2nd Floor Board Rm

MIND Institute

2825 50th Street

Sacramento, CA

95817

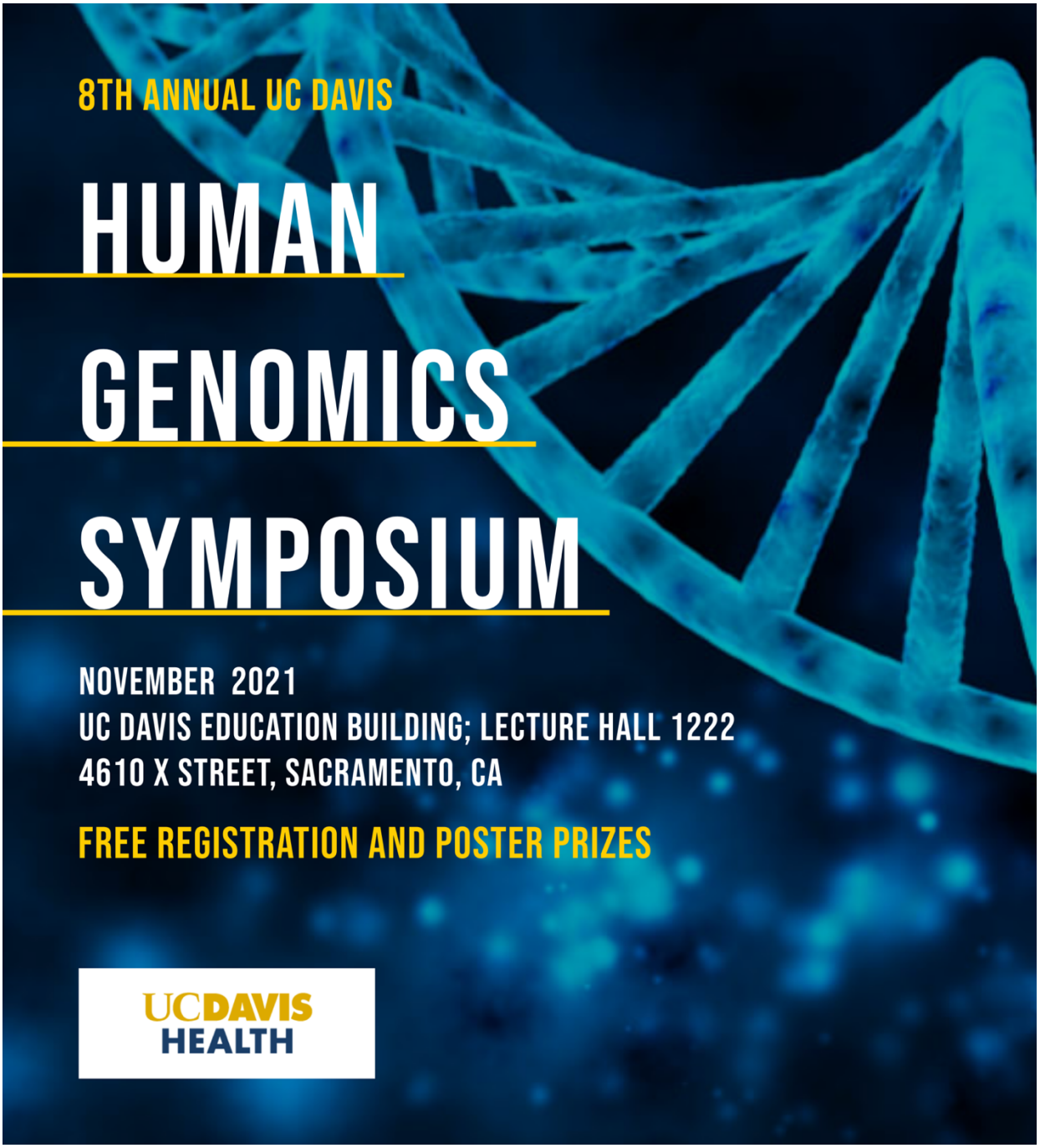
UCDAVIS HEALTH

7TH ANNUAL HUMAN GENOMICS SYMPOSIUM

UNIVERSITY OF CALIFORNIA DAVIS MEDICAL CENTER

NOVEMBER 6, 2020 Virtual Symposium

HUMAN GENOMICS SYMPOSIUM 2021



PLATFORM PRESENTATION ABSTRACTS

1. GENETIC POLYMORPHISMS IN *ACE2*, ARDS-RELATED ENDOTHELIAL DAMAGE MARKERS, INFLAMMATORY FACTORS GENES AND 3P21.31 LOCI ARE ASSOCIATED WITH CASE-FATALITY DIFFERENCES IN THE COVID-19 PANDEMIC.

GRACIELA MOLINA [1,2], Ramón Opazo[3], Paul Lott[1], Ignacio Wichmann[3], Carol Parra[2], Luis Carvajal Carmona[1].

[1]Davis Genome Center. University of California Davis, CA
[2]Facultad de Ciencias de la Salud, Universidad de Playa Ancha. Valparaíso, Chile
[3]Faculty of Medicine. Pontificia Universidad Catolica de Chile, Santiago Chile

e-mail: grmolina@ucdavis.edu

Notable differences in prevalence, mortality, and case-fatality rates of the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) pandemic have been observed among different world regions. Until now, these differences have mainly been attributed to demographics factors. However, genetic diversity may be a disregarded cause of the observed differences. We obtained SARS-CoV-2 epidemiological data from WHO normalized by population and by an equivalent time from the beginning of the pandemic in Europe, Finland, East Asia, South Asia, Latin America and Africa. We selected genetic polymorphisms in *ACE2,* and 50 Acute Respiratory Distress Syndrome (ARDS) risk markers and 7 markers of the new associated loci. The ARDS risk markers were classified as: i) epithelial damage markers, ii) endothelial damage markers, iii) inflammatory factors, iv) coagulation- and fibrinolysis-related markers. The polymorphisms allelic frequencies were taken from the Genome Aggregation Database. A Spearman's rank correlation was used. We found highly significant associations ($p<0.005$) between case fatality differences and the allelic frequencies of and *ACE2*, *ACE*, *VWF*, *TNF*, *INFG,* and *IL10* polymorphisms. As expected, a significant positive correlation between *ACE2* polymorphism and case fatality was observed ($R^2=0.943$; $p=0.0048$). Besides *ACE2* association, most of the remaining significant associations were between the case-fatality rate and polymorphisms of endothelial damage markers and inflammatory factors. We found association with endothelial damage markers in the context of ARDS, while the other associated factors are inflammatory-related. One remarkable observation is that IL-10, a known anti-inflammatory factor, was positively associated with case fatality, indicating that an inflammatory dysregulation is related to ARDS caused by SARS-CoV-2. We also found associations with recently found risk 3p21.31 loci, especially with allelic frequencies of polymorphisms in *SLC6A20* and *LZTFL1* genes. The associations found are consistent with current knowledge about the pathophysiology of SARS-CoV-2 syndrome and associated ARDS. Our findings support the hypothesis that case fatality differences observed in different world regions can be explained, at least in part, by the presence of different genetic backgrounds. Beca Chile Postdoctorado 74190063.

SARS-CoV-2 syndrome, case fatality rate, genetic polymorphisms, Angiotensin-converting enzyme 2, Acute respiratory distress syndrome.

2. DNA METHYLATION EDITING OF GENES ON THE X-CHROMOSOME VIA ADENO-ASSOCIATED VIRUSES

JULIAN A.N.M. HALMAI [1,2], Casiana E. Gonzalez[1,2], Jennifer J Waldo[1,2], Peter Deng[1,2,3], Fiona K. B. Buchanan[1,2], Jasmine Carter[1,2], Chloe Welch[1,2], David Cameron[1,2] and Kyle D. Fink[1,2]*

[1] Department of Neurology, University of California Davis School of Medicine, Sacramento, CA 95817, USA
[2] Stem Cell Program and Gene Therapy Center, University of California, Davis, Sacramento, California
[3] Genome Center and Department of Biochemistry and Molecular Medicine, University of California, Davis, CA, 95616, USA

Despite only containing about 5% of the human genome, there are more than 141 known X-linked intellectual disability genes present on the X-chromosome. In females, monogenic disorders with an X-linked dominant pattern of inheritance result in a mosaic of cells expressing the mutant and wild-type alleles. One of the alleles becomes epigenetically silenced via a dosage compensation mechanism called X-chromosome inactivation. Up to 25% of genes are able to escape from X-chromosome inactivation. These escapees have a specific epigenetic signature associated with them, particularly reduced levels of 5-methylcytosine in CpG island promoters. A potential therapeutic approach previously demonstrated by our group for a gene on the X-chromosome is to activate the silenced wild type allele in cells expressing a loss-of-function mutant allele. Here, we demonstrate reactivation of two genes involved in X-linked intellectual disabilities in patient-derived induced pluripotent stem cells and neural stem cells, *CDKL5* and *MECP2*. We demonstrate that gene reactivation is possible via DNA methylation editing of the promoter regions using CRISPR/dCas9. In addition, our group demonstrates that this approach is readily translatable via the employment of intein-mediated trans-splicing of large dCas9 effector fusion proteins, allowing delivery to the central nervous system *in vivo* via adeno-associated viruses. Our approach holds great promise for those suffering from X-linked dominant disorders of the CNS.

3. CGG ALLELE INSTABILITY AND MOSAICISM IN *FMR1* PREMUTATION CARRIERS

YEHYUN HWANG [1], Jay Kumar[1], Bruce Hayward[2], Karen Usdin[2], Randi Hagerman[3,4], Flora Tassone [1, 3]

[1]Department of Biochemistry and Molecular Medicine, University of California Davis, School of Medicine, Davis, CA
[2]Section on Gene Structure and Disease, Laboratory of Molecular and Cellular Biology, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD USA
[3]MIND Institute, University of California Davis Medical Center, Sacramento CA
[4]Department of Pediatrics, University of California, School of Medicine, Sacramento CA

Background: Individuals with a CGG expansion in the premutation range in the *FMR1* gene (55-200 CGG) are at risk for *FMR1*-associated disorders and can display CGG size instability and mosaicism. However, it is unknown why some carriers present with somatic allelic instability while others do not, and, which are the risk factors that can lead to instability. In this study, we aimed to identify those molecular factors that may affect repeat instability, in both males and female carriers.

Methods: 426 female participants and 454 male participants were included in this study. Genomic DNA was isolated from whole blood collected from participants using standard procedure. CGG repeat number and the presence of AGG interruptions were determined by triplet primed PCR. Methylation status was determined by Southern blot analysis and HpaII digestion. *FMR1* mRNA expression levels were measured by real time qRT-PCR.
Results: In the 426 females, CGG repeat size and AGG interruptions significantly correlated with allelic instability while *FMR1* mRNA and age did not. Females with higher instability displayed significant differences in CGG repeat size, AGG interruptions, Activation Ratio, and *FMR1* mRNA compared to those with lower instability. Unstable expanded alleles were always unmethylated, thus carried on the active X chromosome. Finally, we did not observe any significant changes in CGG repeat size (58%) with time.

In contrast, we observed a remarkable degree of allelic instability in male premutation carriers significantly which significantly correlated with CGG repeat number but not with *FMR1* mRNA. Instability appeared to change with time as, within the 50 male samples analyzed, and with an age gap of on average of 6 years, 14% of the male carriers displayed a change of more than 4 CGG repeats and 24% an expansion of 1-2 CGG repeats.

Conclusions: Allele instability is seen in both male and female carriers of the *FMR1* premutation allele. Our preliminary results indicate that instability is affected by the CGG repeat number and AGG interruptions but not by *FMR1* mRNA. We also demonstrated that the unstable unmethylated alleles in females are on the active X chromosome and that instability is affected by the AR. Our study highlights the importance of the molecular factors that can affect allele instability and somatic mosaicism in carriers of a premutation allele. Our future studies will focus on whether other type of factors (i.e. repair genes) can affect *FMR1* allele instability.

4. AN AUTOMATED SYSTEM FOR EARLY DIAGNOSIS, SEVERITY AND PROGRESSION IDENTIFICATION IN DUCHENNE MUSCULAR DYSTROPHY: A MACHINE LEARNING AND DEEP LEARNING APPROACH

ALBARA AH RAMLI, MS (1), Alina Nicorici, BS(2), Poonam Prasad, BS(2), JaiHui Hou, PhD(1), Craig McDonald, MD(2), Xin Liu, PhD(1), Erik Henricson, PhD, MPH(2)

(1) Department of Computer Science, School of Engineering
(2) Department of Physical Medicine & Rehabilitation, School of Medicine

Background: Duchenne Muscular Dystrophy (DMD) is a fatal X-linked congenital genetic disorder affecting 1/5000 males that is caused by a lack of dystrophin, a protein critical for function of muscle cells. The disorder leads to progressive debilitating muscle weakness and loss of ambulation around the age of 12 with early death due primarily to cardiac and respiratory complications in the late teens or early 20s. Weakness is detectable from infancy, but symptoms are typically recognized during toddler or early childhood years. There is presently no cure for the disorder, but novel gene repair interventions and other preventive therapies are initiated as early as possible to slow progress of the disease and prevent secondary conditions. Tools are needed to 1) facilitate early diagnosis; 2) identify early indicators of clinical severity, and 3) quantify and track progression of muscle weakness across the ambulatory phase of the disease.

Methods: Here, we present an Artificial Intelligence (AI)-based detection of gait characteristics in toddlers and children with DMD and typically-developing peers. Our system collects data from mobile device acceleration sensors remotely and in real time using our novel Walk4Me smart phone application. Our web application extracts temporal/spatial gait characteristics and raw data signal characteristics, and then uses traditional machine learning and deep learning techniques to identify patterns that can 1) identify children with gait disturbances associated with DMD, 2) describe the degree of mobility limitation, and 3) identify characteristics that change over time with disease progression. Results: We have identified several machine learning techniques that differentiate between DMD and typically-developing children with >99% accuracy across the age range studied and have identified corresponding temporal/spatial gait characteristics associated with each group.

Conclusion: Our work manifests how the latest advances in mobile device and machine learning technology can be adapted to measure clinical outcomes regardless of point of care and that may be used to inform early clinical diagnosis, treatment decision making and to monitor disease progression.

5. DIVERSE MOLECULAR MECHANISMS CONTRIBUTE TO DIFFERENTIAL EXPRESSION OF HUMAN DUPLICATED GENES

COLIN J. SHEW [1,2], Paulina Carmona-Mora[1,3,4], Daniela Soto[1,2], Henriette O'Geen[1], Mira Mastoras[1], Joseph Rosas[1,5], Gulhan Kaya[1], Elizabeth Roberts[1], Dhriti Jagannathan[1], David J. Segal[1-6], Megan Y. Dennis[1-6,†]

[1]Genome Center, [2]Integrative Genetics and Genomics Graduate Group, [3]MIND Institute, [4]Autism Research Training Program, [5]Postbaccalaureate Research Education Program, and [6]Department of Biochemistry & Molecular Medicine, University of California, Davis, CA 95616

Emerging evidence links human-specific segmental duplications (HSDs) to unique neurological features and disease phenotypes of our species. Precise spatiotemporal control of gene regulation is critical to both, and regulatory divergence is thought to directly favor duplicate retention over evolutionary time. Strikingly, despite being nearly identical (>98.5%), many HSD genes show evidence of differential regulation across cell and tissue types. No explanation for this has been investigated, primarily due to the difficulty of studying highly similar regions with sequence-based methods. We examined 75 HSD genes in 30 families and determined that, across 2 cell lines and 4 primary tissues, duplicates display expression patterns consistent with non- or neofunctionalization, and some derived paralogs show greater conservation of expression than the ancestral gene. Analysis of 446 human LCLs showed ~75% of HSD genes are differentially expressed with respect to the ancestral paralog. Globally, copy number differences, post-transcriptional regulation, gene truncation status, and sequence divergence did not explain the observed differential expression between HSD paralogs. We next sought to identify active *cis*-regulatory elements (CREs) in HSDs but found that data from ENCODE were significantly depleted for features in segmental duplications. We implemented an alternative bioinformatic method to recover hundreds of candidate CREs in these regions. Finally, we performed large-insert ChIP in LCLs for RNA PolII, H3K4me3, H3K4me1, and H3K27ac to better distinguish active chromatin in HSD paralogous regions. Alignments of longer reads show an increased mapping quailty within HSDs, allowing identification of differentially marked CREs. Some of these duplicated CREs, including promoters and candidate enhancers, are differentially active in a luciferase reporter assay (N=3/9 tested pairs). Overall, we show that the regulatory landscape of HSDs is systematically undercharacterized, present a set of candidate HSD CREs, and demonstrate differential activity in a subset of elements. This work provides evidence that *cis*-regulatory divergence contributes to novel expression patterns of recent gene duplicates in humans.

6. ASSESSMENT OF ALLELE FREQUENCY OF A LATIN AMERICAN-SPECIFIC, BREAST CANCER-PROTECTIVE VARIANT ACROSS BREAST TUMOR SUBTYPES

VALENTINA ZAVALA [1], Tatiana Vidaurre[2], Sandro Casavilca[2], Carlos Castañeda[2], Jeannie Vásquez[2], Fernando Valencia[2], Zaida Morante[2], Monica Calderon[2], Julio Abugattas[2], Henry Gómez[2], Hugo Fuentes[2], Ruddy Liendo Picoaga[2], Jose M. Cotrina[2], Zaida Morante[2], Fernando Valencia[2], C. Monge- Pimentel[2], Silvia Neciosup[2], Bizu Gelaye[3], Laura Fejerman[1].

[1]Department of Public Health Sciences, University of California Davis, Davis, CA;
[2]Instituto Nacional de Enfermedades Neoplasicas, Peru;
[3]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA.

In the United states, the incidence of breast cancer in women of Latin American origin is lower compared to European American and African American women. Among Latinas, a population-specific common polymorphism in the 6q25 chromosomal region near the Estrogen Receptor 1 (*ESR1*), gene (rs140068132A) has been associated with breast cancer risk. The G allele, which is only observed in individuals with Indigenous American Ancestry (IAA), is less common in breast cancer cases compared to unaffected controls. In the discovery genome-wide association study, the G allele was more common in estrogen receptor positive (ER+) compared to ER- patients. In this study we assessed the association of the rs140068132 polymorphism with specific breast cancer subtypes in patients with high IAA.

We genotyped 1,327 BC patients recruited at the Instituto Nacional de Enfermedades Neoplasicas in Lima, Peru, that agreed to participate in the Peruvian Genomics of Breast Cancer (PEGEN-BC) Study. Genotype data for the rs140068132 polymorphism was available for 3,338 women without a breast cancer diagnosis from a study of pregnant women (PrOMIS) from Lima, Peru. After quality controls, 1,312 cases remained. IAA and African ancestry components were estimated using ADMIXTURE v1.3. Four major breast cancer subtypes (ER/PR+ HER2-, ER/PR+ HER2+, ER/PR -HER2+, ER/PR- HER2-) were defined using immunohistochemical markers. Multinomial and binomial logistic regression analyses were performed in R including age at diagnosis, IAA and African ancestry as covariates and the ER/PR+ HER2- subtype was defined as reference.

The average age at diagnosis for the PEGEN-BC Study patients was 50 years (±10.97) and did not differ by tumor subtype. The proportion of IAA component in ER/PR+ HER2-, ER/PR+ HER2+, ER/PR-HER2+ and ER/PR- HER2- tumors was 74% (±0.18), 76% (±0.18), 79% (±0.14) and 76% (±17), respectively (p= 0.007). Fifty percent of cases were ER/PR+ HER2-, 19% ER/PR+ HER2+, 12% ER/PR-HER2+ and 15% ER/PR- HER2-. Overall, the rs140068132-G allele frequency was 14%, and varied by tumor subtype. The G allele was most common in patients with ER/PR+ HER2- tumors (16%), and less so among patients with other subtypes (12%,11% and 12%; respectively). The frequency of the rs140068132 G allele among unaffected Peruvian women from the PrOMIS study was 25%. Multinomial logistic regression models suggested that the AG genotype (vs. AA) was not only associated with reduced odds of developing ER- tumors, but also ER/PR+HER2+ tumors (OR=0.69, 95%CI 0.49-0.97, p=0.04). In a logistic regression model where ER/PR+HER2- tumors where compared to all other subtypes, the odds ratio associated with the AG genotype vs. AA was 0.64 (95%CI 0.49-0.82, p=0.0007). Additionally, we observed that the frequency of GG genotype was higher among patients with ER/PR- HER2- (3% vs.1%) tumors and overall genotype analyses were not consistent with a simple additive effect on tumor subtype for the G allele.

Our results are in line with the previously reported association between breast cancer risk and the rs140068132 polymorphism in Hispanics/Latinas. Additionally, our results strongly suggest that the rs140068132-AG genotype is more common in patients with ER/PR+ HER2- tumors compared to other subtypes. The mechanisms leading to the observed association between this protective variant and the least aggressive ER/PR+HER2 negative breast cancer needs further investigation. The rs140068132 variant is located in an enhancer region close to *ESR1*, suggesting a possible role of this variant on ESR1 expression and as a result, the development or ER-positive tumors. Ongoing gene expression analyses focused on the rs140068132 polymorphism, the ESR1 gene, and other associated genes, will help to elucidate to new approaches in breast cancer chemoprevention.

7. SEXUALLY DIMORPHIC GENE EXPRESSION MOLECULAR CORRELATES OF IMPROVEMENT IN HUMAN ISCHEMIC STROKE

HAJAR AMINI [1], Bodie Knepp[1], Heather Hull[1], Paulina Carmona-Mora[1], Marisa Hakoupian[1], Noor Alomar[1], Glen Jickling[1], Xinhua Zhan[1], Jane Khoury[2], Arthur Pancioli[2], Joseph Broderick[2], Bradley P Ander[1], Frank R Sharp[1*], Boryana Stamova[1*]

* Senior Co-Authors

[1]Department of Neurology, University of California at Davis, Sacramento, CA
[2]University of Cincinnati, Cincinnati, OH

Objective: Ischemic stroke (IS) is sexually dimorphic for risk factors, age, heritability, causes, treatment, and outcome. We identified transcriptional correlates with 90-day outcome that differed between male and female IS subjects.

Methods: RNA from 72 samples from 2 peripheral blood draws (at ≤3 and 24h post IS onset) was analyzed on Affymetrix U133 Plus 2 microarrays. These represented samples from 36 CLEAR trial IS patients treated with tPA with or without eptifibatide after the first blood sample within 3 hours of stroke onset. Changes in gene expression levels (deltaGE) between 3h and 24h were calculated and the association with percent NIH Stroke Scale (NIHSS) improvement from 3h to 90 days (% Improvement) examined. We used mixed-effects linear regression, including Treatment, Age, Sex, Vascular Risk Factors, 3h NIHSS, % Improvement, and a Sex * % Improvement interaction. Sex differences in association of gene expression with % Improvement were determined by examining the Sex * % Improvement interaction term, $p<0.005$ was considered statistically significant.

Results: 577 genes correlated differently with % Improvement in IS males and females. These included matrix metalloproteinases (MMPs), which play a major role in BBB dysfunction and outcomes post IS. *MMP11*, *MMP14* and *MM17* correlated with % Improvement in opposite direction in males and females. Inflammatory genes like *IL-27*, implicated in infarct volume and stroke outcome, and ABC transporters (*ABCC9*) also had opposite correlation with % Improvement in males and females. Calmodulin 1 (*CAML1*) was also sexually dimorphic, and a SNP in *CALM1* has been implicated in IS risk and blood coagulation in female IS patients. EIF2 signaling, a major protein synthesis pathway was activated in males (adj. p = 1e-8), while suppressed in females (adj. p value = 1e-9). Protein synthesis and associated unfolded protein response cascade have previously been implicated in stroke outcome.

Conclusions: The identified sexually dimorphic gene expression associated with 90-day improvement might relate to sex differences in blood immune and clotting pathways. The findings expand our understanding of the genomic underpinnings associated with stroke outcome and may serve as potential sex-specific treatment targets.

8. CASE REPORT OF OLDEST DESCRIBED ADULT WITH SETD2 VARIANT: AN EXPANSION OF PHENOTYPE THAT HIGHLIGHTS THE INTERSECTION OF SETD2 GENE NEURODEVELOPMENTAL AND TUMOR SUPPRESSION ROLES

ALENA EGENSE, MGC, CGC and Katherine Rauen, MD, PhD

Department of Pediatrics, Division of Genomic Medicine, University of California Davis, Sacramento, California

The *SETD2* gene, SET-domain containing 2, encodes a histone H3 lysine 36 (H3K36) methyltransferase and plays a critical role in epigenetic regulation, RNA splicing, and DNA repair[11]. Individuals with autosomal dominant germline variants in *SETD2* have been described to have a disorder (also called Luscan-Lumish syndrome) characterized by a neurodevelopmental phenotype of developmental delay, intellectual disability, autism spectrum disorders (ASD); behavior issues including ADHD; and macrocephaly[2,7,8]. A few individuals have an overgrowth-type presentation also including postnatal overgrowth, obesity, and advanced bone age[4,9] At least six individuals were described to have brain anomalies, one of who was noted to have Chiari I malformation with syringomyelia[4,6]. No reported cases of malignancy. The oldest individual in the current 12 reported cases is a 26-year-old male[6]. To our knowledge, we report the oldest individual with *SETD2* variant, a 40-year-old female. She presented to Genomic Medicine clinic with a multisystem constellation of features not consistent with identifiable syndrome, so broad genetic testing approach was recommended. SNP microarray returned negative. Clinical Exome Sequencing identified a novel nonsense variant in *SETD2* c.1503 C>A (p.Y501X), consistent with previously reported loss of function disease mechanism, and exon 3 location as described in over half of individuals[10]. Parental testing not available.

The patient was born full term after uncomplicated pregnancy and birth, with neonatal course significant for inguinal hernia repair. Consistent with others with *SETD2* variant, she was noted to have global developmental delay. She received academic therapies through high school to complete her GED, and subsequently obtained her associates degree. While her parents never sought formal evaluation, she has history of ADHD- and ASD-like behaviors. No history of seizures. Early childhood parameters are not known but macrocephaly noted as an adult. She is the second case reported to have Chiari I malformation with syringomyelia, diagnosed by MRI at age 21. Our patient also has significant history of benign tumor growth including removal of over 15 colon polyps between ages two and forty and prolactinoma diagnosed at thirty-two by MRI. In addition, she had an unilateral oophorectomy due to ovarian cyst, and has history of obesity, and recurrent pericarditis of unknown etiology. Family history is non-contributory.

This report describes the oldest individual known with a *SETD2* variant, whose presentation is consistent with history of previously reported individuals, and notably the second reported individual with Chiari I malformation with syringomyelia suggesting this may be a more common phenotype associated with SETD2. Interestingly, SETD2 gene also functions as tumor suppressor gene and somatic loss of function variants have been identified in solid tumors including gastrointestinal cancer and leukemia, among others[10]. We propose that this patient's history of early-onset benign tumor growth may be a result of her SETD2 variant and reflect an expansion of the SETD2-gene phenotype. The case highlights the importance of continued study into cancer and tumor risks for individuals with SETD2 variants, and how use of broad approach genetic testing through Exome sequencing can be beneficial even in adults with history of neurodevelopmental features.

References:

1. Chen R, et al. Histone methyltransferase SETD2: a potential tumor suppressor in solid cancers. J Cancer. 2020;11(11):3349-3356.
2. Iossifov I et al. The contribution of de novo coding mutations to autism spectrum disorder. Nature. 2014;515(7526):216
3. Kanu N, et al. SETD2 loss-of-function promotes renal cancer branched evolution through replication stress and impaired DNA repair. Oncogene. 2015;34(46):5699-5708.
4. Lumish HS, et al. Brief Report: SETD2 Mutation in a Child with Autism, Intellectual Disabilities and Epilepsy. J Autism Dev Disord. 2015;45(11):3764-3770.
5. Luscan A, et al. Mutations in SETD2 cause a novel overgrowth condition. J Med Genet. 2014;51(8):512-517.
6. Marzin P, et al. SETD2 related overgrowth syndrome: Presentation of four new patients and review of the literature. Am J Med Genet C Semin Med Genet. 2019;181(4):509-518.
7. O'Roak BJ, et al. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. Science. 2012;338(6114):1619-1622.
8. O'Roak BJ, et al. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. Nature. 2012;485(7397):246-250. Published 2012 Apr 4.
9. Tlemsani C, et al. SETD2 and DNMT3A screen in the Sotos-like syndrome French cohort. J Med Genet. 2016;53(11):743-751.
10. van Rij MC, et al. Two novel cases expanding the phenotype of SETD2-related overgrowth syndrome. Am J Med Genet A. 2018;176(5):1212
11. Xu Q, et al. SETD2 regulates the maternal epigenome, genomic imprinting and embryonic development. Nat Genet. 2019;51(5):844-856.

POSTER PRESENTATION ABSTRACTS

9. PILOT PATHWAY VARIANT ANALYSIS FOR FENTANYL IN PEDIATRIC BURN PATIENTS

KRISTIN N. GRIMSRUD DVM, PHD [1]*, Ryan R. Davis BS[2], Clifford G. Tepper PhD[2] and Tina L. Palmieri, MD, FACS, FCCM[3]

[1]Dept. of Pathology and Laboratory Medicine, School of Medicine, University of California, Davis
[2]Dept. of Biochemistry and Molecular Medicine, School of Medicine, University of California, Davis
[3]Dept. of Surgery, School of Medicine, University of California, Davis

Background: Opioid dosing in patients remains challenging, particularly due to the wide range in interpatient variability, as one size fits all dosing is not effective. While pain control is achieved in some individuals with a standard dose, others may need a much higher dose, and some may experience adverse reactions with even a small dose. To complicate things further, the development of tolerance, dependency or addiction when using opioids long term pose challenges. Despite decades of investigating opioids, we still do not fully understand the underlying mechanisms responsible for the vast variability in response and potential downstream negative consequences contributing to tolerance and addiction. To develop patient-tailored dosing regimens we focused our efforts on patient genetic variability as well as the individual factors contributing to the variable effects, such as drug-drug interactions, clinical procedures, and comorbidities.

Methods: In a small pilot analysis from eight pediatric burn patients, we utilized whole-exome sequencing (WES) to evaluate several genes in the fentanyl pathway encompassing metabolism, response and downstream effects as well as several other genes that may be of clinical significance for other drugs (e.g. cytochrome P450s; CYPs). Results: Interestingly, all eight patients had at least one or more variants in the mu opioid receptor with variants in 62.5% of patients predicted to have functional impact and 25% of patients being heterozygous for a loss of function mutation. Only one patient had a missense variant in the CYP3A4/3A5 pathway, which is more conserved, and other CYPs such as 2D6, 2C9, 2C19 and 2B6 did have several missense variants identified with some predicted to be clinically significant. Moreover, several patients had missense variants in the delta opioid receptor while no notable variants were identified in the kappa opioid receptor. Several missense variants were identified in the SLC22A1 and ABC1 transporters, as well as the COMT, serotonin (HTR2A) and dopaminergic (DRD2) pathways. Interestingly, the potassium channel (KCNJ6) and adrenergic receptor subtype 2A (ADRA2A) seem to be well conserved as no notable variants were identified in these genes. Conclusions: Based on these findings we suggest that the genetic variability contributing to fentanyl efficacy is more likely related to variants in the mu-opioid receptor and not the corresponding metabolism pathway. Large scale studies in diverse patient populations need to be conducted to better characterize the frequency of variants in these genes, as well as other genes throughout the entire fentanyl pathway and downstream pathways in order to predict efficacy outcomes for the patient as a whole. These pathway burden analysis approaches combined with machine learning predictions will be vital to transform standardized dosing approaches to provide precision patient-tailored care.

10. ULTRA RAPID WHOLE GENOME SEQUENCING IMPACTS PATIENT CARE IN A CRITICALLY ILL CHILD

POOJA PARMAR, MD, MHA; Michelle Perez, MD; Jason Carmichael; Aaina Kochhar, MD; Organization: Valley Children's healthcare

Introduction: Pathogenic mutations in the AMT gene cause autosomal recessive glycine encephalopathy or NKH (Nonketotic hyperglycemia), an inborn error of glycine metabolism producing deficient activity of the glycine cleavage enzyme system thereby accumulating large quantities of glycine in all body tissues including the brain. It is considered rare with an estimated prevalence of 1:60,000. Majority of children with NKH have onset in the neonatal period manifesting as hiccups, drug resistant seizures, marked hypotonia, progressive lethargy evolving into profound coma and/or death due to central apnea; 85% have severe NKH which carries a poor prognosis and 15% attenuated NKH. Those with onset > 3months of age have attenuated NKH. Before age six months children with severe NKH begin to develop progressive spasticity and cortical blindness. Many have swallowing dysfunction requiring tube feeding and develop scoliosis or hip dislocation in childhood or adolescence. No treatment is effective to reduce progression of spasticity, developmental delays and intractable epilepsy in severe NKH. However, for attenuated NKH treatment with benzoate to lower glycine improves attentiveness and facilitates seizure management. Here, we present a case of a critically ill 2 month old female with drug resistant seizures diagnosed with NKH using ultra rapid WGS.

Case: 2 month old term female presented with jerking movements of upper body, stiffening and eye rolling with multiple episodes a day since 4 weeks of age. Born to healthy parents from a non-consanguineous marriage, h/o of prior maternal pregnancy losses, newborn screen resulted normal. On admission, video EEG was concerning for infantile spasms therefore keppra and prednisone started, genetic work up initiated including very long chain fatty acids, serum carnitine, urine organic acids, plasma amino acids and acyl carnitine, serum ammonia and lactic acid. Workup was remarkable for plasma amino acid glycine at 960 (normal 80-400). MRI of the brain was concerning for metabolic disease. Considering the presentation and MRI findings of white matter restriction in the supratentorium, occipital and temporal lobes with equivocal restricted diffusion in the ventral corticospinal tracts in the medulla, nonketotic hyperglycemia was suspected. However, diagnosis of NKH requires elevated CSF glycine and CSF glycine/plasma glycine ratio; yet LP initially could not be performed due to acute change in mental status. Ultra rapid whole genome sequencing performed through the CA state funded pilot - Project Baby Bear confirming NKH; showing compound heterozygosity for 2 likely pathogenic mutations in the AMT (aminomethyltransferase) gene. Paternally inherited c.958C>T (p.Arg320Cys) and maternally inherited c.797T>C (p.Leu266Pro). She was started on Dextromethorphan 5mg/kg/day and sodium benzoate 600mg/kg/day. Seizures gradually improved and steroids weaned. LP later showed elevated CSF glycine at 161.3. After detailed discussion with Genetics, Neurology and primary team regarding care goals for the patient, family changed code status to DNR. During ventilatory support wean, she developed increased secretions from Coronavirus infection, subsequently improved and weaned from PS/CPAP to HFNC. Patient did not tolerate wean, family opted for comfort care. Family was also counseled on recurrence risk of 25% for subsequent pregnancy along with discussion of pre implantation genetic diagnosis.

Discussion: Over the last decade DNA sequencing has been increasingly utilized in medicine, especially for suspected genetic disorders in newborns. By using ultra rapid WGS, results are available in 2-3 days vs the 2 weeks it would take for diagnostic CSF neurotransmitter studies allowing the benefits of fewer hospital days, procedures or unnecessary treatments, efficient and timely patient care, saving healthcare dollars. By using ultra rapid WGS sequencing in this case, parents were able to have a realistic perspective regarding their child's prognosis. They had time to discuss this diagnosis with specialists and met with a family of a 16 y/o child with attenuated NKH. This allowed the family time to accept the diagnosis, extended family members were able to fly in from out of state to support the family and a baptism was performed at bedside. In addition parents were able to experience a gamut of emotions from the certainty of having a diagnosis, relief of knowing it was not caused by maternal antidepressants, optimism and hope that she could improve, sadness at realizing the outcome and grief for their daughter not being able to live the life they imagined for her. Within a week she continued to deteriorate and after multiple discussions between family and specialists, the family realized comfort care was the right choice for their daughter and she expired later that night.

11. IDENTIFICATION OF A MATERNALLY INHERITED PATHOGENIC KCNQ2 MUTATION IN A NEONATE WITH SEIZURES

JULIA R FROUD, MD [1], Alena Egense, MGC, CGC[2], Shannon N Liang, MD[3], Celia H Chang[3], MD, and Suma Shankar, MD[2]

[1]UC Davis Medical Center, Department of Pediatrics
[2]UC Davis MIND Institute, Department of Genomic Medicine
[3]UC Davis Medical Center, Department of Pediatric Neurology

Introduction: The genetic differential diagnosis for neonatal seizures is broad and includes neuronal migration disorders, vascular malformations, chromosomal disorders, channelopathies, neurocutaneous syndromes, inborn errors of metabolism, and cell signaling defects. Early identification and diagnosis of neonates with genetic epilepsy can provide important information regarding treatment, prognosis, and recurrence risk. We report a case of a neonate who was found to have KCNQ2 related epilepsy on ultra-rapid whole genome sequencing in the first two weeks of life.

Case Report: A 7-day-old female was transferred to UC Davis Children's Hospital for seizures. She was born via repeat c-section at 39 weeks to a 31-year-old G2P12 mother following a pregnancy complicated by gestational diabetes. Family history was significant for a 3-year-old brother with seizures in the neonatal period and autism. He was successfully weaned off antiepileptic medication around 4 months of age with no subsequent seizures. The patient appeared healthy at birth and was discharged home on DOL 2. On DOL 3 she developed left eye twitching and rhythmic jerking of the left upper and lower extremities in addition to excessive sleepiness and poor feeding. On exam she was non-dysmorphic but had significant hypotonia. Video EEG confirmed seizure activity. A full sepsis workup was negative. Head ultrasound and brain MRI with spectroscopy were normal. Seizures were initially difficult to control with levetiracetam alone but responded well to the addition of phenobarbital. Genetics was consulted on DOL 10. Given the family history, the decision was made to perform ultra-rapid trio whole genome sequencing through Project Baby Bear at Rady Children's Hospital. WGS returned positive for a maternally inherited pathogenic variant, c.1741C>T, p.Arg581Ter, in *KCNQ2*. This variant is known to have incomplete penetrance and is associated with a wide range of phenotypes from benign familial epilepsy to neonatal epileptic encephalopathy.

Discussion: *KCNQ2* mutations are a rare and likely underdiagnosed cause of neonatal seizures. To the best of our knowledge, this is the first reported patient to be diagnosed with KCNQ2-related epilepsy on ultra-rapid whole genome sequencing in the neonatal period. Early genetic testing in neonates with epilepsy has the potential to influence treatment, prognostication, and genetic counseling. The patient described in this report continues to be hypotonic but seizure-free on levetiracetam and phenobarbital. Her development is being closely monitored. Her older brother has been referred for targeted genetic testing to see if he carries the same variant as his sister.

12. ATYPICAL PRESENTATION OF IMPRINTING DEFECT ANGELMAN SYNDROME WITH EXPRESSIVE LANGUAGE

RUCHI PUNATAR, MD [1] Alena Egense, MGC, CGC[2] Suma Shankar, MD, PhD[2]

[1] Department of Pediatrics, Division of Developmental Behavioral Pediatrics, University of California Davis, Sacramento, California
[2] Department of Pediatrics, Division of Genomic Medicine, University of California Davis, Sacramento, California

Introduction: Angelman syndrome (AS) is an imprinting disorder caused by disruption of the maternally inherited UBE3A gene in the 15q11.2-q13 region. AS is usually characterized by severe developmental delay, seizures, absent speech, gait ataxia, happy demeanor, excitability, and hypermotoric behavior. Here we report on an atypical presentation of AS with expressive language.

Case Description: A 16-year-old boy initially presented due to obesity, hyperphagia, and intellectual disability. He was reported to have hypotonia as an infant. At three years of age he developed hyperphagia, and he was obese by seven years. His speech and language milestones were severely delayed with first word approximately at seven years. Currently, he speaks in 3-5 word phrases. He has a no history of seizures or other neurological concerns. His family describes his demeanor as happy, but he has anxiety.

On exam, the patient's weight was 98.2 kg (98^{th} percentile, Z=2.16), height was 176 cm (58^{th} percentile, Z=2.16), BMI was 31.6 kg/m^2 (98^{th} percentile, Z=2.10), and head circumference was 58 cm (97^{th} percentile, Z=1.95). He had intermittent exotropia of his right eye, gynecomastia, obesity, and hyperextendable fingers. Bilateral palm length was 11 cm and bilateral mid finger length was 8 cm. His muscle tone and bulk were normal, and he had a normal gait.
Initial testing included normal cytogenomic SNP microarray with no evidence of uniparental isodisomy, and normal fragile X (FMR1) studies. AS/PWS methylation studies came back positive for AS. However, given that the patient did not match the usually described AS phenotype, repeat DNA methylation studies were sent on a new blood specimen. This was also positive for AS diagnosis. There was no evidence of mosaicism on the repeat testing. He was also referred for an endocrinology evaluation given his overgrowth.

Discussion and Conclusion: More than 80% of individuals with AS have absent or very limited speech, seizures usually starting before age three years, and delayed or disproportionately slow growth in head circumference resulting in microcephaly by age two years. However, individuals with atypical AS with history of hyperphagia, obesity, verbal language ability, and growth parameters >95^{th} percentile similar to this individual have been described.[1] A recent 2019 study of 22 individuals with mosaic AS found similarities which included developmental delay, preserved expressive language skills, and an ability to manage activities of daily living.[2] Mosaicism in AS is believed to occur due to a postzygotic loss of imprinting in some of the cell lines, resulting in a somatic mosaicism.[3] While this individual fits the clinical characteristics of mosaic AS, there was no evidence for mosaicism in the blood sample. To our knowledge this is the first description of an individual with AS with preserved expressive language skills where mosaicism was not identified, although mosaicism in other tissues has not been tested. This case illustrates the atypical phenotypic spectrum of AS, the importance of broader literature review, and need for additional evaluation when confronted with atypical clinical features of a well described genetic syndrome.

References

1. Le Fevre A, Beygo J, Silveira C, et al. Atypical Angelman syndrome due to a mosaic imprinting defect: Case reports and review of the literature. Am J Med Genet Part A. 2017;173(3):753-757. doi:10.1002/ajmg.a.38072
2. Carson RP, Bird L, Childers AK, Wheeler F, Duis J. Preserved expressive language as a phenotypic determinant of Mosaic Angelman Syndrome. Mol Genet Genomic Med. 2019;7(9). doi:10.1002/mgg3.837
3. Horsthemke B, Buiting K. Imprinting defects on human chromosome 15. Cytogenet Genome Res. 2006;113(1-4):292-299. doi:10.1159/000090844

13. A GERMLINE *CTNNA1* VARIANT IN A LARGE CHILEAN FAMILY WITH HEREDITARY DIFFUSE GASTRIC CANCER

GRACIELA MOLINA [1,2], Ana Estrada[1], Alma Poceros[1], Carol Parra[2], Osvaldo Torres[3], Claudia Aguayo[3], Natalia Landeros[4], Andrés Rodriguez[4], Alejandro Corvalán[4,5], Luis Carvajal-Carmona[1].

[1]UC Davis Genome Center. University of California-Davis.[2]LINA. Facultad de Ciencias de la Salud. Universidad de Playa Ancha, Valparaíso, Chile.[3]Servicio de Cirugía Digestiva. Hospital Regional Guillermo Grant Benavente, Concepción, Chile. [4]Departamento de Hematología y Oncología. Facultad de Medicina. Pontificia Universidad Católica de Chile, Santiago Chile. [5]Advanced Center for Chronic Disease (ACCdiS), Santiago, Chile. e-mail: grmolina@ucdavis.edu

Gastric cancer is the leading cause of cancer-related death in Chile. Worldwide, only ten percent of gastric cancer have a familial aggregation and in Hereditary Diffuse Gastric Cancer (HDGC), E-Cadherine 1 gene (*CDH1*) is the most commonly mutated gene (30%). To our knowledge, only one HDGC Chilean family with a pathogenic *CDH1* mutation has been reported, suggesting a low frequency of *CDH1* mutations in this population. *CTNNA1* mutations (alpha-1 catenin gene), also have been found in HDGC cases. E-cadherine 1 and alpha-1 catenin are part of *adherens junction* complex. Here, we describe a large pedigree of an HDGC family with eleven members affected with gastric cancer and two with breast cancer, in two following generations. The index patient was diagnosed with a diffuse gastric tumor at 51 years of age and one of the affected relative (a deceased brother of the index case) at 38 years of age. The index patient´s gastric tumor histology type is diffuse, but one of the affected sister´s gastric tumor is papillary type. Thus, this family fulfills the HDGH clinical criteria. Cancer gene panel sequencing was performed in index patient DNA and cascade genotyping in family members was performed using Kompetitive allele-specific PCR. A germline variant c.293G>A (p.R98Q, rs746832629) in the *CTNNA1* gene was found in the index patient. This variant has been reported two times in ClinVar and is classified as an uncertain significance variant. In TCGA, *CTNNA1* c.293G>A variant has been reported four times in gastric, colon, and uterine tumor samples. This variant maps to the beta and gamma catenin binding domain of the protein, and the mutated residue is conserved from fish to primates. The variant population frequency in the Genome Aggregation Database is in Latinos is 0.0049 and worldwide is 0.000076. We searched the variant in other members of the family and carried out a co-segregation analysis. Based on our preliminary data, we think that this variant could be pathogenic and causative of the HDGC observed in this family. We will search for the second hit in an index patient´s tumor sample and perform a functional analysis of the variant during this semester. Beca Chile Postdoctorado 74190063, CONICYT-FONDAP 15130011, 1R01CA223978-01.

Alpha-1 catenin gene; Familial Diffuse Gastric Cancer.

14. EFFECTS OF CDH1 VARIANTS IN THE ANTISENSE LNCRNA AC099314.1 IN GASTRIC CANCER AND HEREDITARY PREDISPOSING CANCER SYNDROMES

GRACIELA MOLINA [1,2], Paul Lott[1], Ted Toal[1], Ana Estrada[1], Luis Carvajal-Carmona[1].

[1]UC Davis Genome Center. University of California-Davis.[2]LINA. Facultad de Ciencias de la Salud. Universidad de Playa Ancha, Valparaíso, Chile. e-mail: grmolina@ucdavis.edu

INTRODUCTION: Mutations in Cadherine 1 gene (CDH1) are related to Hereditary Diffuse Gastric Cancer, lobular breast cancer and other Hereditary Predisposing Cancer Syndromes. A long non-coding RNA (LncRNA) AC099314.1 is coded in the antisense strand opposite to CDH1 gene and partially overlapping exons 10 and 13. There is evidence that LncRNA antisenses to tumor suppressor genes (TSG) are co-expressed to its TSG, and being underexpressed in cancer.

AIM: To analyze the putative effects over the structure or function of LncRNA AC099314.1 by overlapping CDH1 gene variants.

METHODS: We sequenced tumor or blood of gastric cancer patients from Sacramento, Mexico and Colombia. In the data obtained, we search variants in the CDH-LncRNA AC099314.1 overlapping sequences. We also search for variants in TCGA and Ensemble database. The predicted effect on CDH1 protein was got from Ensembl Variant Effect Predictor. The variant effect over LncRNA AC099314.1 structure was performed using RNAstructure and the putative miRNA binding sites using miRmap. Sequence alignment was performed using BLAST,

RESULTS: We found that LNC-RNA AC099314.1 is complementary to a CDH1 intron two sequence. Thus, an alignment of the variants of the LncRNA to CDH1 intron two sequence was also performed. We found a new CDH1 frameshift gastric cancer somatic mutation that also could be a LncRNA AC099314.1 donor splice-site mutation. This variant could lead to retain intron two-three, changing the secondary structure of the LncRNA dramatically, and creating several new miRNA binding sites. Interestingly, this variant is located precisely at the 5´ terminal binding site of the LncRNA to CDH1 intron two and modifies the annealing sequence inside the CDH1 intron two. Also, we found two reported CDH1 germinal variants (rs786202508 and rs876658378) classified as uncertain or benign significance by ClinVar. These mutations are in the LncRNA AC099314.1 exon two acceptor site and could lead to exon two skipping. Exon two skipping also changes the LncRNA secondary structure and the complementarity to CDH1 intron two. These latest two variants was found in Hereditary Diffuse Gastric Cancer (HDGC) and other Hereditary Predisposing Cancer Syndromes (HPCS) patients.

DISCUSSION: We found that LncRNA AC099314.1 has a complementary sequence to CDH1 intron two. CDH1 intron two has some unknown regulatory elements that lead to express a novel CDH1 transcript involved in invasion and angiogenesis. Despite having pathogenic, uncertain or bening CDH1 effects, the new and described mutations could also have effects in structure or function in LncRNA AC099314.1. This LncRNA could have not only cis-effects, but trans-effects and be related to sporadic gastric cancer, HDGC, and other HPCS. Beca Chile Postdoctorado 74190063, 1R01CA223978-01.

Key words: CDH1, Long non coding RNA, mutations.

15. A ZEBRAFISH 'HUMANIZED' MODEL OF DUPLICATED GENE *SRGAP2* REVEALS NOVEL FUNCTIONS IN BRAIN AND EYE DEVELOPMENT

JOSÉ M. URIBE-SALAZAR[1,2], Brittany Radke[1], Alexandra Colón-Rodríguez[1], KaeChandra Weyenberg[1], Cole Ingamells[1], Gulhan Kaya[1], Eva Ferino[1], Pamela Lein[3], Li-En Jao[4], Megan Y. Dennis[1,2]

[1] Genome Center, MIND Institute, and Department of Biochemistry & Molecular Medicine, University of California, Davis, CA, USA
[2] Integrative Genetics and Genomics Graduate Group, University of California, Davis, CA, USA
[3] Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA, USA
[4] Department of Cell Biology and Human Anatomy, University of California, Davis, CA, USA

Gene duplication is a fundamental source of species innovation that contributes to novel phenotypic features. *SRGAP2* is a notable example of a gene uniquely duplicated in the *Homo* lineage, resulting in three partial paralogs present in modern humans that potentially contribute to neurological features exclusive to humans. Previous studies show that expression of human paralog *SRGAP2C*, a truncated form of the ancestral *SRGAP2A*, impacts cortical neuronal migration and synaptogenesis in mice, by antagonizing the function of the ancestral ortholog *Srgap2*, mirroring the traits observed in *Srgap2* knockdown mice. We sought to use zebrafish (*Danio rerio*) as a higher-throughput model to quickly test the function of *SRGAP2*. *srgap2* CRISPR-generated 'knockout' larvae exhibited a greater head-width to body-length ratio compared to wildtype siblings (3.3 - 4.8% average increase, $p=2.08\text{x}10^{-8}$), suggesting an increase in brain size. Interestingly, *SRGAP2C* mRNA-injected 'humanized' larvae followed the same morphological trend of larger heads (2% average increase, $p=0.045$). Consistent with previous findings that link *SRGAP2A* dysfunction with epilepsy (in humans) and synaptic connectivity (in mice), *SRGAP2C*-humanized or *srgap2*-knockout larvae also exhibited increased seizure activity when exposed to GABA antagonist pentylenetetrazol (12 - 17% average activity increase, $p<0.05$). To understand the nature of these defects on a molecular level, we performed RNA-seq experiments and found that a subset of genes involved in brain development were upregulated in both the *srgap2*-knockout and *SRGAP2C*-humanized larvae ($p<2\text{x}10^{-8}$). Unexpectedly, several gene ontology terms involved in eye development were also upregulated in both lines ($p<1\text{x}10^{-8}$), suggesting that *srgap2* may play a previously unknown role in eye development. Analysis of available human retina and lens RNA-seq data confirmed high expression of *SRGAP2* paralogs in these tissues. Ongoing experiments include a detailed characterization of the role of *srgap2* in eye development by performing morphological detail of the eye regions across mutants. In summary, these studies show that zebrafish represents a feasible model to characterize and discover potentially new functions of a well-studied human duplicated gene and provide a roadmap to understand if/how other genes may contribute to the evolution of novel neurological traits unique to humans.

16. GENOMIC VARIANT DETECTION WITHIN HUMAN SEGMENTAL DUPLICATIONS

DANIELA C. SOTO [1,2], Aarthi Sekar[1,2], Ruta Sahasrabudhe[3], Gulhan Kaya[1], Lutz Froenicke[3], Megan Y. Dennis[1,2†].

[1]Genome Center, MIND Institute, and Department of Biochemistry & Molecular Medicine, [2]Integrative Genetics and Genomics Graduate Group, [3]DNA Technologies Sequencing Core Facility, University of California, Davis, CA 95616

Short-read sequencing (SRS) technologies have been pivotal for genome-wide identification of single nucleotide variants (SNVs) implicated in disease. However, short read lengths (~100-300 bp) lack sequence context to accurately map to highly-similar segmental duplication (SD) regions. Lack of information at these loci could account for missing genetic risk for many well-studied human disorders. In recent years, long-read sequencing (LRS) technologies have overcome limitations of SRS allowing robust detection of structural variants, but high error rates have hindered their utilization in SNV calling. Here, we used whole-genome sequence data from two haploid cell lines (CHM1 and CHM13) and one human individual (HG002) to establish best practices in variant calling across human SD regions. Assessment of an existing benchmarking dataset of the haploid cell lines suggests overestimation of variants at duplicated loci and, as such, we established 52.7 Mbp and 34.8 Mbp high-confidence regions using sequences from bacterial artificial chromosomes of CHM1 and CHM13 respectively. Using this new benchmark and Genome In a Bottle's novel benchmarking dataset for challenging regions, we evaluated long-read (PacBio and ONT) and-range (10X Genomics linked reads) sequencing as tools for SNV detection in nearly identical (≥98%) duplicated regions of the human genome. We found that LRS reads can accurately map to regions previously inaccessible to SRS, including historically concealed genes like SMN1/SMN2; however, about ~5% of the duplicated space remains consistently inaccessible to any technology. Further, when applying a diverse set of variant-calling algorithms to our data, we determined that GATK and DeepVariant performed similarly across technologies, and PacBio HiFi data best detected variants within both unique and SD regions. To assess the utility of a candidate-gene approach coupled with LRS, we performed targeted sequencing of a subset of genes within near-identical duplications (1.7 Mbp), achieving over 50x coverage in regions of interest in CHM1, CHM13, and HG002. To improve variant calling performance further, we devised a novel bioinformatics pipeline to improve targeted long-read base accuracy of sequenced molecules by leveraging PCR duplicates. The results of these efforts will be integral in our use of LRS to uncover the complete variability landscape across the human genome in diverse populations, particularly for SD regions most recalcitrant to standard genetic approaches.

17. UTILIZING NEWBORN SCREENING TO FACILITATE IMMUNOLOGIC DIAGNOSIS OF 22Q11.2 DELETION SYNDROME

ERICA CHERK and VR Dimitriades, Department of Pediatrics, Division of Pediatric Allergy, Immunology and Rheumatology, University of California Davis Health, Sacramento, CA

Rationale: Historically, 22q11.2 deletion syndrome has been underdiagnosed. However, since initiation of Severe Combined Immune Deficiency newborn screening (SCID-NBS) in California in 2010, immunologists have become involved in earlier identification of cases of 22q11.2 deletion syndrome.

Case Description: A clinically well-appearing 10 month-old, ex-28 WGA male diagnosed with 22q11.2 deletion syndrome after a positive SCID NBS prompted further immunological workup. He did not have any of the cardiac (congenital heart disease), facial (cleft palate, hypertelorism, posteriorly rotated ears), or endocrinologic abnormalities (hypoparathyroidism) commonly associated with 22q11.2 deletion syndrome.

Methods: SCID NBS (measuring numbers of T cell receptor excision circles [TRECs]), CBC, flow cytometry, tracking lymphocyte subpopulations (from birth until diagnosis), Invitae Primary Immunodeficiency panel genetic testing

Results: On day 16 of life, the patient was found to have positive SCID newborn screening with low TREC numbers. Subsequent immunologic testing showed mild hypogammaglobulinemia (which resolved over time) and T cell lymphopenia (which persisted over time). Invitae testing identified a pathogenic variant in *TBX1,* a gene associated with 22q11.2 deletion syndrome.

Conclusions: Without SCID newborn screening and immunologic follow-up, this case of 22q11.2 deletion syndrome without typical clinical features may not have been detected. Ultimately, earlier detection of 22q11.2 deletion syndrome will greatly improve health and quality of life for affected children, allowing them to receive appropriate preventative care (e.g. associated disease tracking, developmental assessments, infection prevention) prior to developing complications related to their genetic disorder.

18. DETERMINING THE ROLE OF CTCF LOOPS IN NEURON SPECIFIC PATERNAL IMPRINTING OF *UBE3A*

ORANGEL GUTIÉRREZ FUGÓN [1], Dag Yasui PhD[2], Janine LaSalle PhD[2], David Segal PhD[2]
UC Davis Genetics and Genomics Graduate Group[1], UC Davis Genome Center[2]

Angelman syndrome (AS) is a severe neurogenetic disorder affecting about 1 in 15,000 births that has a phenotypic and genetic overlap with autism spectrum disorder. It is characterized by seizures, poor speech, ataxia, and a happy demeanor. AS is caused by a functional loss of the *UBE3A* gene caused by the maternal deletion of *UBE3A,* an imprinted gene encoding a ubiquitin ligase that regulates synaptic development. *UBE3A* is biallelically expressed in non-neuronal cells but undergoes imprinting in neurons, resulting in exclusive maternal expression. Previous studies have shown that an antisense transcript is responsible for silencing the paternal allele of *UBE3A* in neurons, but it is currently unknown how this neuron-specific transcription is regulated. The transcript originates at an upstream *SNRPN* gene, extends past a noncoding gene *IPW,* and continues to transcribe antisense to *UBE3A* (*UBE3A-ATS*), however in non-neurons it terminates at *IPW*. The presence of binding sites for the loop forming protein CTCF within this region suggest that it may play a pivotal role in performing a boundary function. CTCF has been shown to regulate expression of other parentally imprinted genes (*IGF2/H19*). Several studies published by our lab using the murine model have been successful at unsilencing paternal *Ube3a* using epigenetic editing tools targeted to the *Snrpn* promoter. Epigenetic editing with catalytically inactive Cas9 (dCas) offers great potential as a therapeutic platform because it does not alter the genome permanently. However, our lab's previous strategy also affects critical paternally expressed genes upstream of *IPW*. The design of an improved strategy for *UBE3A* unsilencing to treat AS is partly limited by the lack of understanding of how tissue-specific imprinting is controlled at this locus.

To address this gap in knowledge we have developed an innovative system to study this critical boundary region using epigenetic editing tools and a human neuronal precursor model (LUHMES). Prior studies have shown that CTCF binding at *IPW* is reduced upon neuronal differentiation and that methylation inhibits CTCF's DNA binding sites and subsequent looping activity. Therefore, I hypothesize that CTCF creates a chromatin loop in non-neurons that prevents the extension of *UBE3A-ATS* past *IPW*. Furthermore, CTCF is evicted by DNA hypermethylation in neurons, allowing extension of the antisense transcript past *IPW*. Determining the role of chromatin loops in tissue-specific imprinting will allow for improved paternal *UBE3A* unsilencing strategies. Utilizing this proposed human cell culture model may improve the translation of epigenetic unsilencing strategies for novel AS therapies.

In order to differentiate expression on the maternal allele from expression on the paternal in LUHMES cells, allele-specific SNPs were identified using 10x Genomics linked-read sequencing. To assess the differentially methylated state at *IPW*, pyrosequencing was performed and showed that methylation was approximately 60% in brain tissue while in HEK293T cells it was about 6%. To determine if CTCF is frequently bound at *IPW* in non-neurons, ChIP-PCR was carried out in HEK293T cells demonstrating that DNA from *IPW* bound to CTCF is enriched in comparison to an IgG control. Data harvested from previous ChIP-seq studies also shows that CTCF is more frequently bound at *IPW* in undifferentiated LUHMES than in differentiated LUHMES neurons. qRT-PCR was performed to demonstrate that *UBE3A-ATS* is only expressed in differentiated LUHMES neurons and brain tissue but not in undifferentiated LUHMES and HEK293T cells. A timeline of LUHMES cell *UBE3A-ATS* expression throughout eight days in differentiation media was also completed. Taken together these preliminary findings support the hypothesis that CTCF binding at *IPW* is decreased in neuronal cells in association with increased methylation at its binding site and that LUHMES cells serve as an appropriate model for studying *UBE3A-ATS* expression.

In future planned experiments, to determine if a CTCF chromatin loop is sufficient to inhibit transcription of *UBE3A-ATS* past *IPW* in neurons, we will target a dCas9-CTCF chimeric protein to the *IPW* binding site. To determine if CTCF binding at *IPW* is necessary for the termination of *UBE3A-ATS* in non-neurons we will express a dCas9/DNA methyltransferase DNMT3A fusion protein in undifferentiated LUHMES cells. This research approach may provide insight into how chromatin looping regulates paternal *UBE3A* silencing in neurons and could lead to the development of epigenetic therapeutics for AS.

19. LONG-TERM OUTCOMES OF MEDIUM-CHAIN ACYL-COA DEHYDROGENASE DEFICIENCY (MCADD)

FARIA AHMED, MS, LCGC; Erica Wright, MS, CGC; Peter Baker, MD

Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most common disorder of fatty acid oxidation, and the estimated prevalence is approximately 1 in 15,000 individuals in the United States. This inborn error of metabolism is characterized by metabolic decompensation triggered by periods of prolonged fasting and/or illness. Since its addition to newborn screening (added to Colorado newborn screening in 2006), the clinical variability of MCADD has become more apparent. However, long term data tracking outcomes of this condition remain limited. In this study, we conducted a retrospective chart review of all patients with MCADD followed by the Inherited Metabolic Diseases (IMD) clinic at Children's Hospital Colorado. Of the 90 patients followed for MCADD at CHCO, 64 were born in Colorado, Montana, or Wyoming, the three states that are serviced by the IMD clinic. The measures investigated included: newborn screening octanoylcarnitine (C8) levels, genotype data when available, growth parameters, co-morbidities, patient adherence to following up with the IMD clinic as recommended, and the number of emergency department visits and emergency department to hospital admissions related to MCADD. Individuals homozygous for the common pathogenic variant c.985A>G (pLys329Glu), which is known to confer a more severe phenotype of MCADD, were found to have the most elevated newborn screening C8 levels, as well as the highest number of emergency department visits and hospital admissions.

Additionally, previous research has indicated that parents and caregivers of children with MCADD diagnoses experience elevated levels of stress and anxiety surrounding their child's care and prognosis. In an effort to explore the psychosocial impact a diagnosis of MCADD has on the individual's parents, caregivers, and family as a whole within the CHCO MCADD patient population, an anonymous survey was administered to the parents and caregivers of children with MCADD. The results of the survey identified that parents and caregivers often felt isolated due to their child's MCADD diagnosis, especially during their child's first year of life, which was also characterized by frequent worry and anxiety about ensuring that the child had frequent enough feeds, and was receiving appropriate care when not under the direct supervision of their primary caregivers. Parents and caregivers continue to worry about their child's ability to manage their healthcare independently as they grow older and advocating for themselves appropriately in social situations. The information gleaned from this retrospective chart review and prospective survey indicates that the MCADD patient population cared for at CHCO are being successfully managed medically the majority of the time, and identifies areas in which IMD providers can further support MCADD patient families so as to alleviate avertible stress and anxiety related to the MCADD diagnosis.

20. LAUGHTER-LIKE VOCALIZATIONS IN THE FULL *UBE3A* DELETION RAT MODEL OF ANGELMAN SYNDROME

ELIZABETH L. BERG*, Shekib A. Jami, Stela P. Petkova, Jacob Ellegood, Timothy A. Fenton, John A. Gray, Markus Wöhr, and Jill L. Silverman

*Presenting author

Angelman Syndrome (AS) is a rare neurogenetic disorder caused by the loss of ubiquitin protein ligase E3A (*UBE3A*) expression in the brain, typically due to a deletion of the maternal 15q11-q13 region. Symptoms of the disorder include developmental delay, impaired receptive and expressive communication skills, ataxia, motor and balance deficits, poor attention, intellectual disabilities, microcephaly, seizures, and a happy demeanor characterized by frequent smiling and laughing. While many of these symptom domains have been recapitulated and studied in a single *Ube3a* exon deletion mouse model of AS, a lack of consistent reports has led to the theory that perhaps not all mouse studies were assessing the effects of an absence of all functional UBE3A. Therefore, the recent generation of a full *Ube3a* deletion rat model of AS has opened up new opportunities to (1) study the effects of loss of all isoforms of UBE3A, (2) investigate the more complex AS-relevant behaviors, such as social communication, that have been difficult to study with high signal sensitivity in mice, and (3) do so using an outbred species with greater genetic diversity and therefore improved translational validity. Leveraging the rich behavioral repertoire and sophisticated communication system of rats, we discovered an overexpression of laughter-like vocalizations and aberrant social reciprocal interactions in the rat model of AS. Using high-resolution magnetic resonance imaging and electrophysiological techniques, we also characterized volumetric abnormalities in the brain and identified reduced long-term potentiation as a putative cellular mechanism underlying the learning and memory deficits apparent in the model. Therefore, in addition to revealing unique and robust phenotypes valuable as preclinical outcome measures in the evaluation of therapies for AS, our findings simultaneously help to elucidate the neurological etiology of behavioral abnormalities resulting from loss of *Ube3a*.

21. EXPLORING THE CAUSAL RELEVANCE BETWEEN EARLY-LIFE ADVERSITY, EPIGENETICS AND STRESS VULNERABILITY WITH EDITING TECHNOLOGIES

CHELSEY J. LEE (Graduate Student, Segal Lab, UC Davis Integrative Genetics and Genomics)

Description: Early life adversity (ELA) is highly and universally prevalent, accounting for increased mortality as well as increased rates of mental and physical morbidity across the developmental trajectory. In excess, ELA imparts hyper- or hypo-sensitivity to stress, indelibly shaping an individual's propensity to cope with emotional and cognitive challenges later in life. The vital question regarding the pathophysiological bases of ELA – *how* it "gets under the skin" - remains poorly understood. Previous findings correlated gene expression and epigenomic changes within the brain's hypothalamic-pituitary-adrenal (HPA) axis structures to the stress vulnerability phenotype. Windows of increased HPA axis plasticity may correspond to windows of greater vulnerability – but perhaps such windows offer opportunities for intervention as well.
Epigenetic editing technology offers another opportunity to further investigate *how* and *which* of these alterations in the epigenome are causally or functionally relevant to the disease phenotypes observed.

Using a translational mouse model of chronic early-life adversity, this research proposal will investigate the hypothesis that changes in epigenetic information are an important physical component of how ELA manifests consequences later in life. The Baram Lab's limited bedding and nesting (LBN) paradigm of fractured caregiving will be utilized to first establish a robust stress vulnerability phenotype to which distinct epigenetic "signatures" can be assigned. A consistent phenotypic hallmark readily observed in mice was persistent impairment of hippocampus-dependent learning and memory function, particularly in males. This proposal will study glucocorticoid receptor (GR) regulation in the dorsal hippocampus – whether specific gene expression changes induce specific structural and functional changes has not yet been ascertained. Using DNA methylation analysis and RNA-seq of GR in male LBN mouse hippocampi, molecular profiles in the hippocampus will inform targets prioritized for editing.
Epigenetic effectors of the neuronal plasticity agent Neuron Restrictive Silencer Factor (NRSF) will also be characterized. Transient NRSF-dependent mechanisms have been observed in reprogramming of stress-sensitive hypothalamic neurons involved in care-induced cognitive and behavioral outcomes. Along with GR, NRSF may serve as a complementary and interrelated driver of the stress vulnerability phenotype that can unveil useful downstream targets. We will probe further into what events *specifically* (and perhaps *quantifiably*) produce change in phenotypes by delivering CRISPR-based epigenetic editing tools to dorsal hippocampal neurons, exploring circumstances under which implicated genes may be persistently activated or silenced.

Demonstrating through proof-of-concept the capacity of targeted editing technologies to establish causal relevance between ELA-related epigenetic events in the HPA axis and disease phenotypes related to stress vulnerability offers a potential solution to the long-standing absence of treatments precisely targeting HPA axis components. We aim to close this current gap in the field, elucidating potential diagnostic and therapeutic value to inform future interventive efforts that mitigate – and perhaps even reverse – the effects of early life insults.

22. TEMPORAL GENE EXPRESSION DYNAMICS AFTER ISCHEMIC STROKE: HOW GENE EXPRESSION TRAJECTORIES CAN GUIDE DISCOVERY AND TREATMENT STRATEGY

PAULINA CARMONA-MORA, Glen C Jickling, Xinhua Zhan, Marisa Hakoupian, Heather Hull, Noor Alomar, Hajar Amini, Bodie Knepp, Frank R Sharp, Boryana S Stamova, Bradley P Ander

Almost 800,000 stroke cases are recorded each year in the United States representing a leading cause of long-term disability. Prompt detection and treatment is critical for improved outcomes. Ischemic stroke (IS) is the most common type of stroke and its etiologies are broad but include cardioembolic and large or small vessel causes. Peripheral leukocytes infiltrate the damaged region of brain and modulate response to injury. We previously showed peripheral blood cells display distinct gene expression profiles after IS which reflect the immune responses. However, temporal dynamics of gene expression after IS are unknown and would help improve our understanding of shifting molecular and cellular pathways involved in acute brain injury.

RNA-sequencing was performed to identify differentially expressed genes from isolated monocytes, neutrophils, and whole blood. We analyzed the transcriptomic profiles of 104 IS samples and included control participants with vascular risk factors (diabetes and/or hypertension and/or hypercholesterolemia – 42 samples). Individuals were split into time points (TPs) from stroke onset (TP1= 0-24 h; TP2= 24-48 h; and TP3= >48 h), and controls were assigned TP0. A linear regression model including time and the interaction of diagnosis × TP with cutoff of $p<0.02$ and fold-change $>|1.2|$ was used. Time dependent changes were analyzed using artificial neural networks to identify clusters of genes that behave in a similar way across TPs.

Unique gene trajectories were revealed in the three sample types. These include genes not expressed in TP0 that peak only within the first 24 h, others that peak or decrease in TP2 and TP3, and more complex patterns. Genes that peak at TP1 in monocytes and neutrophils are related to cell adhesion and leukocyte differentiation/migration, respectively. Early peaks in whole blood occur in genes related to transcriptional regulation. In monocytes, interleukin pathways are enriched across all TPs, whereas there is a trend of suppression after 24 h in neutrophils. The inflammasome pathway is enriched in the earlier TPs in neutrophils, while not enriched in monocytes until over 48 hours. Different gene profiles in whole blood allow to dissect the contributions of other cell types to the acute immune response in acute IS.
Our analyses on gene expression dynamics and cluster patterns allow identification of key genes and pathways at different time points following ischemic injury. These will be valuable to validate as panels of IS biomarkers and point to possible treatment targets that are important in the acute time window.

23. ADAPTING CRISPR-BASED EPIGENETIC EDITING FOR TARGETED ESCAPE FROM X CHROMOSOME INACTIVATION (XCI) TO A MOUSE MODEL OF CDKL5 DEFICIENCY DISORDER (CDD)

BUCHANAN, F. K. B. , Halmai, J. A. N. M., Gonzalez, C., Cameron, D., Waldo, J. J.

CDKL5 Deficiency Disorder (CDD) is a rare neurodevelopmental disorder that causes global developmental delay and severe impairment of gross motor skills. The condition is characterized by seizures beginning at a median age of 6 weeks, with 69.3% of patients experiencing seizures daily. Despite a necessity for medical intervention, tested pharmacotherapies have failed to demonstrate comprehensive and lasting amelioration of symptoms and are complicated by deleterious side effects. At present, there is no medical treatment that addresses the underlying cause of the disease, de novo loss-of-function mutations in the X-linked CDKL5 gene. Essential for normal brain development, the CDKL5 protein is a serine/threonine kinase that is involved in processes including neuronal growth and migration. Due to the disease severity and etiology, the majority of patients are females who are heterozygous for pathogenic CDKL5 mutations. The process of random X chromosome inactivation (XCI) means that while these patients demonstrate loss of CDKL5 expression in about half of their neurons, the healthy allele is present in all neurons. These healthy alleles represent an opportunity to restore endogenous CDKL5 alleles if they are remodeled to mimic an active state.
A 2020 publication from our lab demonstrates artificial escape from XCI by editing DNA methylation on the promoter of CDKL5 in silenced alleles in human neuronal-like cells. We were able to show 67% reactivation of the healthy CDKL5 allele using the epigenetic editor CRISPR-dCas9-Tet1-VP64. To advance this approach as a putative therapeutic, it needs to be evaluated in a relevant disease model. To accomplish this, we have screened 10 novel gRNAs with VP64 and Tet1 in two mouse cell lines to demonstrate efficacy in modulating gene expression and remodeling methylation at the Cdkl5 promoter. Additionally, we have demonstrated transduction of primary cortical neurons derived from CDD model mice with in-house AAV9 vectors, which also demonstrate strong transduction in live mice. This platform will be evaluated in vivo using the identified lead gRNA and has the potential to inform translational epigenetic interventions for CDD.

24. DUAL DIAGNOSIS IN AN INDIVIDUAL WITH INTELLECTUAL DISABILITY AND HEARING LOSS

ELIJAH MEYERS; Alena Egense, MGC; Suma Shankar, MD, PhD

1) Division of Genomic Medicine, Department of Pediatrics, UC Davis, Sacramento, CA

We report a 17 year old male that was referred to our clinic by his neurologist to identify a genetic etiology for his multisystemic abnormalities including intellectual disability. The patient was the product of an uncomplicated pregnancy, however he failed the newborn hearing screen due to congenital sensorineural hearing loss. He was also noted to have right cryptorchidism and within the first year of life began having seizures. He has significant developmental delay, is nonverbal, and exhibits autistic behaviors. On exam the patient was noted to have hypotonia, nystagmus, microphthalmia, degenerative myopia, bilateral colobomas, bilateral ptosis, downslanting palpebral fissures, low placed ears, smooth philtrum, and wide bulbous tip of nose. Family history is significant for hearing loss present in one sibling. Ancestry is mixed European and consanguinity was denied. Patient was evaluated by genetics after birth and underwent genetic testing for 22q11.2 deletion via FISH study, Smith Lemli Opitz Syndrome, karyotype, and a chromosomal microarray, all of which returned negative. Subsequent testing identified two pathogenic variants identified in GJB2 gene (Connexin 26) testing which explains the patient's sensorineural hearing loss but does not explain the patient's complete medical history. Given a broad list of differential diagnoses, whole genome sequencing was recommended. Results revealed a heterozygous pathogenic missense variant in PACS1 :c.607C>T (p.Arg203Trp). Pathogenic variants in PACS1 are associated with an ultra rare autosomal dominant syndrome called Schuurs-Hoeijmakers syndrome characterized by intellectual disability, craniofacial abnormalities, and other congenital abnormalities. There are less than 70 cases reported worldwide but all individuals exhibit intellectual disability, speech and language problems, and distinct facial features that include ptosis, and downslanting palpebral fissures. Therefore, the genetic test result, along with the previously identified pathogenic GJB2 variants, are consistent with the patient's presentation, and confirm a diagnosis of Schuurs-Hoeijmakers syndrome. Given that hearing loss and intellectual disability are heterogeneous conditions that can be isolated or syndromic, the patient may have benefited from receiving broader genetic testing earlier in life. However, when using a tiered testing approach, this case exemplifies the importance of receiving additional genetic testing to rule out a dual diagnosis if previously identified pathogenic variants do not explain a patient's phenotype.

25. "ATYPICAL" FINDING ON NON-INVASIVE PRENATAL SCREENING RESULT LEADS TO PREVIOUSLY UNDIAGNOSED UNIPARENTAL DISOMY

ELYSE LOVE [1], Laila Rhee[1], Kristin Herman[2]

[1] University of California Davis, Department of OB/GYN, Division of Maternal-Fetal Medicine
[2] University of California Davis, Department of Pediatrics, Division of Genomic Medicine

Background: Uniparental disomy (UPD), occurs when both copies of a chromosome, or of part of a chromosome, are derived from a single parent. The mechanism of UPD include monosomy and trisomy rescue. UPD can cause clinical consequences by disrupting genomic imprinting or by unmasking autosomal recessive conditions. Past estimates of UPD prevalence include rates of 1 in 3,500 and 1 in 5,000; however, were based on extrapolation from UPD events causing clinical presentation and not reflective of the incidence in the general population. A recent research study from 23andMe, Inc. estimated that the incidence of UPD is more common than was previously estimated and occurred in approximately 1 in 2,000 births. Most cases of UPD in this study did not demonstrate any adverse clinical consequences. Interestingly, of their 199 case subjects of UPD, chromosome 18 was the only chromosome for which no cases were documented.

Case: A 36-year-old woman was referred to our Prenatal Diagnosis clinic for a chorionic villus procedure (CVS) at 14 weeks due to an "atypical finding" on non-invasive prenatal screening results. The report noted that there was a suspected finding outside the scope of the test, which may include, but is not limited to, fetal mosaicism, fetal chromosome abnormality, maternal chromosome abnormality, or normal variation. The lab report indicated that genetic counseling with the option of comprehensive ultrasound evaluation and *diagnostic testing* should be considered. A genetic counselor at the performing laboratory was contacted, who elaborated that the atypical finding was suspected to be of maternal origin and involved chromosome 18.

After extensive discussion and careful consideration, our patient ultimately declined any invasive fetal testing, but elected to undergo peripheral blood chromosome analysis and cytogenomic SNP microarray for herself. The karyotype was resulted out as normal female, 46,XX; however, the microarray analysis demonstrated homozygosity (absence of heterozygosity), across the entire length of chromosome 18. This result is consistent with UPD of chromosome 18. She subsequently had a video visit with a medical geneticist due to concern about clinical features concerning for Marfan syndrome and for further discussion about these results. Due to technical limitation of the video visit, physical examination was not able to be completed and overall assessment was that UPD of chromosome 18 in this individual was likely of no relevant clinical significance.

Discussion: This case illustrates many relevant points, including the classification of "healthy" individuals with previously undiagnosed UPD will continue to increase with expansion of direct-to-consumer studies and increasing utilization of non-invasive prenatal screening technologies. Patients electing to undergo non-invasive prenatal screening should be informed of the possibility of uncovering an underlying maternal condition and appropriately counseled regarding the follow-up recommendations. Testing laboratories should increase transparency about the origin of their "atypical" findings to allow for more informed decision-making to prevent unnecessary invasive diagnostic procedures for maternal findings. Interestingly, our case was found to have UPD of chromosome 18, which was the only chromosome not previously reported in 199 cases within the 23andMe Inc. database. This seems counterintuitive, as chromosome 18 is one of the few chromosomes that can survive in a live born conception and seems more likely to be affected due to a trisomy rescue event.

26. RAPID CELL COUNTER: AUTOMATED AND HIGH-THROUGHPUT ESTIMATION OF CELL DENSITY WITHIN DIVERSE CORTICAL LAYERS

AARTHI SEKAR [1-3], Thiago Sanches[1,4], Keiko Hino[5], Matangi Kumar[3], Juliann Wang[3], Elisa Ha[3], Blythe Durbin-Johnson[3], Sergi Simó[5], Megan Y. Dennis[1-3]

[1]Integrated Genetics and Genomics Graduate Group, [2]Department of Biochemistry & Molecular Medicine, MIND Institute, [3]Genome Center, [4]Department of Animal Sciences, [5]Department of Cell Biology & Human Anatomy, University of California, Davis, CA, USA

Researchers have long used transient transgenic mouse models to experimentally test the role and function of human genes over the course of neurodevelopment. Tracking and quantifying the abundance and location of cells in the developing brain in transgenic mice has enabled researchers to develop a greater understanding of mechanisms underlying nervous system morphogenesis. Widely used experimental methods to quantify cells labeled with fluorescent markers—such as immunohistochemistry, *in situ* hybridization, and expression of transgenes via stable lines or transient *in utero* electroporations—rely upon accurate and consistent quantification of images. Current methods to quantify fluorescently-labeled cells rely on labor-intensive manual counting or semi-automated approaches, such as the Fiji plugin *Cell Counter*, which requires custom macros to enable higher-throughput analyses. In response to the need for a faster method to analyze images, we have created RapID Cell Counter, an automated cell-counting tool with an easy-to-implement graphical user interface, that facilitates quick and consistent quantifications of cell density within user-defined boundaries that can be divided into equally-partitioned segments. Compared to the standard manual counting approach, we show that RapID matches accuracy and consistency, and only requires ~10% of user time, when quantifying the distribution of fluorescently-labeled neurons in *in utero* electroporation experiments. Using RapID, we recapitulated previously published work focusing on two genes, *Srgap2* and *Cul5*, important for projection neuron migration in the neocortex. Additionally, we have used RapID to quantify projection neuron displacement in a novel knockout model of *Rbx2*. We propose RapID as an efficient method for neuroscience researchers to process fluorescently-labeled brain images in a consistent, accurate, and high-throughput manner.

27. EXPANDING THE PHENOTYPIC SPECTRUM OF KBG SYNDROME: 3 PATIENTS WITH SEVERE LANGUAGE DELAYS

STACEY COLE, Alena Egense, Collette DeFilippo, Suma Shankar

Introduction: KBG syndrome is a rare syndrome most significant for key facial features, macrodontia, intellectual disability and short stature, with significant variability among individuals. KBG, which is named for the families initially identified with this syndrome, is caused by mutations in the ANKRD11 gene.

We report 3 individuals with mutations in ANKRD11 who presented with varying spectrums of KBG syndrome, all whom have severe language delays.

Methods: All patient met criteria for the Precisions genomics in nonverbal/minimally verbal and neurodevelopmental delayed children. All patients had whole genome sequencing performed through this study.

Results: Three difference gene mutations in ANKRD11 were identified among the study patients, one was a 2 base pair deletion, one was a variant of uncertain significance (VUS) and the last was a stop mutation.

Our 1st patient was found to have a heterozygous pathogenic variant in *ANKRD11* at c.2288_2289del. Her features included short stature, delayed bone age, bilateral mixed sensorineural hearing loss, synophrys, broad eyebrows, full cheeks, intermittent exotropia, prominent incisors and bilateral brachydactyly of the 5th fingers.

Our 2nd patient's genetic variation was a VUS *ANKRD11* C.4798C>G, p.Arg1600Gly. His features included autism and developmental delay, large upper incisors (10mmx10mm), oral abnormalities, feeding issues in early childhood, delayed gastric emptying, exotropia with nystagmus, mild synophrys, and abnormal hair whorls. While his genetic change was classified as a VUS he was noted to have several characteristic features of the syndrome.

Our final patient was identified as having a nonsense mutation ANKRD11 c.7825C>T (p.Gln2609Ter). Her features included speech apraxia, developmental delay, severe ADHD, history of short stature, and broad eyebrows.

Conclusion: KBG is inherited in an autosomal dominant manner, yet over half of the pathologic variants are de novo mutations. Key features included macrodontia, specific facial features and postnatal height < 10th percentile. All of our patients were non-verbal/severely speech delayed. While speech delay is mention as a minor finding in some presentations of this disorder, severe speech delay was one of the distinguishing features for each of our patients. As we perform more whole genome sequencing many of the traditional phenotypes for many syndromes may begin to look different. In the case of KBG in our small group of patients, we identified severe speech delay as a major finding.

28. GENE EXPRESSION DECONVOLUTION IN SINGLE-CELL 5' RNA-SEQ REVEALS CELL-TO-CELL HETEROGENEITY IN RETT SYNDROME MOUSE BRAIN

OSMAN SHARIFI, Dag Yasui and Janine LaSalle

Mutations in MECP2 gene encoding Methyl CpG Binding Protein 2 (MeCP2) has been found to be the cause of 80-90% of Rett Syndrome (RTT) cases. MECP2 is an X-linked gene containing two isoforms (MeCP2-e1 and MeCP2-e2). MeCP2 protein isoforms bind to methyl groups in DNA and act as transcriptional regulators for normal neurological function. RTT primarily affects females who are heterozygous and mosaic for expression of *MECP2* due X-chromosome inactivation. The mosaic nature of *MECP2* expression creates a mixture of mutant and wild-type (WT) MeCP2 brain cells that lead to cell non-autonomous “bad neighborhood” effects. Previously, these mosaic effects have been investigated in mice using microscopy image analysis; however, these effects have not been studied throughout development. While it is clear that MeCP2e1 function is critical for normal neurological function, the molecular phenotypes in distinct brain cell types have not been investigated over the time course of disease progression. The goal of this study is to investigate the gene expression of the cortex and hypothalamus; two regions of the brain responsible for cognitive/executive functions and metabolism, respectfully, that are dysfunctional in RTT temporally at the single cell level. The gene expression deconvolution method established an approach to identify the brain cell types in the RTT mice validated by the Allen Brain Atlas database.

29. THE POTENTIAL OF RNA-DIRECTED THERAPY FOR ANGELMAN SYNDROME

ULRIKA BEITNERE[1], Oran Gutierrez[1], Miranda Palomares[1], Henriette O'Geen[1], David J. Segal[1]

[1]Genome Center, MIND Institute and Department of Biochemistry and Molecular Medicine,
UC Davis, Davis, CA, USA
Contact: Ulrika Beitnere ubeitnere@ucdavis.edu

Since the discovery of nucleic acids, RNA has been considered an extremely delicate molecule, prone to degradation, and therefore dismissed in therapeutic applications. Today, however, major developments, including CRISPR, have made RNA a focus of medical research with incredibly exciting applications.

More recently, a new class of CRISPR family nucleases: Cas13, targeting RNA directly was characterized (Cox et al, 2017, Konermann et al, 2018), demonstrating the potential of using it in the development of RNA targeting therapeutics.
A neurogenetic disorder that could benefit from a therapeutic aimed at the RNA level is Angelman Syndrome (AS). It arises from the genetic loss of the maternal *UBE3A* gene in the brain neurons, causing severe mental and physical impairments.

Due to a brain-specific long non-coding RNA transcript, known as the UBE3A-antisense (*UBE3A-ATS*), paternal *UBE3A* remains silenced. Reactivation of the paternal allele could therefore restore UBE3A expression in the brain.
Preliminary data will be presented on the safety and distribution of the Cas13 nuclease in vivo to the brain in a mouse model for Ube3a screening. The results are compared with an antisense oligonucleotide (ASO) that is targeting the same *UBE3A-ATS*. For the delivery of Cas13 to the brain AAV-PHP.eB is used and injected either intravenously or intracranially. The most promising approach will be tested for the duration of *Ube3a* activation and behavioral rescue in a mouse model of AS.

30. 3D GENOME ORGANIZATION IN THE DEVELOPING RHESUS MONKEY BRAIN

AEDRIC LIM [1,2], Colin Shew[1,2], Michele Martinez[3], Alice Tarantal[3], Megan Y. Dennis[1,2]

[1] Genome Center, MIND Institute, and Department of Biochemistry & Molecular Medicine, University of California, Davis, CA, USA
[2] Integrative Genetics and Genomics Graduate Group, University of California, Davis, CA, USA
[3] Departments of Pediatrics and Cell Biology and Human Anatomy, School of Medicine, and California National Primate Research Center, University of California, Davis, CA, USA

Most genetic variation underlying differences in behavioral traits and neurodevelopmental disorders remains unknown despite considerable effort. Much work has focused on protein- coding regions, which comprise ~1.5% of primate genomes, compared to functional noncoding elements comprising ~40%. Transcriptional analysis of the developing brain in distantly related primates, such as humans and rhesus monkeys, show that spatiotemporal expression patterns are largely conserved across lineages, with minor differences likely contributing to species divergences. To delve into genomic mechanisms underlying gene regulation in the developing primate brain, we have generated bulk (n=3 fetuses) and single-cell (n=1 fetus) RNA-seq from diverse regions of fetal rhesus monkey brains (60 days gestation, late first/early second trimester) representing a time of early neurogenesis.. The single-cell RNA-seq data has revealed a number of different cell types, the majority of which are neuronal. These data will facilitate deconvolution of downstream results to specific cell types. We have also begun to query the 3D genome organization within these samples using PLAC-seq to identify DNA interactions within chromatin enriched at active promoters in rhesus neural tissue (150 days gestation). Our preliminary data with a single sample of the parietal lobe in the cerebral cortex shows significant enrichment of putative *cis*-acting regulatory elements with genes previously found to be important in brain development. Using this approach on a greater number of samples, regions, and developmental timepoints will allow us to hone in on noncoding drivers of gene expression important in the development of the brain. Ultimately, our goal is to connect variants identified in children with autism spectrum disorder discovered from patient whole-genome sequencing data with identified *cis*-acting regulatory elements to hone in on previously undiscovered causes of neurodevelopmental disorders.

31. EVALUATION OF EFFECTOR DOMAINS FUSED TO DCAS9 FOR ALLELE-SPECIFIC SILENCING IN HUNTINGTON'S DISEASE PATIENT-DERIVED CELLS

JENNIFER WALDO [1,2], Peter Deng[1,2,3], Julian Halmai[1,2], Jasmine Carter[1,2], Casiana Gonzales[1,2], Fiona Buchanan[1,2], David Cameron[1,2], Jan Nolta[1], David Segal[3] and Kyle Fink[1,2].

[1] Stem Cell Program and Institute for Regenerative Cures, University of California Davis Health Systems, Sacramento, CA, 95817, USA
[2] Department of Neurology, University of California Davis Health Systems, Sacramento, CA, 95817, USA
[3] Genome Center, MIND Institute, and Biochemistry and Molecular Medicine, University of California, Davis, CA, 95616, USA

Huntington's disease (HD) is a rare, autosomal dominant neurodegenerative disorder caused by a trinucleotide expansion in exon 1 of the Huntingtin gene, which leads to neuronal dysfunction and cell toxicity. Targeted allele-specific reduction is important, as it is not known what the consequences of knockdown of both mutant and healthy Huntingtin protein could be long term. This study aimed to target single nucleotide polymorphisms (SNPs) found in the patient population to reduce expression of mutant HTT using repressive domains fused to dxCas9 in HD patient-derived cells. Heterozygous SNPs near regulatory regions of the Huntingtin promoter were confirmed in HD patient cells, allowing for the design of allele-specific gRNAs. A novel vector (dxiCas9) was cloned containing repressive effector domains, which allowed for broader PAM site coverage and higher binding specificity compared to spCas9. dxiCas9 and gRNA plasmids were introduced to a HD patient fibroblast line and qRT-PCRs were performed to assess knockdown of total HTT transcript levels to screen for gRNA selection. Knockdown was assessed at 4 different loci in the HTT gene and significant downregulation was achieved using several of our gRNAs that were designed to be allele specific. Targeting of SNP 3 resulted in significant downregulation compared to a non-treated control, while gRNAs targeting SNP5 did not show any effect on gene expression, suggesting the importance of the SNP location and chromatin landscape on gene regulation. gRNAs targeting SNP1 showed significant downregulation in patient derived iPSCs and in primary cortical neurons from a transgenic mouse model. The ability to downregulate mutant HTT has a large translational application and could lead to a one-time treatment in the future. Our future directions are to assess and optimize the allele-specificity along with moving into an in vivo model to optimize delivery of our therapeutic.

32. REGULATION OF SINGLE-CARBON UNITS IN FOLATE METABOLISM THROUGH SERINE-GLYCINE BIOSYNTHESIS IS ESSENTIAL FOR EARLY EYE FORMATION

JUSTINE M. CHEE [1], Louise Lanoue[2], Dave Clary[2], Kendall Higgins[3], International Mouse Phenotyping Consortium, K. C. Kent Lloyd[4,6], Henrik Westerberg[5], Ala Moshiri[6]

[1]Oakland University William Beaumont School of Medicine, Rochester, MI, United States.
[2]Mouse Biology Program, University of California Davis, Davis, CA, United States.
[3]University of Miami School of Medicine, Miami, FL, United States.
[4]Department of Surgery, School of Medicine, University of California Davis, Sacramento, CA, United States.
[5]Medical Research Council Harwell (Mammalian Genetics Unit and Mary Lyon Centre), Harwell, Oxfordshire OX11 0RD, UK.
[6]Department of Ophthalmology & Vision Science, School of Medicine, University of California Davis, Sacramento, CA, United States.

The purpose of this study was to identify novel genes and pathways required for early eye development. The International Mouse Phenotyping Consortium (IMPC) database was consulted to look for mouse embryos with ocular defects. The vast majority of the eye abnormalities observed in mouse embryos were small or absent eyes, findings which are most relevant to microphthalmia, anophthalmia, and coloboma (MAC) spectrum disease in humans. Screening of the IMPC database yielded 63 unique knockout lines with embryonic eye defects. A literature search was then performed to determine if mouse knockouts of these 63 genes existed and if there were eye abnormalities associated with them. The results showed that 41 of the 63 had previously published knockout mouse models, and 22 did not. Out of the 41 published knockout mouse models, only 13 of them noted ocular defects in the original publication, and 28 of them did not. Therefore, the 28 published knockouts which did not previously detect ocular abnormalities and the 22 unpublished knockouts together represent 50 novel genes that contribute to early eye development in mice. Protein-protein interactions between the 63 genes were predicted using STRING software analysis. Gene Ontology analysis was used to determine the cellular functions of these genes and the pathways in which they function. Another literature search was conducted to identify established genes associated with congenital MAC spectrum disease in humans, which yielded 114 “gold-standard” genes. Bioinformatic tools were used to analyze the predicted relationship between the 63 IMPC genes and 114 gold-standard MAC spectrum disease genes. This analysis showed that several of the IMPC genes point to serine-glycine biosynthesis and signaling pathways regulating pluripotency of stem cells as potential important pathways in early eye development. However, the genetic underpinnings of MAC spectrum disease are incompletely understood. Identification of novel genes and pathways associated with early eye formation using genome-wide screening of mammalian animal models may reveal new developmental mechanisms required for eye formation and also hasten the diagnosis and treatment of this congenital blinding disease.