



Mount Sinai *The Mindich Child Health and Development Institute*

MCHDI Developmental Outcomes

Spring 2016

Research Advancements: Neurodevelopment

Hospital-based Environmental Exposures and Preterm Infant Neurodevelopment

Preterm infants are at elevated risk of developmental delay that is incompletely explained by degree of prematurity or severity of illness in infancy. The specific mechanism of neurological injury resulting from preterm birth has not been elucidated, although alterations in cortical developmental trajectory following preterm birth — as opposed to focal brain injury — are strongly implicated.

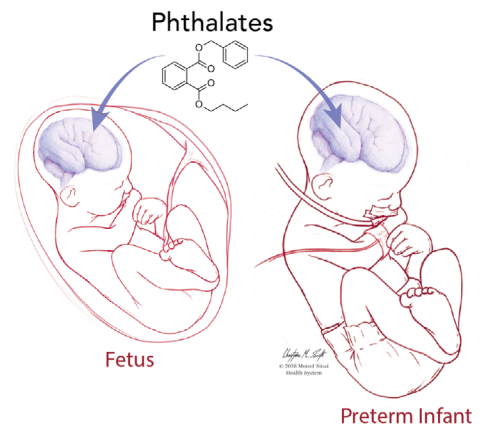
Annemarie Stroustrup, MD, MPH, Associate Professor in the Departments of Pediatrics and Preventive Medicine, is investigating the hypothesis that modifiable environmental chemical exposures in the neonatal intensive care unit (NICU) — exposures associated with adverse neurodevelopmental profiles in term cohorts — contribute to the behavioral difficulties seen in preterm infants. Dr. Stroustrup initiated the NICU-Hospital Exposures and Long-Term Health (NICU-HEALTH) cohort, a “first of its kind” environmental health cohort of preterm infants. NICU-HEALTH focuses on environmental exposures in the NICU that could contribute to cognitive, motor, and behavioral morbidities common among preterm infants.

The initial focus of NICU-HEALTH is to assess the impact of NICU-based phthalate exposure on neurodevelopmental outcomes of preterm infants. Phthalates, a ubiquitous family of industrial chemicals, are added as non-covalently bonded adjuvants to enhance the physical properties of plastic material. As they are not chemically integral to plastic materials, phthalates are easily liberated from the plastic over time, particularly in conditions of high heat or humidity. These conditions, common inside neonatal incubators and in respiratory support circuits, allow for ready leaching of phthalates from plastic.

Associations between prenatal or early childhood exposure to phthalates and alterations in multiple domains of neurodevelopment are well documented. Elevated third trimester *in utero* exposure to mixtures of phthalates found in medical equipment has been associated with poorer performance on measures of infant executive function, attention, and motor reflexes in multiple studies. Preterm infants spend a critical period of development — equivalent to the fetal third trimester — *ex utero* in the NICU where they face elevated, direct exposure to these same chemicals.

As relevant exposures are those linked to clinical outcomes, and as concurrent exposure to multiple different phthalates occurs at any given time, a weighted quantile sum (WQS) approach — developed by Dr. Chris Gennings, Professor of Preventive Medicine and a Mindich Institute member — was used to evaluate phthalate mixtures. WQS regression allows for analysis of weighted indices of phthalate metabolites. WQS weighting is based on the potency of each individual chemical entity to be included in the mixture analysis for a specific outcome measure of interest. In preliminary studies, the outcome used was the NICU Network Neurobehavioral Scale (NNNS), a standardized physical exam of motor and behavioral function administered in infancy.

Appropriately adjusted multivariable regression models indicate a significant association between NICU-based exposure to specific phthalate mixtures and NNNS quality of movement ($\beta=-0.12$, $p=0.01$), implying a negative impact of phthalate exposure on gross motor performance. There was also an association between phthalate exposure and both increased arousal ($\beta=0.07$, $p=0.02$) and decreased lethargy ($\beta=-1.97$, $p=0.05$). An indication of



Preterm infants face exposures in the NICU that are associated with neurodevelopmental morbidities in term-born children. The contribution of hospital-based environmental exposures to neurobehavioral morbidities of prematurity is unknown.

an association between phthalate exposure and attention was also identified; the inclusion of a quadratic term in this model provided a better fit than a simple linear model (two degrees of freedom contrast test, $p=0.08$). Interestingly, poor performance specifically on NNNS quality of movement, arousal, lethargy, and attention summary scales has been most strongly associated with poor long-term neurodevelopmental outcomes in other studies of preterm infants. Future results from NICU-HEALTH will address the role of phthalates and other NICU-based environmental exposures on long-term neurodevelopmental outcomes of preterm infants.



Annemarie Stroustrup, MD, MPH

Associate Professor of Pediatrics

Metabolic Pathways of Embryogenesis Lend Insights into Cancer Cell Growth

Cancer cells acquire a characteristic metabolic signature, known as the Warburg Effect, which is the increased reliance on glycolysis and lactate production for rapid cell division. The mechanisms that promote and maintain this aberrant glycolytic dependency in cancer remain unclear. The laboratory of Jaime Chu, MD, Assistant Professor of Pediatrics, approaches these questions from the vantage point of the embryo, and the emerging concept that metabolic programs of rapid cellular proliferation in embryogenesis are shared with cancer cells.

Metabolic enzymes have been traditionally regarded as bystanders in cancer, subservient to oncogenes and tumor suppressor genes. However, recent discoveries have shifted this paradigm to show that glycolytic enzymes such as *phosphofructokinase (PFK)* and *TP53-inducible glycolysis and apoptosis regulator (TIGAR)* are themselves essential for driving tumor growth, and that cancer genes, such as the tumor suppressor p53, have alternate, but key functions in regulating energy production to support cell growth.

The Chu lab focuses on the function of Mannose Phosphate Isomerase (MPI),

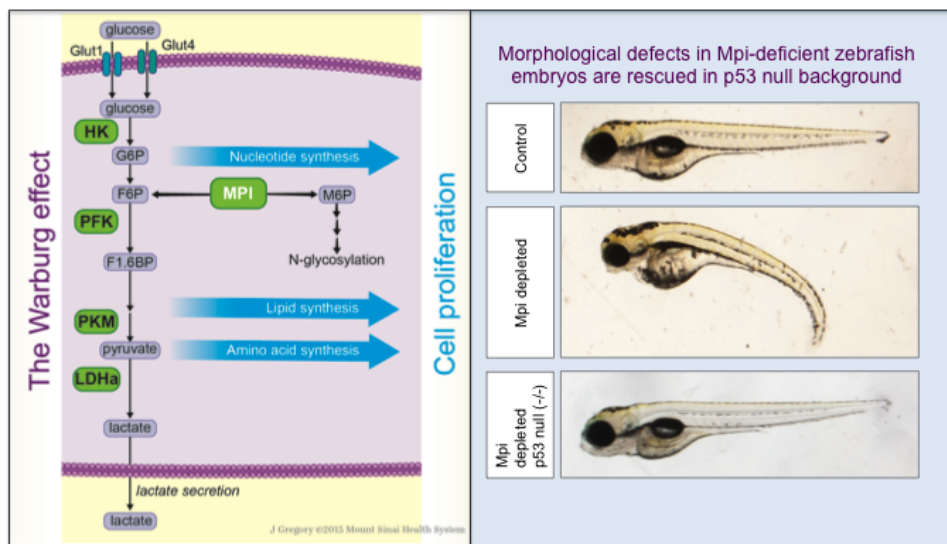
a metabolic enzyme that has long been understood to play a role in protein N-glycosylation, which is the building of sugar modifications on proteins that direct protein function and stability. MPI mutations in humans results in a syndrome classified among the congenital disorders of glycosylation (CDG; MPI-CDG), but the clinical presentation of MPI-CDG is inexplicably unique to the other 40+ types of CDG, which calls to question whether MPI plays an additional role in a different cellular process. The Chu lab has generated a zebrafish model to study the pathogenesis of MPI-CDG, and has discovered new roles of MPI, distinct from that in glycosylation. Their findings point to MPI as a novel activator of p53, and both function together to regulate Warburg metabolism and cell survival.

Using an unbiased approach of RNA-sequencing and metabolite profiling of Mpi-deficient zebrafish embryos, Dr. Chu's group found that loss of MPI activates p53 and subsequently suppresses glycolysis, leading to apoptosis. This new finding that loss of MPI induced p53-mediated cell death prompted the question of whether overexpression of MPI may be protective to promote cell survival. Previously

published data have shown MPI depletion can augment radiation-induced cell death in cancer cells. Dr. Chu's group found that ultraviolet (UV) radiation to zebrafish embryos led to cell death largely mediated by decreased MPI activity. Zebrafish embryos were exposed to UV radiation, and the group with MPI overexpression had significantly less p53-mediated cell death, suggesting that MPI expression has a protective effect against genotoxic stress. The lab has shown that these functions of MPI are conserved in primary mouse embryonic fibroblasts, as well as human colon cancer cells (HCT116). These data point to MPI as a central promoter of cell survival through p53 to maintain the fuel stores necessary for glycolytic metabolism both during development and in response to genotoxic stress, and uncovers MPI as a potent target for future cancer therapies.



Jaime Chu, MD
Assistant Professor of Pediatrics



Left: The Warburg Effect is characterized by increased glycolysis to generate precursors necessary for rapid cell proliferation. MPI has roles in both glycosylation and glycolysis. (Illustration by Jill Gregory, ISMMS Department of Academic Medical Illustration)

Right: 24 hour post-fertilization zebrafish embryos showing morphologic phenotypes with Mpi depletion and p53 knockout.

Faculty Highlights

MCHDI Faculty Receive \$20 Million in Funding from Child Health Environmental Assessment Resource (CHEAR) Grants

The Icahn School of Medicine at Mount Sinai has been awarded 2 new grants from the National Institutes of Health for the newly formed CHEAR (Child Health Environmental Assessment Resource) research program. The mission of CHEAR is to provide the extramural research community access to the laboratory and data management expertise needed to add measures of environmental exposures to their children's health research. CHEAR is designed to develop new methodologies and provide state of the art tools for researchers to assess a

full array of environmental exposures which affect children's health. CHEAR will also lead the extramural community in defining and developing the nascent science of "Exposomics" — the totality of environmental exposures from conception through development, including chemical, physical and biological stressors as well as lifestyle and social environments. *Mount Sinai is the only Institution to receive 2 components of CHEAR.* (a Lab Network Hub and the Data Center). These 2 grants represent over 20 million dollars of new NIH funding over the next 4 years and

will put Mount Sinai at the forefront of efforts to define and analyze the exposome. CHEAR is designed to incorporate environmental measures into Clinical and Epidemiologic Research studies around the world, including much needed studies of gene-environment interaction. Robert O. Wright, MD, MPH led the Lab Hub application along with MCHDI members Manish Arora, PhD, BDS, MPH and Rosalind J. Wright, MD, MPH while Susan Teitelbaum, PhD led the Data Center application along with MCHDI member Chris Gennings, PhD.



Robert O. Wright, MD, MPH

Professor and Ethel H Wise Chair, Preventive Medicine
Director, Lautenberg Laboratory



Manish Arora, PhD, BDS, MPH

Associate Professor, Preventive Medicine & Dentistry



Rosalind J. Wright, MD, MPH

Dean, Translational Biomedical Sciences
Director, Clinical and Translational Science Award
Horace W. Goldsmith Professor, Children's Health Research
Professor, Pediatrics & Preventive Medicine



Susan Teitelbaum, PhD

Associate Professor, Preventive Medicine



Chris Gennings, PhD

Professor, Preventive Medicine & Population Health Science and Policy

Pilot Projects: 2015-2016 Awardees

Project Title: "Nsun Family RNA Methyltransferases in Pluripotency, Reprogramming and Development"

Investigators: *Martin J. Walsh, PhD, MCHDI Investigator and Associate Professor of Pediatrics, Structural & Chemical Biology, and Genetics and Genomics Sciences; Jianlong Wang, PhD, MCHDI Investigator and Associate Professor of Developmental and Regenerative Biology*

Abstract: The NOL1/NOP2/Sun domain-containing genes encode for a family of the RNA methyltransferases encompassing the genes for NSun1, 2, 3, 4, 5, 6 and 7. Cytosine methylated RNA is pervasive throughout the transcriptome, yet the function imparted through these novel RNA methyltransferases remains poorly understood. The NSun family proteins predominantly encode for enzymes capable of N5 cytosine methyltransferase catalytic activity and display expression during early embryogenesis. Recently, we have shown that specific non-genic and enhancer-associated RNA transcripts

are decorated and enriched with N5-methylcytosine (m5C). Some of these non-coding RNA transcripts are directly associated with the transcriptional co-activator functions of the PPAR γ coactivator 1 alpha (PGC1 α) in adapting to metabolic stress. Moreover, many of the NSun family members are highly expressed during early development and cell differentiation closely corresponding with cell proliferation and self-renewal. An emerging presence of RNA transcript modification (epi-transcriptome) seems to run in parallel with the epigenetic alterations in genomic DNA and chromatin to impose control of global gene expression patterns. The goal of the collaboration between Drs. Walsh and Wang will be to explore the role of the NSun family members in shaping stem cell self-renewal, pluripotency and the response to metabolic stress during cellular reprogramming by regulating the "epi-transcriptome" of mouse embryonic and induced pluripotent stem cells (ESCs/iPSCs). The Walsh and Wang laboratories

have demonstrated the combined expertise in genomics, epigenetics, biochemistry, and stem cell biology to ensure the transition of this pilot program to gain support through funding mechanisms by the NIH. The short-term goal will be to identify responsible Nsun genes that are critical for stem cell pluripotency and somatic cell reprogramming. We will perform both loss and gain of function of Nsun genes to determine their influence in stem cell self-renewal, differentiation, and somatic cell reprogramming. Our long term goal, which requires additional funding mechanisms, will be to determine genome-wide scope of genic and non-genic RNA transcript substrates as targets of responsible NSun RNA methyltransferases in self-renewing ESCs and ESCs iPSCs undergoing reprogramming, and study how such RNA transcript substrates' epitranscriptome may have contributed to mammalian development and epigenetic/metabolic reprogramming.

Pilot Projects, continued on next page

Pilot Projects: 2015-2016 Awardees, continued

Project Title: “Maternal Effects in Childhood-Onset Psychiatric Disorders”

Investigators: *Dorothy E. Grice, MD, MCHDI Investigator and Professor of Psychiatry; Joseph D. Buxbaum, PhD, MCHDI Investigator and Professor of Psychiatry, Neuroscience and Genetics & Genomic Sciences; Sven Sandin, PhD, Assistant Professor of Psychiatry*

Abstract: Childhood-onset psychiatric disorders are associated with significant public health burden but novel insights into the causes and treatment of such disorders are sparse. Maternal effects, defined as effects of environmental and/or genetic influences at the maternal level that affect phenotypes of the child, are a critical source of variance for many traits but are understudied in psychiatric disorders. Multiple maternal conditions in pregnancy have been associated with adverse short-term and long-term outcomes in the offspring, conditions including maternal smoking, diabetes, and infections during pregnancy that have been associated with autism, schizophrenia, and other psychiatric disorders. We propose to develop a national epidemiological database suited to study maternal effects and, as a first study, examine the role of maternal effects in obsessive-compulsive disorder (OCD). OCD is a life-long, serious psychiatric disorder that affects up to 3% of the population and incurs high personal and societal costs. Our recent epidemiological studies suggest that maternal effects might be of particular importance in the etiology of OCD and the related tic disorders, Tourette disorder and chronic tic disorder, (TD). This knowledge, and, in particular, identifying environmental factors that underlie the maternal effects, could be especially significant. For example, Down syndrome was one of the first conditions to be consistently associated with maternal factors, specifically older age of the mother. An understanding of the effect of maternal age on risk has helped impact the occurrence of Down syndrome in several ways. A similar story, probably not as extreme, could unfold for OCD and TD, which our preliminary data also suggest have a maternal component to risk. Understanding which are bona fide risk factors will open the door to prevention. In addition, the approaches and cohort that we develop will be appropriate for studies on other disorders in children for



2015-2016 Pilot Program Recipients (from left to right): *Martin J. Walsh, PhD; Dorothy E. Grice, MD; Hirofumi Morishita, MD, PhD*

which there is Swedish register data (e.g., asthma, obesity, etc.).

Project Title: “Novel Tooth Matrix Biomarker For Neurodevelopmental Susceptibility”

Investigators: *Hirofumi Morishita, MD, PhD, MCHDI Investigator and Assistant Professor of Psychiatry, Neuroscience, and Ophthalmology; Manish Arora, PhD, BDS, MPH, MCHDI Investigator and Associate Professor of Preventive Medicine and Dentistry*

Abstract: Environmental stimuli, including chemical toxicants exposure, during development may result in life-long neurodevelopmental impairment. However, due to lack of suitable molecular human biomarkers, it is not known which toxicants impact specific neurodevelopmental processes that ultimately lead to neurodevelopmental disorders. Consequently, work in this area has been limited to animal models with little clinical translation, or conversely human studies with minimum mechanistic insight. Arora has pioneered a dental biomarker that directly measures perinatal molecular targets and environmental chemicals. Morishita has discovered molecular regulators of neurodevelopmental critical periods. Due to the shared evolutionary origins of tooth dentine and the brain, dentine is well positioned

to capture not only the developmental timing of toxicant exposure, but also concurrent variations in expression of neurodevelopmental regulators. By combining the expertise of both PIs in human exposure and mechanistic animal studies, we will establish novel human biomarkers to predict environmentally driven disruptions of specific neurodevelopmental events associated with neurodevelopmental disorders. Our preliminary human study (Arora) shows a link between lead exposure and autism. Our mouse study (Morishita) predicted that lead can disrupt critical developmental periods for brain plasticity. Based on these preliminary data, we will test a hypothesis that teeth can be a valuable human biomarker to assess if perinatal toxicant exposure disrupts brain plasticity and impacts on neurodevelopmental clinical outcomes associated with the autism. First, we will apply novel teeth metal biomarkers to determine if environmental toxicant exposures are associated with increased risk of autism in a unique cohort of monozygotic and dizygotic twins discordant for autism. Next, we will assess the molecular and functional impact of toxicant exposure on developmental critical periods for cortical plasticity in mice. Finally, we will undertake the first-ever human study that determines if brain plasticity and environmental toxicants interact to alter the risk of autism.

New Internal Faculty

Susan Teitelbaum, PhD

Susan Teitelbaum, PhD is an Associate Professor of Preventive Medicine. As an environmental and cancer epidemiologist, her research accomplishments have been in two important health areas, breast cancer and childhood growth and development. Involved as a key investigator from its inception, the Long Island Breast Cancer Study Project has become one of the most successful and productive breast cancer case-control studies funded by NCI/NIEHS; there have been over 80 peer-reviewed publications and numerous spinoff grants, including several she has received. In an effort to better understand the early risk factors of breast cancer, her research has expanded to include childhood growth and development since earlier pubertal development is an established breast cancer risk factor. This led to her involvement in another groundbreaking epidemiologic investigation, Growing Up Healthy, an NIEHS/NCI funded prospective cohort study, part of a nationwide consortium investigating the environment's role in girls' early pubertal development. They have confirmed that the age of pubertal development has decreased compared to reports of only a decade ago and identified associations between endocrine disrupting chemicals and perturbations in girls' growth and development. Beyond her personal research program, she works with the World Trade Center (WTC) Health Program Data Center as Deputy Director; her work with this group has resulted in several important publications on WTC responder health. Most recently, she leads the NIEHS Data Center (DC) for the Children's Health Exposure Analysis Resource (CHEAR). The CHEAR DC provides a data repository and statistical analysis, data integration, and interpretation services to CHEAR researchers.



Susan Teitelbaum, PhD
Associate Professor,
Preventive Medicine

Jianlong Wang, PhD

Jianlong Wang, PhD is an Associate Professor of Developmental and Regenerative Biology. He received his PhD from the University of Massachusetts and completed his post-doctoral fellowship in Stem Cell Biology at the Harvard Medical School and Dana-Farber Cancer Institute. He is the recipient of the Irma T. Hirsch and Weill-Caulier Trusts Career Scientist Award and one of the recipients of the MCHDI pilot grant award this year along with co-PI, and MCHDI faculty member, Martin J. Walsh, PhD. His lab studies the molecular mechanisms underlying pluripotency and reprogramming. They employ both proteomic and genomic approaches to identify and study pluripotency protein-protein interaction and transcriptional regulatory networks that govern stem cell pluripotency and somatic cell reprogramming. Insights from these studies will facilitate efficient derivation/generation and optimal propagation of embryonic/induced pluripotent stem cells (ESCs/iPSCs) for their safe application in disease therapeutics and regenerative medicine. His current research includes transcriptional/post-transcriptional control and epigenetic regulation of stem cell pluripotency and somatic cell reprogramming. As a PI on several New York state-funded stem cell grants and the existing NIH-funded R01 grant, Dr. Wang laid the groundwork for his proposed pilot research by developing novel biochemical affinity purification approaches for studying pluripotency protein complexes in embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), and by discovering many novel pluripotency transcription factors and epigenetic regulators including miRNAs that are critical for maintenance and/or establishment of ESCs/iPSCs.



Jianlong Wang, PhD
Associate Professor,
Developmental and
Regenerative Biology

Trainee Highlights

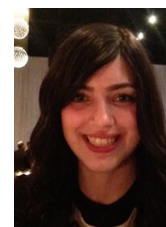
Elena Sanchez-Rodriguez, PhD is currently a postdoctoral fellow in the laboratory of Coro Paisan-Ruiz, PhD under the department of Neurology. She is the winner of the Young Investigator's Competition in the post-doctoral division. The focus of her work is to elucidate the genetics and molecular basis underlying Early Onset Parkinson Disease and other movement disorders using genetics approaches and zebrafish as a model system. She is modeling two genes causing Atypical Juvenile Parkinsonism in zebrafish using genome-editing Cas9/CRISPR system.



“This is a great honor for me to be awarded this year with the Young Investigator Competition (Post-doctoral Division) at the MCHDI annual retreat. As usual all the work presented were of a high level and very competitive and it will be a pleasure to continue hearing about all this amazing works in the coming retreats.”

Elena Sanchez-Rodriguez

Kathryn Manheimer is a PhD candidate in the departments of Pediatrics and Genetics and Genomic Sciences. She is the winner of the Young Investigator's Competition in the predoctoral division. Her work with mentor Bruce Gelb, MD is investigating the genetic architecture of congenital heart disease using whole genome and whole exome sequencing data as part of the Pediatric Cardiac Genomics Consortium.



Faculty Highlights

Awards/Honors

- Dusan Bogunovic**, PhD, International Cytokine and Interferon Society, Milstein Award for Young Investigators
- Dusan Bogunovic**, PhD, American Society for Microbiology, Young Investigator Award
- Adolfo García-Ocaña**, PhD, Icahn School of Medicine at Mount Sinai, Organizer of Fifth Annual NYC Regional Diabetes Meeting
- Adolfo García-Ocaña**, PhD, American Diabetes Association Scientific Sessions Meeting Planning Committee (Islet Biology/Insulin Secretion), Co-Chair, 2016-2018
- Philip J. Landrigan**, MD, MS, DIH, Juntendo University, Tokyo, Japan, Araki Foundation Award for Social Medicine Promotion 2016
- Philip J. Landrigan**, MD, MS, DIH, Asbestos Disease Awareness Association, Dr. Irving Selikoff Lifetime Achievement Award 2016
- Xiu-Min Li**, MD, Future of Health Technology Summit, Massachusetts Institute of Technology, Future of Health Technology Award 2016
- Nadia Micali**, MD, MRCPsych, PhD, FAED, National Alliance for Research on Schizophrenia and Depression, Independent Investigator Award
- Donald Scott**, PhD, Juvenile Diabetes Research Foundation, Innovation award: Enhanced transplant efficiency and function of human beta cells with ChREBP-alpha

Grants

- Dusan Bogunovic**, PhD, March of Dimes, “Genetic defects leading to persistent IFN- α/β inflammation in children”
- Joseph Buxbaum**, PhD, Simons Foundation Autism Research Initiative, “Integrating Large Scale Whole Exome Data with Whole Genome Data”
- Joseph Buxbaum**, PhD, NIMH, Supplement, “The Autism Sequencing Consortium: Autism Gene Discovery in >20,000 Exomes”
- Joseph Buxbaum**, PhD, NIMH, Supplement, “Population-based Autism Genetics and Environment Study”
- Nadia Micali**, MD, MRCPsych, PhD, FAED, NIMH, R01, “Neurobiological and Behavioral Risk Mechanisms of Youth Avoidant/Restrictive Eating Trajectories”
- Nadia Micali**, MD, MRCPsych, PhD, FAED, NIMH, R21, “Polygenic and trans-diagnostic risk for anorexia nervosa and obsessive-compulsive disorder: Novel associations with emotional, behavioral, and cognitive dysfunction in a population-based cohort”
- Andrew Sharp**, PhD, March of Dimes, “Folate Pathway Neural Tube Defects”
- Susan Teitelbaum**, PhD, and **Chris Gennings**, PhD, NIEHS, CHEAR Center for Data Science
- Jianlong Wang**, PhD, NICHD, R21, “Catalytic Activity Independent Functions of TET Proteins in Pluripotency”
- Robert O. Wright**, MD, MPH, **Manish Arora**, PhD, BDS, MPH, and **Rosalind J. Wright**, MD, MPH, NIEHS, CHEAR Center for Laboratory Network Hub

Faculty Highlights

Publications

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Website: www.mountsinai.org/mchdi

Email: mchdi@mssm.edu

Facebook: www.facebook.com/mindichchdi

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Contact: Tel: (212) 824-8938 Fax: (212) 241-3310

Address: 1470 Madison Avenue, 8th Floor
Hess Center for Science and Medicine at Mount Sinai
New York, NY 10029-6542