Department of Genetics
2025-2026

GENETICS FACULTY

2025-2026

	Room	Building	Phone
Gil Atzmon, Associate Professor (primary appointment, Medicine/Endocrinology)	502C	Golding	430-3628
Nicholas E. Baker, Adjunct Professor	1001 Health Sciences Road Irvine, CA 92697-3950		949-824-1969
Nir Barzilai, Professor (primary appointment, Medicine/Endocrinology)	701A	Belfer	430-3144
Renata A. Batista-Brito, Associate Professor (primary appointment, Neuroscience)	203	Kennedy	430-8507
Hannes Buelow, Professor (secondary appointment, Neuroscience)	709	Ullmann	430-3621
Roy S. Chuck, Professor (primary appointment, Chair, Ophthalmology and Visual Sciences)	3332 Rochambeau Ave., MMC		920-6665
Ales Cvekl, Professor (primary appointment, Ophthalmology and Visual Sciences)	123	Ullmann	430-3217
Meelad Dawlaty, Associate Professor (secondary appointment, Developmental and Molecular Biology and Stem Cell Institute)	419	Price	678-1224
Winfried Edelmann, Professor (primary appointment, Cell Biology)	277	Price	678-1086
Scott Emmons, Distinguished Professor Emeritus (secondary appointment, Neuroscience)	703	Ullmann	430-3130
John Greally, Professor (secondary appointment, Pediatrics)	322	Price	678-1234
William R. Jacobs, Jr., Professor (primary appointment, Microbiology & Immunology)	577	Price	678-1075
Andreas Jenny, Professor (primary appointment, Developmental and Molecular Biology)	503	Chanin	430-4183
Ganjam V. Kalpana, Professor (secondary appointment, Microbiology & Immunology)	823	Ullmann	430-2354
Peri Kurshan, Assistant Professor (primary appointment, Neuroscience)	810	Kennedy	430-3631

Herb Lachman, Professor   103   Forchheimer   430-2428
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    (secondary appointment, Medicine/Hematology)
    (tertiary appointment, Genetics)
    (tertiary appointment, Neuroscience)

Jack Lenz, Professor   717   Ullmann   430-3715
    (secondary appointment, Microbiology & Immunology)

Wei Liu, Associate Professor   117A   Ullmann   839-7926
    (primary appointment, Ophthalmology and Visual Sciences)

Sridhar Mani, Professor   302D-1   Chanin   430-2871
    (primary appointment, Medicine/Oncology)

Sofiya Milman, Associate Professor   702B   Belfer   430-3462
    (primary appointment, Medicine/Endocrinology)

Cristina Montagna, Adjunct Professor   Department of Radiation   732-235-6430
                                             Oncology
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Bernice Morrow, Professor   475   Price   678-1121
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    (tertiary appointment, Pediatrics)

Xiao Peng, Assistant Professor   Department of Pediatrics   741-2323
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                                             Bronx, New York 10467

Srilakshmi Raj, Assistant Professor   422A   Price   678-1245

Deepa Rastogi, Professor   6B36   Van Etten   839-7653
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Namita Roy-Chowdhury, Professor   523   Ullmann   430-2254
    (primary appointment, Medicine/Liver Diseases)

Julie Secombe, Professor   809   Ullmann   430-2698
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Milan Sen, Associate Professor   Jacobi Medical Center   718-918-4921
    (primary appointment, Orthopaedic Surgery)   1400 Pelham Parkway South
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Frank Soldner, Assistant Professor (primary appointment, Neuroscience)	235	Kennedy	839-7770
Simon D. Spivack, Professor (primary appointment, Medicine/Pulmonary Medicine) (secondary appointment, Epidemiology & Population Health) (tertiary appointment, Genetics)	301	Price	678-1040
Yousin Suh, Adjunct Professor	Columbia University 630 West 168th Street Room 10-518 New York, NY 10032		212-305-6832
Masako Suzuki, Adjunct Professor	Texas A&M University 214A Cater-Mattil Hall 373 Olsen Blvd. College Station, TX 77843		979-847-8714
Anne Van Arsdale, Associate Professor (primary appointment, Obstetrics & Gynecology and Women's Health (Gynecological Oncology)	1695 Eastchester Road Room 601 Bronx, New York 10461 and		718-405-8086
	469	Price	678-1159
Vladislav Verkhusha, Professor (Co-Director of the Gruss-Lipper Biophotonics Center)	1217	Ullmann	430-8591
Jan Vijg, Professor and Chair (secondary appointment, Ophthalmology & Visual Sciences)	450	Price	678-1151
Tao Wang, Professor (primary, Epidemiology & Population Health)	1303A	Belfer	430-4007
Melissa Wasserstein, Professor (primary appointment, Pediatrics)	3411 Wayne Avenue, MMC		718-741-2318
Daniel Weiser, Associate Professor (primary appointment, Pediatrics)	813	Ullmann	430-2181
Zhengdong Zhang, Professor	353A	Price	678-1139
Deyou Zheng, Professor (primary appointment, Genetics and Neurology) (tertiary appointment, Neuroscience)	320	Price	678-1217
Bin Zhou, Adjunct Professor	The University of Chicago KCBD 5112 E. 57th Street Chicago, IL. 60637		773-702-4340

RESEARCH FACULTY

Department of Genetics

2025-2026

Name (Mentor)	Title	Room	Building	Phone
Mikhail Baloban (Verkhusha)	Research Asst. Prof.	1217	Ullmann	430-2127
Anna Burton (Greally)	Associate	314	Price	678-1570
Robert Dubin (Greally)	Staff Scientist	553	Price	678-1226
Swati Haldar (Kalpana)	Staff Scientist	823	Ullmann	430-2404
Amit Kumar (Buelow)	Staff Scientist	709	Ullmann	430-3622
Jhih-Rong Lin (Zhang)	Staff Scientist	353	Price	678-1147
Shahina Maqbool (Greally)	Research Assoc. Prof.	157	Price	678-1163
Alexander Maslov (Vijg)	Research Assoc. Prof.	468	Price	678-1135
Rajiv Pathak (Kalpana)	Staff Scientist	823	Ullmann	430-2404
Nathaniel Pearson (Greally)	Associate	314	Price	678-1570
David Reynolds (Morrow)	Staff Scientist	1203	Ullmann	929-246-6735
Karman Saini (Greally)	Associate	314	Price	679-1570
Daria Shcherbakova (Verkhusha)	Research Asst. Prof.	1217	Ullmann	430-2127
Jidong Shan (Morrow)	Research Assoc. Prof.	401	Price	678-1155
Lijie Shi (Morrow)	Research Asst. Prof.	469	Price	678-1122
Selvin Soby (Mirhaji)	Research Asst. Prof.	1P-59	MMC/Yonkers	914-457-6052
Shixiang Sun (Vijg)	Staff Scientist	478	Price	678-1135

POSTDOCTORAL FELLOWS

Department of Genetics

2025-2026

Name (Mentor)	Telephone	Lab Location
Asmaa Abdullah (Dawlaty)	678-1210	413 Price
Mohd Murshad Ahmed (Zhang)	678-1147	353 Price
Natalia Barykina (Verkhusha)	430-2127	1217 Ullmann
Arunima Debnath (Buelow)	430-3622	709 Ullmann
Sara Fielder (Dawlaty)	678-1210	413 Price
Harrison Ho (Zheng)	678-1166	320 Price
Mariko Isshiki (Raj)	678-1570	314 Price
Giriraj Lokhande (Vijg)	678-1135	455 Price
Kyrylo Manoilov (Verkhusha)	430-2127	1217 Ullmann
Rohan Misra (Zheng)	678-1166	320 Price
Micaela Parra (Dawlaty)	678-1210	413 Price
Bethany Terry (Secombe)	430-4463	809 Ullmann
Peng Zhang (Shan)	678-1161	407 Price

GRADUATE STUDENTS

Department of Genetics

2025-2026

Name (Mentor)	Telephone	Lab Location
Olivia Albert (Vijg/Montagna)	678-1135	468 Price
Zipporiah Bush (Soldner)	839-7770	235 Kennedy
Michael Camerino (Cvekl)	430-3219	123 Ullmann
Melissa Castiglione (Secombe)	430-4463	809 Ullmann
Wenna (Yen Chun) Chen (Zheng)	678-1166	353 Price
Ronald Cutler (Vijg/Sidoli)	678-1135	468 Price
Blake Ebert* (Dawlaty)	678-1210	413 Price
Emilie Ernst (Kalpana)	430-2404	823 Ullmann
Elisha Fogel (Morrow)	678-1122	469 Price
Michael Gilmore (Vijg)	678-1135	468 Price
Hilledna Gregoire* (Secombe)	430-4463	809 Ullmann
Hersh Gupta (Raj)	678-1570	314 Price
Maja Haerle* (Soldner/Zheng)	839-7770	235 Kennedy
Jacquelin Ho (Buelow)	430-3622	709 Ullmann
Gordon Huang (Zheng/Vijg)	678-1135	468 Price
Kevyn Jackson (Morrow/Zheng)	678-1122	469 Price
Richard Daniel Kelly (Secombe)	430-4463	809 Ullmann
Harmony Ketchum (Dawlaty)	678-1210	413 Price
Kassidy Lundy (Greally)	678-1570	314 Price
Dana Luong (Chang/Raj)	678-1570	314 Price
Marliette Matos-Rodriguez (Greally)	678-1570	314 Price
Ian MacArthur* (Dawlaty)	678-1210	413 Price
Daniella Miller (Morrow)	678-1122	469 Price
Amira Millette (Secombe)	430-4463	809 Ullmann
Tinaye Mutetwa (Raj)	678-1570	314 Price
Kevin O'Leary* (Zheng)	678-1166	353 Price
Elizabeth Pan (Vijg/Zheng)	678-1135	468 Price
Megan Russell (Zhou/Zheng)	678-1551	408 Price
Aubrey Siebels (Secombe)	430-4463	809 Ullmann
Chynna Smith (Raj/Greally)	678-1570	314 Price
Eric Sosa (Greally)	678-1570	314 Price
Jacob Stauber (Greally)	678-1570	314 Price
Gloria Stoyanova (Morrow)	678-1122	469 Price
Jada Summerville (Kurshan)	430-3646	810 Kennedy
Esteban Uceda Arias-Stella (Weiser)	430-2181	813 Ullmann
David Yang (Raj/Greally)	678-1570	314 Price
Xiang Yu Zheng (Zheng)	678-1166	353 Price

*M.D./Ph.D. Students

GIL ATZMON, Ph.D.

Epigenetic Profiling in Healthy Aging and Exceptional Longevity

Examples of epigenetic regulation of genome architecture and gene expression are paved across the evolutionary lineage. Even if only a small proportion of human genes are subject to similar effects, they may still play a major role in the phenotypic variation and susceptibility to diseases. My long-term research goal is to investigate changes in the epigenetic control of gene expression which may be one of the central mechanisms by which aging predisposes to many age-related diseases and therefore lifespan.

Despite some early work, the role of epigenetics in human life span and age related diseases has remained unexplored. Discovering that methylated loci are involved in the genetic control of cellular existence, modify the risk for age-related disease and influence mortality is a novel and extremely important concept that would significantly enhance our understanding of the biology of aging. In addition, accumulating evidence supports the notion that major age-associated diseases (such as diabetes, metabolic syndrome and cancer) are regulated by epigenetic alterations. Epigenetic changes may provide information on the pivotal points between healthy and sick stages in the lifespan of an individual. Hence, epigenetic changes could serve as landmarks of events such as onset of disease and these events can be tracked long after its occurrence (forms of epimutation and the Barker theory).

To test our hypothesis, we employed a novel high-throughput genome-wide methylation assay, HELP-tag and EPIC arrays (Illumina). Additionally, we utilized a combination of large-scale epigenomic analysis (EWAS) to identify the most distinctive epigenetic loci that show greatest differential methylation. We then performed Multi-locus validation for methylation status using MassARRAY. We applied both RNAseq and candidate loci expression to explore possible mechanisms of methylation regulation. We combined these results with whole genome sequences to assess the interaction between the genetic blueprint and the environment as it manifested through epigenetic changes.

This research furthered our understanding of the complexity of healthy lifespan process by identifying loci that when altered epigenetically have important ramifications for age-related diseases and lifespan. Validating the genes whose function is modulated epigenetically could lead to interventions to delay or even prevent the development of age-associated diseases.

<u>Recent Publications:</u>

Briller S *et al.*. A computational framework for detecting inter-tissue gene-expression coordination changes with aging. Sci Rep. 2025, 15(1):11014.

Falah G *et al.*. CRISPR-Cas9 mediated d3GHR knockout in HEK293 cells: Revealing the longevity associated isoform stress resilience. Exp Gerontol. 2024, 196:112586.

Andrawus M *et al*.. Genome integrity as a potential index of longevity in Ashkenazi Centenarian's families. Geroscience. 2024, 46(5):4147-4162.

Miller B *et al*.. Humanin variant P3S is associated with longevity in APOE4 carriers and resists APOE4-induced brain pathology. Aging Cell. 2024, 23(7):e14153.

Falah G *et al*. The d3GHR carrier epigenome in Druze clan longevity. Sci Rep. 2024,14(1):21419.

NICHOLAS BAKER, Ph.D.

Cell competition, ribosomopathy, and neuronal development

Cell-cell communication is the key process that makes complex life possible, enabling cells to follow different fates. We use *Drosophila* and more recently mice to identify and characterize new genes involved in these crucial processes. Currently we study novel molecular mechanisms of signaling and regulation in three main areas.

Cell competition and aneuploidy Competition is a process that can occur when cells within tissues differ, for example due to somatic mutation. A specific pathway of cell competition selectively removes cells that have become aneuploid, or acquired other large-scale genetic changes. This is thought to suppress tumorigenesis and promote healthy aging. We have begun to identify the molecular pathways involved. Our current goals include understanding how differences in genome content between cells are recognized, how cell competition participates in tumor suppression, including the potential cell competition roles of p53, one of the most important human tumor suppressors, and how changes in cell competition might increase or be exploited to decrease cancer incidence, both in fruitflies and in mammals.

Ribosomopathy Ribosomes are essential for growth. Their biogenesis and assembly are elaborate, regulated processes. Ribosome biogenesis and function is affected during growth and in neurodegenerative diseases. Mutations in ribosomal protein genes unexpectedly seem to be causal in many cancers. We are interested in the molecular signaling mechanisms activated by ribosomes, and their roles in cancer and neurological diseases.

Neural cell fate determination Commitment to neural development requires the expression of particular transcriptional master regulatory genes, of which the proneural basic helix-loop-helix (bHLH) proteins are most important. Their activities appear to be highly regulated. Our studies use genetic screening, modern genome resequencing methods and multidisciplinary studies to characterize how proneural bHLH proteins are regulated.

Selected recent publications and preprints

Khan, C. and N.E. Baker. The DNA damage response and cell competition are p53- and Xrp1-dependent processes that suppress hyperplastic aneuploidy. *bioRxiv*. **https://doi.org/10.1101/2022.06.06.494998**.

Kiparaki, M. and Baker, N.E. (2023) Ribosomal protein mutations and cell competition: autonomous and nonautonomous effects on a stress response. *Genetics* **224**: iyad080

Folgado-Marco, V., Ames, K., Chuen, J., Gritsman, K., and Baker, N.E. (2023) Haploinsufficiency of the essential gene RpS12 causes defects in erythropoiesis and hematopoietic stem cell maintenance. *Elife*,12: e69322.

Baker, N.E. and Montagna, C. (2022). Reducing the aneuploid cell burden – cell competition and the ribosome connection. *Dis Model Mech* **15**: dmm049673.

Kumar, A. and Baker, N.E. (2022). The CRL4 E3 ligase Mahjong/DCAF1 controls cell competition through the transcription factor Xrp1, independently of polarity genes. *Development* **149**: dev200795

Kiparaki, M., Khan, C., Folgado Marco, V., Chuen, J., and Baker, N.E. (2022) The transcription factor Xrp1 orchestrates both reduced translation and cell competition upon defective ribosome assembly or function. *Elife*, **11:** e71705.

Ji, Z., Chuen, J. Kipakai, M., and Baker, N.E. (2021) Cell competition removes segmental aneuploid cells from *Drosophila* imaginal disc-derived tissues based on ribosomal protein gene dose. *Elife*, **10**:e61172.

Quiquand, M., Rimesso, G., Qiao, N., Suo, S., Zhou, C., Slattery, M., White, K.P., Han, J.J., and Baker, N.E. (2021) New regulators of *Drosophila* eye development identified from temporal transcriptome changes. *Genetics*, **217(4)**: iyab007.doi: 10.1093/genetics/iyab007.

Baker, N.E. Emerging mechanisms of cell competition. (2020) *Nature Reviews Genetics,* **21**; 683-697.

Blanco, J., Cooper, J.C., and Baker, N.E. (2020) Roles of C/EBP class bZip proteins in the growth and cell competition of Rp ("Minute") mutants in Drosophila (2020). *Elife,* **9** 9:e50535

Baker, N.E., Kiparaki, M., and Khan, C. (2019) A potential link between p53, cell competition and ribosomopathy in mammals and in *Drosophila*. *Dev Biol* **446**: 17-19.

NIR BARZILAI, M.D.

Searching for Longevity Genes in Humans

Why do some people live much longer than others? What allows these individuals to escape age-associated diseases that contribute to mortality in the elderly? Is this a result of favorable genes or merely a healthy life style? If the genome does play a role, what are the mechanisms?

To address these questions, we recruited over 1500 Ashkenazi Jews. The Ashkenazi Jewish population is unique as it is derived from a small number (several thousands) of founders and therefore it is genetically homogeneous. This population has been utilized for identification of several genes, a prominent example being the breast cancer gene. The subjects fall into three groups; probands, subjects with exceptional longevity (1:10000 in the general population); their offspring; and a control group consisting of spouses of the offspring and other Ashkenazi Jewish people recruited from the Einstein Aging Study.

Studying the clinical and metabolic phenotype, revealed certain physiological characteristics in the centenarians and their offspring such as high levels of high-density lipoprotein (HDL), high adiponectin levels, and high IGF-1 levels. In collaboration with Dr. Atzmon and Suh, we showed that each of those phenotypes is now associated with a genotype that has a functional meaning, and each of those genotypes have been validated independently in at least one other population of centenarians. One of the genotypes is also specifically protective from cognitive decline, and this was also validated as an Alzheimer's protective gene. We studied telomere length demonstrating longer telomeres in our longest living subjects and their offspring compared to control. These findings may indicate longer telomeres at birth or slower attrition rate in their length, and this was associated with a specific haplotype of the telomere gene. Most important, since the trait of longer telomeres is associated with protective lipoprotein profile and less age-related disease, this test may be used as a predictor for longevity.

Using an un-biased approach, we have employed an Affimetrix 6.0 platform with almost 2MM markers and across the genome. Comparing the centenarian genotype to a younger un-related control, we established 35 genotypes that increase monotincally with aging (from age 60 to 112)) and were linked significantly ($p<10-6$) to genes that have not been previously linked to aging. In collaboration with Drs. Greally and Atzmon, we have used high throughput methylation assay (HELP) to demonstrate that centenarians methylation pattern across the genome is significantly different than in younger un-related subjects.

Our lab has trained many graduates and post-docs, and the latest graduate, Reid Thompson, MD/Ph.D. student, can be a reference. We offer a clinical platform for variety of genomic studies in collaboration with many of the Einstein faculty.

<u>Recent Publications</u>:

Kato K, Zweig R, Schechter CB, Verghese J, Barzilai N, Atzmon G. Personality, self-rated health, and cognition in centenarians: Do personality and self-rated health relate to cognitive function in advanced age? Aging (Albany NY). 2013 Mar 23. PMID: 23524310

Han J, Ryu S, Moskowitz DM, Rothenberg D, Leahy DJ, Atzmon G, Barzilai N, Suh Y. Discovery of novel nonsynonymous SNP variants in 988 candidate genes from 6 centenarians by target capture and next-generation sequencing. Mech Ageing Dev. 2013 Jan 31. doi:pii: S0047-6374(13)00020-1. PMID: 23376243

Barzilai N and Ferrucci L. Insulin Resistance and Aging: A Cause or a Protective Response? J Gerontol A Biol Sci Med Sci. August 2012; PMID: 22859390

Gombar S, Jung HJ, Dong F, Calder B, Atzmon G, Barzilai N, Tian XL, Pothof J, Hoeijmakers JH, Campisi J, Vijg J, Suh Y. Comprehensive microRNA profiling in B-cells of human centenarians by massively parallel sequencing. BMC Genomics. 2012 Jul 31;13(1):353 PMID: 22846614

Barzilai N, Guarente L, Kirkwood TB, Partridge L, Rando TA, Slagboom PE. The place of genetics in ageing research. Nat Rev Genet. 2012 Jul 10;13(8):589-94. doi: 10.1038/nrg3290. PMID: 22777128

Huffman DM, Deelen J, Ye K, Bergman A, Slagboom EP, Barzilai N, Atzmon G. Distinguishing Between Longevity and Buffered-Deleterious Genotypes for Exceptional Human Longevity: The Case of the MTP Gene. J Gerontol A Biol Sci Med Sci. 2012 Apr 10. PMID: 22496539

Conneely KN, Capell BC, Erdos MR, Sebastiani P, Solovieff N, Swift AJ, Baldwin CT, Budagov T, Barzilai N, Atzmon G, Puca AA, Perls TT, Geesaman BJ, Boehnke M, Collins FS. Human longevity and common variations in the LMNA gene: a meta-analysis. Aging Cell. 2012 Feb 16. PMID: 22340368

Rajpathak SN, Liu Y, Ben-David O, Reddy S, Atzmon G, Crandall J, Barzilai N. Lifestyle Factors of People with Exceptional Longevity. J Am Geriatr Soc. 2011 Aug 3. PMID: 2181276

RENATA A. BATISTA-BRITO, Ph.D.

The Batista-Brito lab investigates how inhibitory circuits shape sensory representation and perception at critical developmental ages. We use a powerful combination of methods, including single-cell genetic profiling, mouse genetics, cell-type specific manipulation of neuronal activity, *in vivo* electrophysiology, *in vivo* 2-photon imaging, and behavioral analysis, in order to functionally dissect the developmental impact of specific sources of inhibition on cortical processing and perception. This research illuminates how cortical functions are altered in neurodevelopmental disorders, with particular focus on schizophrenia and autism.

We investigate how postnatal development of inhibitory function shapes the way sensory information is processed in the brain in the context of health and disease. Perception depends on the adaptive function of brain areas comprised of many types of cells and synaptic connections that develop on a long timescale. During development, neural networks must grow from a state of zero connectivity to the precisely interconnected circuits characteristic of the adult brain. The activity of GABAergic inhibitory neurons during postnatal development is likely to mediate synaptic refinement, reducing synchrony and enhancing precision in the mature network. Accordingly, dysregulation of GABAergic interneurons has been linked to several neurodevelopmental disorders. Addressing these questions will identify key developmental processes, elucidate fundamental mechanisms by which sensory information guides behavior, and potentially provide new biomarkers for neuropsychiatric diseases.

We investigate the mechanisms by which contextual modulations are implemented within local cortical circuits and impact behavior. Contextual influences of global behavioral/arousal state (e.g. how alert am I?), sensory predictions (e.g. which stimuli do I expect?), and top-down attention (what is relevant to me?) are implemented throughout sensory cortices and have massive impact on perception. Such contextual influences are mediated by excitatory and inhibitory local circuits, however the specific nature of those circuits remain largely unknown. In this line of research we ask the basic science question - what are the essential neuron classes responsible for translating neuromodulatory signals into changes in sensory processing and perception? We hope that investigating the circuit mechanisms of contextual modulation operations, we will not only shift current research paradigms by opening new avenues for studying the role of inhibition in sensory behavior, but will also enhance our understanding of how mutations in GABAergic inhibitory neurons and alterations in neuromodulatory signaling lead to specific deficits of information processing in neuropsychiatric diseases.

Papers:
1. Batista-Brito R, Vinck M, Ferguson KA, Chang JT, Laubender D, Lur G, Mossner JM, Hernandez VG, Ramakrishnan C, Deisseroth K, Higley MJ, Cardin JA. Developmental dysfunction of VIP interneurons impairs cortical circuits. ***Neuron***, 2017, 95(4):884-895. PMCID: PMC5595250.
2. Batista-Brito R, Mujandar A, Nuno A, Cardin JA. Developmental loss of EbbB4 in PV interneurons disrupts state-dependent cortical circuit dynamics. *BioRxiv*, 2020. doi: 10.1101/2020.12.09.418590. *Under* evaluation in *Molecular Psychiatry*.

3. Mayer C, Hafemeister C, Bandler RC, Machold R, **Batista Brito R**, Jaglin X, Allaway K, Butler A, Fishell G, Satija R. Developmental diversification of cortical inhibitory interneurons. ***Nature.*** 2018 Mar 22; 555(7697):457-462. PMID: 29513653.

4. Vormstein-Schneider DC, Lin JD, Pelkey KA, Chittajallu R, Guo B, Arias Garcia M, Sakopoulos S, Stevenson O, Schneider G, Zhang Q, Sharma J, Franken TP, Smith J, Vogel I, Sanchez V, Ibrahim LA, Burbridge T, Favuzzi E, Saldi GA, Xu Q, Guo L, Yuan X, Zaghloul KA, Sabri E, Goldberg EM, Devinsky O, **Batista-Brito R**, Reynolds J, Feng G, Fu Z, McBain CJ, Fishell GJ, Dimidschstein J. Viral manipulation of functionally distinct neurons from mice to humans. ***Nature Neuroscience***, 2020, *23*, 1628-1636.

5. Mossner J, **Batista-Brito R**, Pant R, Cardin JA. Developmental loss of MeCP2 from VIP interneurons impairs cortical function and behavior. ***eLife***, 2020; Apr 28;9:e55639. PMID: 32343226.

6. Munguba H, Chattopadhyaya B, Nilsson S, Carriço JN, Memic F, Oberst P, **Batista-Brito R**, Munoz-Manchado AB, Wegner M, Fishell G, Di Cristo G, Hjerling-Leffler. Postnatal Sox6 regulates synaptic function of cortical parvalbumin-expressing neurons. ***Journal Neuroscience***, 2021 Sep 9:JN-RM-0021-21. doi: 10.1523/JNEUROSCI.0021-21.2021. Online ahead of print.PMID: 34503995

7. Ratliff J, & **Batista-Brito, R**. The interneuron class struggle. ***Cell***, 2020, *183*(4), 845–847.

8. Batista-Brito R, Fishell G. Interneurons: Learning on the Job. ***Neuron.*** 2019, 102(5):905-907. PMCID: PMID:31170396

9. Batista-Brito R, Vinck M, Zagha E, Ratliff J. Top down modulation of cortical circuits in health and disease. ***Current Opinion in Neurobiology***, issue 52. 2018.

For full paper list see:
http://batista-britolab.com/index.php/papers-2/

HANNES E. BUELOW, Ph.D.

Genetics of Nervous System Development and Function

My lab uses the small nematode C. elegans with its simple and well characterized nervous system as a genetic model. We are trying to understand how a nervous system forms and how it functions. Specifically, we are interested in how intercellular communication regulates how growing axons and dendrites navigate to connect to their partners and be appropriately patterned. Second, we are investigating how an animal's experience affects connectivity, i.e. how experience impinges on hardwiring of the nervous system and the molecules that mediate these processes.

In the first project we are focused on understanding the development of dendrites of somatosensory neurons. These dendrites, which resemble menorah-like candelabras are patterned by a novel pathway we have discovered and named the Menorin pathway. It comprises conserved genes that either promote or restrict the growth of dendrites both cell-autonomously and non-cell-autonomously. Since many neuropsychiatric conditions are characterized by changes in neuronal connectivity and patterning, we believe that our work in *C. elegans* could lay the foundation for understanding these processes in humans as well – as the worm has done so many times! In a second project, we are investigating how an animal's experience can influence and change neuronal circuits. We have developed methods to visualize cell-specific synaptic connections and are now using a combination of genetic, behavioral, and imaging approaches to test how specific connections change in response to the environment. We have discovered that specific insulin-like molecules coordinate this rewiring of the nervous system in response to experience. Since, again, insulin-like peptides and their receptors are expressed in the human brain in highly specific spatial patterns (of unknown significance), we anticipate that our work will lay the foundation to understand basic principles of how insulin-like peptide function in the brain. In a final project, we are studying the role of extracellular sugars such as heparan sulfate (HS) proteoglycans in development and disease. We believe that the molecular diversity of these sugars provides information and generates a molecular map that helps shape the nervous system and intercellular communication in other organs as well. Our goal is to decipher the information contained in HS, determine the factors that create and modulate it and describe the genes that respond to it. To this end, we use both worm models and, in a collaboration with Drs Steidl (Cell Biology) and Almo (Biochemistry) the mouse hematopoietic system.

Selected Recent Publications:
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ALES CVEKL, Ph.D.

Genetic and Epigenetic Regulatory Mechanisms in Mammalian Eye Development and Diseases

We are studying mammalian eye as a model system to elucidate basic molecular mechanisms of tissue-specific gene expression during cell type specification, determination and differentiation. Gene control is regulated a) at the level of *cis*-regulatory grammar of promoters and enhancers mediated by sequence-specific DNA-binding transcriptional factors (TFs), b) at the level of *cis*-sites in 5'- and 3'-UTRs in mRNAs mediated by RNA-binding proteins (RBPs) and microRNAs, and c) 3D-organization of chromatin regulated by promoter-enhancer interactions and formation of topologically associated domains (TADs) involving DNA-binding protein CTCF and cohesin complex. We use mouse loss-of-function, transgenic models, and engineered proteins as well as human eye organoids differentiated from ES/iPS cells toward the lens, retina, and complex eye structures.

PAX6 encodes a sequence-specific DNA-binding TF that plays multiple pivotal roles in the earliest stages of lens and retinal development. Mutations in PAX6 cause aniridia, characterized by the absence of iris, as well as early onset cataract, corneal abnormalities, foveal hypoplasia, and glaucoma. Genetic data of the microphthalmia-anophthalmia-coloboma (MAC) human syndrome identified prominent roles of TFs PAX6 and SOX2 and retinoic acid (RA) signaling. Pax6 directly regulates expression of DNA-binding TFs c-Maf and Prox1. Together, these genes form gene regulatory networks (GRNs) comprised of multiple feed-forward loops governing crystallin gene expression in the lens. These GRNs represent excellent models to study principles of tissue morphogenesis. Tissue-specific transcription of lens crystallin genes is the highest compared to other mammalian tissues and only comparable to globin genes in red blood cells. Lens morphology also allows precise spatial and temporal correlation between gene expression and terminal differentiation not found anywhere else. Ongoing studies measure chromatin resident times of Pax6 and c-Maf and generate novel insights into the mechanisms underlying the transcriptional bursts. Pax6 also plays important roles in the formation of brain and pancreas.

We study enhancers via their genomic deletions coupled with analyses of transgenic EGFP reporters. We examine interactions of PAX6 and BAF complexes with chromatin at single molecule resolution using super-resolution microscopy. We employ isogenic human iPS cells carrying both heterozygous and homozygous mutations in PAX6 to generate *in vitro* lens and retinal organoids to elucidate the cellular and molecular mechanisms underlying aniridia. The unbiased multi –omics approaches used in the lab include RNA-seq, scRNA-seq, ATAC-seq, scATAC-seq, ChIP-seq/CUT&RUN, whole-genome DNA methylation, and Hi-C. Nascent transcription/transcriptional bursting, mRNA splicing and transport, crystallin mRNA stability control, and their translational regulation via RBPs are probed via RNA FISH and MCP-MS2 system to visualize mRNAs at single molecule levels. All these projects are highly collaborative and involve computational biology and bioinformatics.

Our interest in age-related ocular diseases is focused on age-related macular degeneration and cataract. Using eye, retinal, retinal pigmented epithelium (RPE) and lens organoids differentiated from human ES/iPS cells we aim to develop human models to understand disease mechanisms through CRISPR-based genome engineering and use these systems for discovery of novel therapeutic interventions.

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MEELAD DAWLATY, Ph.D.

Epigenetic regulation of stem cells, development and cancer

We seek to understand the epigenetic mechanisms governing the biology of stem cells, development and cancer. We study how the DNA methylation and demethylation machineries reshape the epigenome and regulate stem cell biology during development and in disease. We integrate mouse genetics with cellular, molecular, biochemical and bioinformatics approaches to define epigenetic pathways and mechanisms regulating stem cell specification, self-renewal, pluripotency and multipotency. We focus on the Tet family of DNA dioxygenases (Tet1, Tet2 and Tet3) which promote DNA demethylation by converting 5-methylcytosine (5mC) to 5-hdyroxy-methylcytosine (5hmC) and other derivatives. Tet enzymes also partner with chromatin regulatory complexes to promote gene activation and repression independent of their catalytic activity. We study how these dual functions of Tets regulate gene expression, and define their molecular and biological requirements in development and their implications in human diseases like cancer.

- *Embryonic stem cell (ESC) biology:* We study the enzymatic dependent and independent roles of Tets in gene regulation in ESCs and dissect their biological significance in pluripotency and development. We have identified noncatalytic roles of Tet enzymes in partnering with Sin3a and PRC2 for H3K27 modification. This is essential for establishing bivalency at developmental genes and is critical for silencing mesodermal and trophectodermal genes as well as cell cycle progression in ESCs.

- *Neural and hematopoietic lineage specification during development:* We study how Tet enzymes regulate lineage specification and organogenesis during embryogenesis with a focus on the hematopoietic and neural lineages. We have shown that Tets regulate hematopoietic and neural stem cell specification, self-renewal and multipotency.

- *Hematopoietic stem cells (HSCs) and cancer:* We investigate how Tet2, which is commonly mutated in human blood malignancies, regulates HSCs. We have identified enzymatic and nonenzymatic requirements for Tet2 in regulating the myeloid and lymphoid lineages, respectively.

Our work defines novel mechanisms of epigenetic regulation by Tet enzymes and 5hmC in development. It has implications in identifying new markers and targets for stem cell applications and for treatment of diseases.

For more details on our research please visit our lab website: https://www.dawlatylaboratory.com

Selected publications:

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5. Flores JC, Sidoli S, and Dawlaty MM, Tet2 regulates Sin3a recruitment at active enhancers in embryonic stem cells, ***iScience***, June (2023), PMID: 37456851

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WINFRIED EDELMANN, Ph.D.

Genomic Instability and Cancer in Murine Models

The maintenance of genomic integrity in all organisms requires multiple DNA repair pathways that are involved in the processes of DNA replication, repair and recombination. Perturbations in these pathways can lead to increased mutation rates or chromosomal rearrangements that ultimately result in cancer. DNA mismatch repair (MMR) is one of the repair systems that mammalian cells employ to maintain the integrity of its genetic information by correcting mutations that occur during erroneous replication. Mutations in MMR genes are linked to one of the most prevalent human cancer syndromes, Lynch syndrome and a significant number of sporadic colorectal cancers. At the molecular level tumors that develop in these patients display increased genomic mutation rates as indicated by increased mutations at microsatellite repeat sequences (termed microsatellite instability, MSI). MMR in eukaryotes is complex and involves several homologs of the bacterial MutS and MutL proteins. In mammals, the initiation of the repair process requires two complexes formed by three different MutS homologs (MSH): A complex between MSH2-MSH6 for the recognition of single base mismatches and a complex between MSH2-MSH3 for the recognition of insertion/deletions. The repair reaction also requires a complex between the two MutL homologs MLH1 and PMS2 that interacts with the MSH complexes to activate subsequent repair events which include the excision of the mismatch carrying DNA strand and its re-synthesis. In addition to correcting DNA mismatches, the MMR system mediates an apoptotic response to DNA damage and both of these functions are thought to be important for genome maintenance and tumor suppression. We have generated gene targeted mouse lines with inactivating mutations in all the different MutS and MutL homologs, and also in genes that function in the later MMR steps to study their roles in genome maintenance and tumor suppression. In addition, we have generated knock-in mouse lines with missense mutations and conditional knockout mouse lines that inactivate specific MMR functions and/or model mutations found in humans. Our studies indicate that specific MMR functions play distinct roles in maintaining genome stability and that defects in these functions have important consequences for tumorigenesis. These studies have also revealed that MMR proteins play essential roles in class switch recombination and somatic hypermutation during antibody maturation and the control of meiotic recombination in mammals. We are currently studying the functions of MMR in intestinal stem cells (ISCs) and cancer stem cells (CSCs) in preclinical mouse models and how loss of MMR in stem cells affects tumorigenesis and the response of tumors to novel anticancer treatments including immune therapeutic approaches.

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SCOTT W. EMMONS, Ph.D.

Genetic Encoding of Neural Circuits, Connectomics

How complex neural circuits form and how they function are major unsolved problems in neurobiology. We use the nematode *Caenorhabditis elegans* to study these questions at the cellular and molecular levels. Connectivity in the *C. elegans* nervous system is assayed by serial section electron microscopy. *C. elegans* is the only animal species for which the complete nervous system wiring diagram, now available for both male and hermaphrodite adults, is known, providing an unprecedented foundation for *C. elegans* neuroscience research.

The *C. elegans* nervous system is a complex neural network that is genetically specified. To investigate how the patterns of connectivity are encoded in the genome, we make use of transgenes that express fluorescent proteins targeted to specific classes of synapses. We use these synapse-specific labels to identify mutants and genes that affect formation of particular cellular synaptic contacts. We are determining the expression patterns of genes that encode neural cell adhesion proteins in the neural network that governs the mating behavior of the adult male. This class of transmembrane proteins is thought to include the molecular cell labels by which appropriate pre- and post-synaptic cells recognize each other. By correlating the expression of these molecules with connectivity, we hope to uncover the molecular code that determines the wiring diagram of the nervous system.

Visit our websites: http://worms.aecom.yu.edu. http://wormwiring.org

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JOHN M. GREALLY, D.Med., Ph.D.

Medical Genomics

The Greally lab combines interests in both basic science and clinical research focused on the human genome. Our goal is to use genomic information to understand human diseases. Typical disease diagnostics involve understanding sequence variants at coding regions of the genome. Our interest extends beyond the ~3% of the genome encoding genes, and focuses instead on DNA sequence variability at *cis*-regulatory loci, and DNA sequence-independent processes involving reprogramming of transcription and cell fate changes.

To make progress in these areas sometimes requires developing new assays and analytical techniques. Our group has a long track record and ongoing interests in genome-wide assays and software development. Lab members typically combine both wet bench and programming approaches in their projects.

Specific examples of ongoing projects include a cohort study of human T lymphocyte ageing, a study of *de novo* variants in intellectual disability, a project (with the Steidl lab) on clonal haematopoiesis, cellular reprogramming studies of obesogenic endocrine-disrupting chemicals, and stem cell-based systems for studying hepatic fibrosis and normal osteogenesis.

We work in the most diverse county in the US, and include as part of our research mission the goal to provide better health care to Bronx residents through genomic information. The basic science studies supporting this are focused on studying the effects of genetic polymorphism on transcriptional regulation, developing these new approaches on diverse Asian populations in collaboration with Singaporean colleagues. We describe this research program, co-developed with Departmental colleague Dr. Srilakshmi Raj, as Population Epigenetics. We are also involved with a Bronx Cancer Genomics initiative, and are beginning to work with the community to understand how to develop responsible and ethical genomic studies of the Bronx population served by the Montefiore Health System.

Dr. Greally is also a clinical geneticist specializing in dysmorphology, seeing patients at Montefiore Medical Center. His Montefiore transformation initiative is the New York Center for Rare Diseases (NYCRD), involving innovations in genomic diagnostics as well as patient care and therapeutics. He has led the development of clinical software tools like MADSEQ (to identify mosaic aneuploidies from sequencing data) and GenomeDiver (to enhance genomic diagnostics). His clinical goal is to improve rare disease diagnostics in patients of all genetic ancestries.

<u>**Recent Publications:**</u>

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WILLIAM R. JACOBS JR., Ph.D.

Sterilizing Chemotherapies and Immunotherapies against Tuberculosis, Herpes, and Influenza

Tuberculosis:
Tuberculosis (TB) was the single leading infectious cause of death in the world in 2018, causing over 10 million new cases per year and accounting for 1.5 million deaths annually. The onset of the HIV epidemic worsened the TB global health burden leading to increases in incidences, reactivated disease, and the emergence of drug resistance. The worsening problem of TB is surprising because both a vaccine and sterilizing chemotherapy exist to treat this disease. A major reason for the ineffectiveness of these therapies, is TB's ability to persist; persistence is the capacity of *Mycobacterium tuberculosis* (*Mtb*) to survive sterilization in animals and humans. Persistence is also an epigenetic process found in all bacteria and cancer cells. Recently, the Jacobs Lab demonstrated populations of *Mtb* have a subpopulation of *Mtb* cells that are phenotypically resistant to bactericidal antibiotics. They have identified specific transcriptional patterns that regulate phenotypic resistance and developed dual reporter mycobacteriophages to rapidly identify this subpopulation of cells. Moreover, they discovered the addition N-acetylcysteine or Vitamin C to cultures of *Mtb* prevent the formation of persisters and allow for rapid sterilization in the presence of bactericidal drugs. Current efforts are focused on characterizing the mechanisms by which persisters are formed and identifying relevant targets to eliminate these persisters.

Herpes and Influenza:
In collaboration with Dr. Betsy Herold, the Jacobs lab has generated a precise deletion of the gene encoding *gD* of Herpes Simplex Virus (HSV) 2, termed ΔgD-2, that upon immunization in mice elicits sterilizing immunity against challenge with HSV-1 and HSV-2. This unprecedented protection results from the induction of a special type of antibodies that mediate antibody dependent cell mediated killing (ADCK) of herpes infected cells. They have subsequently found that many pathogens do not elicit ADCK antibodies but they hypothesized that by cloning genes encoding important antigens into our herpes viral vector, they could elicit protection against other pathogens such as influenza. Recently, the Jacobs lab generated recombinant ΔgD-2 herpes virus expressing genes encoding flu antigens and demonstrated that we can confer complete protection against the homologous influenza challenge. This proof of principle suggests that by cloning antigens from other pathogens, such as *Mtb*, it is possible to make novel vaccines and elicit ADCK antibodies. Thus, other efforts in the Jacobs lab focus on characterizing the mechanisms by which ADCK antibodies facilitate the collaboration of innate immunity with adaptive immune responses.

Lab Website: http://williamrjacobs.org/

Select Publications:

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ANDREAS JENNY, Ph.D.

A genetic model for Endosomal Microautophagy

Aging-associated diseases are an increasing socio-economic burden despite efforts to improve healthspan. Pathologies that cause degeneration of the nervous system are particularly devastating, and in many cases are associated with decline in proteostasis and lysosomal malfunction. Prime examples are Parkinson and Alzheimer's disease that are characterized by accumulation of insoluble protein aggregates that lead to neuronal decay. Autophagy delivers cytosolic components to lysosomes for degradation and is thus essential for cellular homeostasis and to cope with different stressors. Macroautophagy (MA) can selectively degrade organelles or aggregated proteins, but selective degradation of single proteins has only been described for Chaperone-mediated autophagy (CMA) and endosomal Microautophagy (eMI). These two autophagic pathways, described to date only in mammals, are specific for proteins containing KFERQ-related targeting motifs. In collaboration with the Cuervo lab, we have established a genetic model for eMI in *Drosophila in vivo*. We are thus for the first time able to perform genetic screens for regulatory components of eMI, this only recently identified form of autophagy about which barely anything is known.

Lysosomes are also important regulatory hubs that integrate nutritional signals and participate in lipid metabolism. We have recently characterized *Drosophila* Lamp1, a bona fide homolog of the mammalian LAMP1/2. *Lamp1* deficiency results in an increase in the number of acidic organelles in the fat body, strongly suggesting defects in the regulation of the pH of the endolysosomal system. Furthermore, *Lamp1* mutant larvae have elevated levels of sterols and diacylglycerols, indicating functions of Lamp1 in lipid transport beyond sterols. Significantly, these phenotypes are similar to loss of glucocerebrosidase, the gene causing Gaucher disease and a major risk factor for Parkinson. Indeed, *Lamp1* mutations enhance fly PD models!

Rho kinase and its effector Cmb in spermiogenesis

During development, genetic and molecular programs control the differentiation of various cell types and orchestrate their morphogenetic behaviors to form organs with specific functions. Organogenesis requires the coordination of cell polarity, cellular movement, and cell shape, driven by intercellular signaling and the tissue-specific interpretations of these signals. Traditionally, Rho kinase (Rok) functions as effector of the non-canonical Wnt/Frizzled PCP pathway during gastrulation and neural tube formation. In a systematic, genome-wide screen, we have identified the previously uncharacterized Combover, an intrinsically disordered protein as novel Rok substrate. Significantly, our follow up studies have identified a novel and unanticipated role of Rok and Cmb during spermiogenesis. We currently address how, downstream of Rok, Combover orchestrates the transition between axoneme elongation and sperm individualization by coordinating the actin and microtubule cytoskeletons with the plasma membrane, thus ensuring proper resolution of the syncytial spermatids into functional sperm that are encapsulated by their own plasma membrane. The biomedical significance of sperm individualization is further exemplified by the presence of multiciliate spermatozoa and spermatids with unresorbed cytoplasm in infertile men.

It is our goal to use *Drosophila* as model system to address fundamental questions that are relevant for development and disease in general.

Lab homepage: https://www.jenny-lab.org   **@Jenny_FLYlab**

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GANJAM V. KALPANA, Ph.D.

Molecular Genetic Analysis of INI1/hSNF5 in HIV-1 Replication, HIV-1 latency and Cancer: Single molecule analysis to Organismal studies

INI1/hSNF5/SMARCB1/BAF47 is a component of the chromatin remodeling SWI/SNF complex. This complex influence replication of HIV-1 and SARS Corona Virus-2. *INI1/SMARCB1* was discovered as a HIV-1 IN binding protein and is a tumor suppressor biallelically mutated/deleted in many human cancers. The goal of our laboratory is to determine how INI1 affects viral replication, tumor suppression, and general cellular function.

(i) RNA mimicry of IN-binding Rpt1 domain of INI1 and its Implications for developing novel anti-HIV therapies: Our laboratory is pioneering the studies of INI1 in HIV-1 replication. We have demonstrated that INI1 plays essential riles during HIV-1 late events and during transcription. However, the mechanism by which INI1 mediates these effects on HIV-1 was not clear till recently. By solving the NMR structure of IN-binding INI1 Rpt1 domain and combining this analysis with biochemical and virological assays, we have discovered that this domain structurally mimics TAR RNA. This unprecedented information has led us to develop novel therapeutic strategies to combat HIV-1 replication in both acutely and latently infected cells. Using the structural information of the IN-INI1 binding interface, we have we have generated a stapled peptides from the interface alpha helix and have demonstrated that it potently inhibits HIV-1 replication by selective targeting of IN:INI1 and IN:TAR interactions. Furthermore, by using high-throughput virtual screening we have identified a few small molecules that target IN/INI1 interaction and have established that these molecules are effective in inhibiting HIV-1 replication. The stapled peptide and the small molecules are "first-in-class" inhibitors that target host-virus interactions and can be advantageous as it may be harder for the virus to develop resistance to these molecules.

(ii) Application of a single cell and single molecule RNA-FISH and IF method to study HIV-1 latency and SARS-CoV2 replication: A final hurdle to eradicate HIV-1 is the persistence of the virus in latent reservoirs, which are transcriptionally suppressed and low in number. We have developed a Single Cell Single Molecule Immuno-fluorescence and RNA-FISH assay (SMIRA) in collaboration with Dr. Robert Singer to study latency. This novel assay will be applied to characterize latent reservoirs in various reservoirs in blood, brain, gut etc. and the effect of various Latency Reversing Agents (LRA, that are in clinical trials) and drugs of abuse.

(iv) Mechanism of tumor suppression by *INI1/hSNF5* and developing novel and effective therapeutic strategies to combat INI1-deficient tumors: By using a series of genetic systems developed in our laboratory (knock-out, knock-in mouse models, cell culture models), we are dissecting the mechanism of INI1-mediated tumor suppression and are developing molecularly targeted therapies. Previously we discovered that INI1 harbors a masked nuclear export signal. Recently, in collaboration with international team of neuropathologists, we have found that mutations that affect the regulated nuclear export is a tumorigenic event that it offers a novel therapeutic target. In addition, we are investigating novel downstream targets of INI1, i.e. FoxM1 and GBP1, and their effect on senescence and their therapeutic potential.

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PERI KURSHAN, PH.D.

Towards a molecular understanding of synapse development

Defects in the proper development and function of synapses lead to neurodevelopmental disorders such as Autism and Intellectual Disability, however the molecular mechanisms underlying these processes are still largely unknown. We use the nematode *C. elegans*, which has a simple and stereotyped nervous system, to investigate the conserved molecular mechanisms of synapse development and function. On the developmental side of the lab, we study how presynaptic components including cell adhesion molecules, active zone scaffold proteins, calcium channels and synaptic vesicles arrive at the synapse and form a mature and fully functional presynaptic compartment. The translational side of the lab characterizes conserved patient-derived variants in presynaptic calcium channels by assessing their impact on synaptic function. We combine the power of worm genetics with high resolution imaging and molecular computational modeling to elucidate the role of key synaptic genes and how their function goes awry in disease.

For more information on the lab and current projects visit: www.KurshanLab.org

Selected publications:

1. Frankel EB, Tiroumalechetty A, Su Z, Henry PS, Mueller BD, Jorgensen EM, Wu Y, Kurshan PT. Intracellular protein-lipid interactions drive presynaptic assembly prior to neurexin recruitment. *Neuron* (**2025**) Mar 5; 113, 1– 17.

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HERB LACHMAN, M.D.

Induced pluripotent stem (iPS) cells for disease modeling in schizophrenia and autism

A significant obstacle in studying the molecular basis of schizophrenia (SZ), autism spectrum disorders (ASD) and other neuropsychiatric disorders is the inaccessibility of the human brain, which has restricted molecular studies, such as gene expression profiling and epigenetic analysis, to autopsy samples. While some interesting findings have been made using postmortem brain, interpreting the data is associated with numerous confounding factors. In addition, since SZ is believed to be a developmental disorder, studying molecular events in postmortem samples is limiting. The discovery of iPS cells, which essentially allows investigators to reprogram somatic cells into pluripotent stem cells capable of differentiating into neurons and other cell types, provides an opportunity to create patient-specific neurons *in vitro*. The Lachman lab develops patient-specific iPS cells from controls and patients with SZ who have 22q11.2 deletions, which is found in ~1% of patients. Recently, an iPS cell model has been generated for Lowe Syndrome, a rare X-linked disorder that is associated with learning disability and epilepsy. In addition, an iPS cell model relevant to neuropsychiatric disorders is being generated using CRISPR-Cas9 gene editing targeting the ASD candidate gene CHD8. Gene expression profiling using RNA-seq showed that CHD8 haploinsufficiency leads to an increase in expression of genes involved in GABAergic differentiation in cerebral organoids, a property shared with another ASD candidate gene, FOXG1. CHD8 haploinsufficiency also leads to alterations in WNT/β-catenin signaling. Both GABAergic differentiation and WNT/β-catenin signaling are druggable targets; translating basic science findings into novel drug treatments for SZ and ASD is a major objective of the Lachman lab.

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JACK LENZ, Ph.D.

Molecular Genetics of Viruses in Cancer and Evolution

Viruses have etiological roles in about 12% of human cancers and in many other instances throughout nature. Viral tumorigenesis entails a variety of molecular genetic mechanisms. Some viruses encode genes that evolved to support virus replication that can also drive host cell proliferation, manipulate host cell survival, and evade host immune responses. Others lack genes that directly drive oncogenic processes, but instead cause tumors by insertion of their DNA into the host cell genome resulting in transcriptional activation of host oncogenes that flank viral DNA insertion sites, most commonly by an enhancer mechanism. Some viruses use all of these mechanisms. Current research efforts are focused on insertional oncogenesis by human papillomaviruses (HPVs) in collaboration with Drs. Cristina Montagna and Anne Van Arsdale. Key issues being addressed include identification of host oncogenes targeted by these viruses, mechanisms underlying transcriptional effects on flanking oncogenes, genetic interactions between viral and host oncogenes in tumorigenesis, and mechanisms of virus induced instability in host genomes.

Retrovirus DNA comprises about 8% of the genome. Human endogenous retrovirus K (HERV-K) is the most recent of all the retroviruses to enter the germline DNA of humans that is transmitted from parents to children. All humans are born with about 20 distinct, full-length HERV-K proviruses (i.e. integrated retroviral DNA) in their germline DNA, although all of these have mutations that inactivate their infectivity. We were the first to show that most of these proviruses formed relatively recently in human evolution, long after the divergence of the human and chimpanzee lineages approximately 6 million years ago, including some that formed so recently that they are not yet fixed in the human genome. Current interests are under what circumstances HERV-K proviruses are transcribed, whether human cells can recognize HERV-K transcripts, and whether infectious HERV-K ever regenerates from the proviruses in the human genome today.

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WEI LIU, Ph.D.

Retinal cell differentiation, inherited degenerations, and regeneration

- Elucidate the molecular and cellular mechanisms of retinal cell differentiation using engineered mice.

- Model human retinal cell differentiation and inherited degenerations using stem cell-derived organoids.
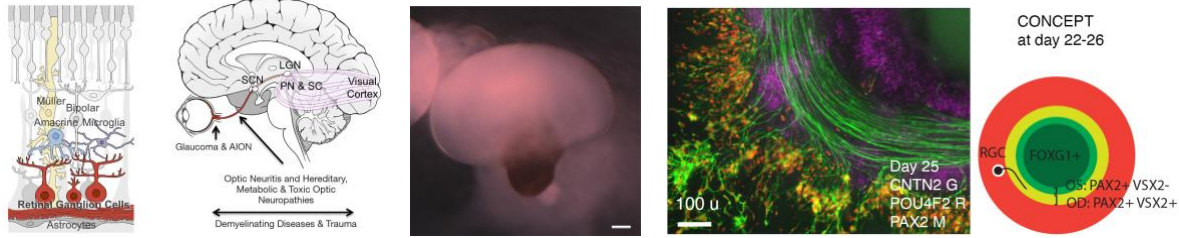
The neuroretina, retinal pigment epithelium (RPE), ciliary body, and iris are structurally and functionally connected in the human adult retina. Inherited degenerations of any tissue affect the others, leading to blinding retinal diseases such as retinitis pigmentosa, age-related macular degeneration, and glaucoma. Macular degenerations affect vision the most, as the macula is responsible for central vision and visual acuity. The human adult neuroretina does not naturally regenerate. Regenerative medicine of the retina holds the promise to save and restore vision.

Elucidating the mechanisms of retinal cell differentiation is a prerequisite for retinal regeneration. Embryonic development of the neuroretina, RPE, ciliary body, and iris is an integrated process regulated by transcription factors and signal transduction pathways. In mice, morphogenesis of optic cups leads to the specification of neuroretinal and RPE progenitor cells in the inner and outer layers of E10.5 optic cups, respectively. The neuroretina is continuous with RPE via tissue bending. Close to the bending region, the peripheral neuroretina gradually reduces its thickness to form a tapered zone, which is specified as the ciliary margin at E12.5. Neuroretinal progenitor cells are multipotent, producing all retinal neurons and Müller glial cells. The ciliary margin differentiates into the ciliary body and iris. How embryonic retinal cells regionalize and differentiate in concert to generate multiple cell types in the functional retina remains unclear. We address the knowledge gap by dissecting the functions of transcription factors, chromatin remodeling proteins, and signaling transduction pathways during retinal development using engineered mice.

The macula is enriched for cone photoreceptors and is unique to primates. The availability and high cost of non-human primates limit their use in retinal disease studies. Macular degenerations are often not closely recapitulated in mouse models because mice do not have the macula. Notably, we recently generated and characterized cone-rich human retinal organoids reminiscent of the macula based on the ratio of cones to rods and single-cell transcriptomes. As a recognition by the field, we recently received an NEI prize for progress toward developing lab-made retinas. We now utilize retinal organoids to model human retinal cell differentiation and inherited degenerations.

We recently generated CONCEPT organoids that exhibit directional axon growth and pathfinding of human retinal ganglion cells (RGCs). Our CONCEPT organoids are the first-in-class organoids in studying early differentiation, axon growth, and pathfinding of human RGCs, making an impact on RGC regeneration and reconnection.

Current projects:

1) Chromatin mapping of transcription factors and chromatin remodeling proteins in retinal cells during development;

2) Molecular characterizations of retinal phenotypes in mutant mice;

3) Protein-protein interactions between transcription factors and chromatin remodeling proteins in development;

4) Integrated studies of single-cell ATAC-seq and single-cell RNA-seq of mutant mouse retinas;

5) Retinal organoid models of human retinal cell differentiation and inherited retinal degenerations;

6) Modeling axon growth and pathfinding of human retinal ganglion cells using CRISPR-edited CONCEPT organoids.

Recent publications

1. **Liu, W.**[¶], Shrestha, R., Lowe, A., Zhang, X., and Spaeth, L. (2023). Self-formation of concentric zones of telencephalic and ocular tissues and directional retinal ganglion cell axons. **Elife** *12*. 10.7554/eLife.87306. PMID:37665325. PMC10476969. [¶]Corresponding author.
2. **Liu W.** METHODS FOR THE GENERATION OF HUMAN RETINAL GANGLION CELLS AND COMPOSITIONS, ASSAYS, DEVICES, AND KITS COMPRISING SAME. **International PCT Application** PCT/US22/41843.
3. Ferrena, A., Zhang, X., Shrestha, R., Zheng, D., and **Liu, W.** (2024). Six3 and Six6 jointly control diverse target genes in multiple cell populations over developmental trajectories of mouse embryonic retinal progenitor cells. **PLoS One** *19*, e0308839. 10.1371/journal.pone.0308839.
4. Kim S, Lowe A, Dharmat R…Zhou Z, Chen R, **Liu W** (2019). **Proc Natl Acad Sci U S A**, 116(22):10824-10833.
5. Diacou R, Zhao Y, Zheng D, Cvekl A, **Liu W** (2018). **Cell Reports**, 25: 2510-2523.
6. **Liu W**[¶], Cvekl A (2017). **Dev Biol**, 428(1):164-175. [¶] Corresponding author.
7. Lowe A, Harris R, Bhansali P, Cvekl A, **Liu W** (2016). **Stem Cell Reports**, 6(5):743-756.
8. **Liu W**, Lagutin O, Swindell E, Jamrich M, Oliver G (2010). **J of Clin Invest**,120: 3568-77

SRIDHAR MANI, M.D.

Host-Microbial genetics, orphan nuclear receptors, metabolites, and disease

Pregnane X Receptor (PXR) [a.k.the Steroid and Xenobiotic Receptor (SXR)] is a master nuclear receptor regulator of host xenobiotic/endobiotic metabolism, detoxification, and inflammation. More recently, we have shown that the receptor plays a major role in relaying host microbial signals in the intestines with innate immunity. Specifically, we have shown that microbial metabolites of L-tryptophan, indoles, and indole propionates, activate PXR in intestinal epithelial cells to promote intestinal immune homeostasis via a TLR4 specific pathway[*]. This discovery has led our laboratory into new directions primarily focused on molecular mechanisms governing host-microbial and microbial-microbial relationships in the intestines (similar approaches could be used elsewhere or for other physiologic-pathophysiologic conditions).

1.   Discovery of endobiotic PXR ligands and use of microbial metabolite mimicry to design drugs combating intestinal inflammation and cancer. Here we are investigating how different microbial metabolites bind and activate and/or antagonize PXR function in the intestines with the hopes of establishing a physiologic role for microbial metabolites in mammals. In parallel, we have embarked on chemical biological approaches to discovery of indole propionate analogs as potent PXR ligand agonists, with the eventual hope of designing small molecule drugs (microbial metabolite mimicry) combating intestinal inflammation and inflammation-induced cancer[**]. More recently, the role for indole metabolites connects the gut microbes to neuronal function partly via PXR[***]. We are also interested in covering all other human receptors in regard to microbial metabolite effects.

2.   Discovery of new (novel) microbes and mechanisms governing its regulation of innate immunity. Here we are interested in deciphering molecular mechanisms of indole metabolites as well as small molecule indole mimics as they interact with the host microbiome (e.g., biofilms, drug resistance etc.) in conditions of homeostasis and intestinal stress. These types of investigations have led to the identification of a novel bacterial strain with a unique community phenotype that alters intestinal inflammation. We have diversified our interests to the study of how and why these novel bacterial strains arise during inflammation, what regulates their swarming behavior, and how they execute a phenotype in mice[$]. Finally, our goal is to derive probiotic approaches, harnessing our internal microbiome, for the treatment of a variety of health conditions. These projects have led to multidisciplinary approaches of designing probiotic drug delivery systems using physics of bacterial spreading, molecular microbiology, and host biology[$$]. We are also interested in bar coded recording of transcriptional events in probiotics and pathogens.

<u>**Selected Publications:**</u>

[*] Venkatesh M, Mukherjee S, Wang H, Li H, Sun K, Benechet AP, Qiu Z, Maher L, Redinbo MR, Phillips RS, Fleet JC, Kortagere S, Mukherjee P, Fasano A, Le Ven J, Nicholson JK, Dumas ME, Khanna KM, <u>Mani S</u>. Symbiotic Bacterial Metabolites Regulate Gastrointestinal Barrier Function via the Xenobiotic Sensor PXR and Toll-like Receptor 4. Immunity 41(2): 296-310 (2014**)**

[**] Dvorak Z et al (40 authors), <u>Mani S*.</u> Targeting the Pregnane X Receptor Using Microbial Metabolite Mimicry. EMBO Molecular Medicine (Cover Page Citation) 12(4):e11621(2020)

[**] Dvorak Z, Sokol H, <u>Mani S</u>. Drug Mimicry: Promiscuous Receptors PXR and AhR, and Microbial Metabolite Interactions in the Intestine. Trends Pharm Sci (Cover Page Citation) 41(12): 900-908 (2020)

[***] Serger E et al (primary work from the Giovanni lab, Imperial College, London). The gut metabolite indole-3 propionate promotes nerve regeneration and repair. Nature Jun 22 doi:10.1038/s41586-022-04884-x (online ahead of print 2022)

[$] De A, Chen W, Li H, Wright JR, Lamendella R, Lukin DJ, Szymczak WA, Sun K, Kelly L, Ghosh S, Kearns DB, He Z, Jobin C, Luo X, Byju A, Chatterjee S, San Yeoh B, Vijay-Kumar M, Tang JX, Prajapati M, Bartnikas TB, Mani S. Bacterial Swarmers Enriched During Intestinal Stress Ameliorate Damage. Gastroenterology 161(1):211-224. doi: 10.1053/j.gastro.2021.03.017 (2021)

[$$] Chen W, Mani N, Karani H, Li H, <u>Mani S</u>, Tang JX. Confinement discerns swarmers from planktonic bacteria. Elife 10:e64176 (2021)

Ondrová K, Zůvalová I, Vyhlídalová B, Krasulová K, Miková E, Vrzal R, Nádvorník P, Nepal B, Kortagere S, Kopečná M, Kopečný D, Šebela M, Rastinejad F, Pu H, Soural M, Rolfes KM, Haarmann-Stemmann T, Li H, <u>Mani S*</u>, Dvořák Z*. Monoterpenoid aryl hydrocarbon receptor allosteric antagonists protect against ultraviolet skin damage in female mice. Nat Commun. 2023 May 11;14(1):2728. doi: 10.1038/s41467-023-38478-6.

SOFIYA MILMAN, M.D., M.Sc.

Genetic Regulation of Endocrine Axes in Human Aging and Longevity

Our research focuses on a unique population of centenarians and their families who are generally free of age-related diseases. Healthy longevity runs in most of these families, suggesting a heritable basis for this phenomenon. Our team conducts translational research focused on the discovery of genomic mechanisms that regulate endocrine and metabolic pathways that protect against common age-related diseases, like diabetes, cardiovascular disease and Alzheimer's disease.

Many of the discovered longevity genes and phenotypes are related to hormonal pathways. Changes in most hormones are observed throughout the human lifespan, but it remains unknown whether the observed changes cause aging, are associated with age but are not the cause of aging, or are protective for the aging body. Identification of protective endocrine parameters will inform our understanding of the mechanisms of healthy aging and lead to the discovery of interventions that protect from age-related diseases.

A biological pathway that has been repeatedly implicated in aging is the somatotropic axis that involves signaling via growth hormone and insulin-like growth factor-I (IGF-I). Substantial evidence exists that diminished signaling via this pathway delays aging, resulting in longer lifespan and health-span, not only in animal models, but also in humans. We demonstrated that lower IGF-I levels were associated with longer survival, better cognitive function, and protection from multi-morbidity in older adults and people with exceptional longevity. Our team uses computational approaches to integrate genetic data with rich phenotypic data in families with inherited longevity to understand the genetic and molecular mechanisms that lead to protective effects of low IGF-I.

<u>**Selected Publications:**</u>

1. Aleksic S, Desai D, Ye K, Duran S, Gao T, Crandall J, Atzmon G, Barzilai N, Milman S. Integrity of Hypothalamic-Pituitary-Testicular Axis in Exceptional Longevity. *Aging Cell* 2022, *in press*

2. Lin JR, Sin-Chan P, Napolioni V, Torres GG, Mitra J, Zhang Q, Jabalameli MR,Wang Z, Nguyen N, Gao T, Regeneron Genetics Center, Laudes M, Görg S, Franke A, Nebel A, Greicius MD, Atzmon G, Ye K, Gorbunova V, Ladiges WC, Shuldiner A, Niedernhofer LJ, Robbins PD, Milman S, Suh Y, Vijg J, Barzilai N, Zhang ZD. Rare genetic coding variants associated with human longevity and protection against age-related diseases. *Nat Aging* 2021; *in press*

3. Zhang WB, Ye K, Barzilai N, Milman S. The antagonistic pleiotropy of insulin-like growth factor 1. *Aging Cell* 2021; 20(9), e13443; PMCID: PMC8441393

4. Gutman D, Lidzbarsky G, Milman S, Gao T, Sin-Chan P, Gonzaga-Jauregui C, Regeneron Genetics Center, Deelen J, Shuldiner AR, Barzilai N, Atzmon G. Similar burden of pathogenic coding variants in extremely long-lived individuals and individuals without exceptional longevity. *Aging Cell* 2020*, in press*

5. Zhang ZD, Milman S, Lin J, Barzilai N, Gorbunova V, Ladiges WC, Niedernhofer LF, Suh Y, Robbins PD, Vijg J. Genetics of extreme human longevity to guide drug discovery for healthy ageing. *Nat Metab* 2020*, in press*

6. Zhang WB, Aleksic S, Gao T, Weiss EF, Demetriou E, Verghese J, Holtzer R, Barzilai N, Milman S. Insulin-like Growth Factor-1 and IGF Binding Proteins Predict All-Cause Mortality and Morbidity in Older Adults. *Cells* 2020; 9(6):E1368

7. Lehallier B, Gate D, Schaum N, Nanasi T, Lee SE, Yousef H, Moran Losada P, Berdnik D, Keller A, Verghese J, Sathyan S, Franceschi C, Milman S, Barzilai N, Wyss-Coray T. Undulating changes in human plasma proteome profiles across the lifespan. *Nat Med* 2019; 25(12):1843-1850

8. Gubbi S, Quipildor GF, Barzilai N, Huffman DM, Milman S. 40 Years of IGF-1: IGF-1: the Jekyll and Hyde of the aging brain. *J Mol Endocrinol* 2018; 61(1):T171-T185. PMCID: PMC598899

9. Gubbi S, Schwartz E, Crandall JP, Verghese J, Holtzer R, Atzmon G, Braunstein R, Barzilai N, Milman S. Effect of Exceptional Parental Longevity and Lifestyle Factors on Prevalence of Cardiovascular Disease in Offspring. *Am J Cardiol* 2017; 120(12):2170-2175. PMCID: PMC5698168

10. Milman S, Huffman DM, Barzilai N. The Somatotropic Axis in Human Aging: Framework for the Current State of Knowledge and Future Research. *Cell Metab* 2016; 23(6):980-9. PMCID: PMC4919980

11. Milman S, Atzmon G, Huffman DM, Wan J, Crandall JP, Cohen P, Barzilai N. Low Insulin-like Growth Factor-1 Level Predicts Survival in Humans with Exceptional Longevity. *Aging Cell* 2014; 13(4):769-71

CRISTINA MONTAGNA, Ph.D.

Genetic, epigenetic and ploidy changes during cell differentiation in development and disease.

Project 1- Role of Septin 9 in Breast Carcinogenesis.
A comparative cytogenetic approach aimed to identify recurrent DNA copy number variations in a panel of murine models for breast cancer resulted in the identification of Septin 9 (Sept9) as potential novel oncogene. The septin family of genes codes for a highly redundant and conserved family of GTP-binding proteins that assemble into filaments and bind to microfilaments and microtubules. At the locus of genomic amplification deregulation of Sept9 expression occurs by a complex pattern of genetic and epigenetic alterations affecting several Sept9 isoform variants. Our hypothesis is that during malignant transformation, breast epithelial cells undergo genomic amplification of the Sept9 locus and over-express Sept9 mRNA and protein. Additionally, aberrant cytosine methylation occurs at specific alternative promoters within the Sept9 locus resulting in an abnormal pattern of Sept9 isoform variants. We are currently studying how the expression of various Sept9 isoforms is regulated in normal and cancer cells and the functional differences between these isoforms.

Project 2- Stage- and Cell Subtype-Specific Epigenetic Regulation of Mammary Gland Development and breast tumorigenesis.
We are interested in investigating the DNA methylation changes occurring in the development of the normal mammary gland during puberty, adult age, pregnant, lactating and undergoing mammary gland involution. This approach has the final goal of dissecting the molecular processes that mediate methylation changes in the morphogenesis and differentiation of the normal breast and to identify "hot spot" loci for gene silencing in breast carcinogenesis.

Project 3- Aneuploidy in aging.
Polyploidy and aneuploidy are the most frequent cytogenetic events observed in mammalian cells. Polyploidization is a widely accepted mechanism for increasing genetic variation in unicellular organisms and for the acquisition of new properties in a variety of cell types (e.g., osteoclast fusion in bone resorption and myoblast fusion in muscle development) and is considered a physiological process. Aneuploidy on the contrary is linked to pathological states. It is a hallmark of spontaneous abortions and birth defects and is observed virtually in every human tumor. While the catastrophic consequence of high levels of aneuploidy observed in abortions is self-explanatory, the role of aneuploidy under physiological conditions is a question waiting for answers. The major goal of this project is to explore a possible correlation between age-associated genome instability in a variety of tissues and functionality of these cells.

Recent Publications:

Acosta, D., M. Suzuki, D. Connolly, R. F. Thompson, M. J. Fazzari, J. M. Greally and C. Montagna (2011). "DNA methylation changes in murine breast adenocarcinomas allow the identification of candidate genes for human breast carcinogenesis." Mammalian genome : official journal of the International Mammalian Genome Society 22(3-4): 249-259.

Connolly, D., I. Abdesselam, P. Verdier-Pinard and C. Montagna (2011). "Septin roles in tumorigenesis." Biological chemistry 392(8-9): 725-738.

Connolly, D., Z. Yang, M. Castaldi, N. Simmons, M. H. Oktay, S. Coniglio, M. J. Fazzari, P. Verdier-Pinard and C. Montagna (2011). "Septin 9 isoform expression, localization and epigenetic changes during human and mouse breast cancer progression." Breast cancer research: BCR 13(4): R76.

Downing, T. E., M. H. Oktay, M. J. Fazzari and C. Montagna (2010). "Prognostic and predictive value of 16p12.1 and 16q22.1 copy number changes in human breast cancer." Cancer genetics and cytogenetics 198(1): 52-61.

Faggioli, F., M. G. Sacco, L. Susani, C. Montagna and P. Vezzoni (2008). "Cell fusion is a physiological process in mouse liver." Hepatology 48(5): 1655-1664.

Faggioli, F., P. Vezzoni and C. Montagna (2011). "Single-cell analysis of ploidy and centrosomes underscores the peculiarity of normal hepatocytes." PloS one 6(10): e26080.

Faggioli, F., J. Vijg and C. Montagna (2011). "Chromosomal aneuploidy in the aging brain." Mechanisms of ageing and development 132(8-9): 429-436.

Weaver, B. A., A. D. Silk, C. Montagna, P. Verdier-Pinard and D. W. Cleveland (2007). "Aneuploidy acts both oncogenically and as a tumor suppressor." Cancer Cell 11(1): 25-36.

BERNICE E. MORROW, Ph.D.

Understanding genetic risk factors for birth defects on a single cell level

Our lab is interested in discovering genes required for human embryonic development to understand the cause of birth defects. Our research begins with collecting DNA samples from affected individuals with genetic disorders having known chromosomal gains or losses, and moves to looking at gene function in vertebrate model organisms. The reason for studying chromosomal disorders is that affected regions in the genome will pinpoint the location of causative genes whose function in organogenesis is sensitive to copy number.

Our main focus is on a disorder termed chromosome 22q11.2 deletion syndrome (22q11.2DS). Most affected individuals have a similar sized 3 million base pair (Mb) deletion encompassing 60 genes. The deletion occurs by a mistake during meiosis in forming the egg or sperm. Individuals with the syndrome have learning disabilities, psychiatric illness, cleft palate, hearing loss and cardiovascular defects. Many of these defects occur commonly in the general population in non-syndromic forms. This is why molecular genetic studies of this syndrome are particularly relevant to human health and disease.

One key gene in the 22q11.2 region is termed *TBX1* and it encodes a transcription factor that is responsible for many of the defects in patients with the syndrome. Using knockout and gain-of-function mutant mice, we have made headway to understand its function. Since it's a transcription factor, we are interested in genes it can regulate. Part of our mission is to understand the role of *Tbx1* in making cell fate decisions in mammalian embryos. We are doing this by taking single cell RNA-sequencing, chromatin accessibility and chromatin immunoprecipitation followed by genome sequencing from microdissected tissues from wildtype and mutant embryos followed by bioinformatics analysis.

Although most individuals with 22q11.2DS have the same sized deletion, the severity of malformations varies dramatically. For example, 60% have heart defects, many requiring surgery, while the rest have a normal heart. We hypothesize that the 22q11.2 deletion is the first hit in the genome and it uncovers other mutations that act as second hits to modify the overall phenotype of the disorder. We are taking candidate gene and unbiased whole genome sequencing approaches to identify genetic "modifiers" in subjects with 22q11DS. We are identifying common and rare, copy number and single nucleotide variants. In order to interpret the genomic data, we are taking systems biology approaches. In this way, we will extract biologically important gene networks in a holistic sense. At the same time, genes in the networks will be tested for functional significance in mouse models.

Recent Publications:

Nomaru H, Liu Y, De Bono C, Righelli D, Cirino A, Wang W, Song H, Racedo SE, Dantas AG, Zhang L, Cai CL, Angelini C, Christiaen L, Kelly RG, Baldini A, Zheng D, Morrow BE. Single cell multi-omic analysis identifies a *Tbx1*-dependent multilineage primed population in murine cardiopharyngeal mesoderm. Nat Commun. 2021 Nov 17;12(1):6645. doi: 10.1038/s41467-021-26966-6. PMID: 34789765; PMCID: PMC8599455

Song H, Morrow BE. *Tbx2* and *Tbx3* regulate cell fate progression of the otic vesicle for inner ear development. Dev Biol. 2022 Dec 12;494:71-84. doi: 10.1016/j.ydbio.2022.12.003. Epub ahead of print. PMID: 36521641.

Yingjie Zhao, Yujue Wang, Lijie Shi, Donna M. McDonald-McGinn, T. Blaine Crowley, Daniel E. McGinn, Oanh T. Tran, Daniella Miller, Elaine Zackai, H. Richard Johnston, Eva W. C. Chow, Jacob A.S. Vorstman, Claudia Vingerhoets, Therese van Amelsvoort, Doron Gothelf, Ann Swillen, Jeroen Breckpot, Joris R. Vermeesch, Stephan Eliez, Maude Schneider, Marianne B.M. van den Bree, Michael J. Owen, Wendy R. Kates, Gabriela M. Repetto, Vandana Shashi, Kelly Schoch, Carrie E. Bearden, M. Cristina Digilio, Marta Unolt, Carolina Putotto, Bruno Marino, Maria Pontillo, Marco Armando, Stefano Vicari, Kathleen Angkustsiri, Linda Campbell, Tiffany Busa, Damian Heine-Suñer, Kieran C. Murphy, Declan Murphy, Sixto García-Miñaúr, Luis Fernández, International 22q11.2 Brain and Behavior Consortium, Elizabeth Goldmuntz, Raquel E. Gur, Beverly S. Emanuel, Deyou Zheng, Christian R. Marshall, Anne S. Bassett, Tao Wang, Bernice E. Morrow. Chromatin regulators in the *TBX1* network confer risk for conotruncal heart defects in 22q11.2DS and sporadic congenital heart disease. NPJ Genome Medicine, In press.

De Bono C, Liu Y, Ferrena A, Valentine A, Zheng D, Morrow BE. Single-cell transcriptomics uncovers a non-autonomous *Tbx1*-dependent genetic program controlling cardiac neural crest cell development. Nat Commun. 2023 Mar 21;14(1):1551. doi: 10.1038/s41467-023-37015-9. PMID: 36941249; PMCID: PMC10027855.

XIAO PENG, M.D./Ph.D.

Translating the mechanistic understanding of immune and inflammatory diseases into patient outcomes

Dr. Peng's translational research is a synthesis of her training in basic science, interest in evolutionary biology, and daily motivation to provide patients with holistic, thoughtful, and personalized clinical care. She advises providers at Montefiore Einstein and around the world on diagnosis and management of complex conditions, particularly those involving suspected blood and immune drivers. She also spearheads many institutional endeavors on the clinical, educational, and research fronts to deliver high-yield, longitudinal, molecular medicine-informed patient care.

She recently joined the Montefiore faculty and co-directs the New York Center for Rare Diseases (NYCRD) with Dr. John Greally, with a focus on leading infrastructure, tool and resource development for culturally sensitive, ancestry-conscious undiagnosed disease discovery. Her combined training in the study of Central Dogma processes, developmental gene regulation, immunity and inflammation, and human genetics and genomics enabled her to found and direct the Genetics of Blood and Immunity (GBI) Service, formerly at Johns Hopkins and now at Montefiore, one of the only ones of its kind in the US and the world to personalize disease management via the integrated interpretation of state-of-the art comprehensive immunophenotyping and genotyping data.

Dr. Peng's PhD was focused on the Smc5/6 complex, an anciently conserved protein complex that lies at the interface between DNA replication/repair, transcriptional regulation and post-translational modifications, specifically SUMOylation and ubiquitination. Dr. Peng showed how this complex and its collaborating proteins contribute important roles to replication-associated repair for defense against endogenous genotoxic stress, but interestingly, it has since also been found to play important roles in cell-intrinsic immune defenses against a variety of viruses.

Specific examples of ongoing projects include:
- Development of novel approaches to clinical phenotype extraction and encoding, raw sequencing data processing and analysis through leadership of the NYCRD Advanced Diagnostics Team
- Genotype- and phenotype-driven approaches to novel gene-disease discovery for conditions arising from mutations in genes that have roles in cell-intrinsic genome defenses, using novel genomics strategies in combination with functional studies in yeast and humans
- Discovery of immune and molecular endotypes within heterogeneous clinical diagnostic categories still poorly understood in terms of genetic architectures and pathobiological subtypes that may inform therapeutic choice, such as juvenile idiopathic arthritis (JIA) and autoimmune hepatitis/primary sclerosing cholangitis (AIH/PSC)
- Detailed characterization of under-recognized immune and blood complications of syndromic Mendelian diseases, particularly disorders of gene regulation and metabolism, through longitudinal immune profiling and elucidation of clinical and cellular responses to therapies
- Identification and addressing of gaps in the current understanding of fertility and pregnancy issues for individuals with known or suspected GBIs. We are gathering information on current practices, pain points and concerns from population health databases, patients and providers, gathering quantitative and qualitative natural history data to address the issues identified above, and developing educational programs and multi-disciplinary guidelines from this data for dissemination to the greater community
- Many Ataxia Telangiectasia (A-T)-related projects from using AI/ML to correspond neuropathology findings from autopsy specimens to pathobiological mechanisms to longitudinal natural history studies and early clinical biomarker discovery to the development of therapeutic screening systems and gene therapies

Dr. Peng also has a long track record of bringing disparate groups to the table and leading community and consortium efforts to improve the sharing, collection, harmonization and synthesis of rare disease data for improved natural history and therapeutic studies. She is a co-founder and co-lead of the GenIA (Genetic Immunology Advisor) database. She serves as Research and UNHIDE Program Co-Chair for the NICER Consortium, as Antibody Deficiency VCEP Co-Chair for the ClinGen Immunology CDWG, and as Co-Chair for the Diagnostic Immune Lab Committee, Prenatal and Reproductive Investigations Task Force, and Newborn Screening by Genome Sequencing Task Force of the Clinical Immunological Society. She is also the geneticist for the international A-T community and the Genetic Disorders of Mucociliary Clearance Consortium.

Select Publications:
1. Caballero-Oteyza A, Crisponi L*, Peng XP*, Yauy K, Volpi S, Giardino S, Freeman AF, Grimbacher B, Proietti M. GenIA, the Genetic Immunology Advisor database for Inborn Errors of Immunity. *J Allergy Clin Immunol.* 2023. 2023 Nov 29:S0091-6749(23)01513-0. doi: https://doi.org/10.1016/ j.jaci.2023.11.022.
2. Peng XP, Zhao XL. The multi-functional Smc5/6 complex in genome protection and diseases. Review. *Nat Struct Mol Biol.* 2023 Jun;30(6):724-734. doi: 10.1038/s41594-023-01015-6. Epub 2023 Jun 19.
3. Peng XP, Caballero-Oteyza A, Grimbacher B. Common Variable Immunodeficiency: More Pathways than Roads to Rome. *Annu Rev Pathol.* 2023 Jan 24;18:283-310. doi: 10.1146/annurev-pathmechdis-031521-024229. Epub 2022 Oct 20.
4. Rivière JG, Saba RC, Carot-Sans G, Piera-Jiménez J, Butte MJ, Soler-Palacín P, Peng XP. Current perspectives and challenges of using AI in immunodeficiencies. J Allergy Clin Immunol. 2025 Jun 28:S0091-6749(25)00691-8. doi: 10.1016/j.jaci.2025.06.015. Epub ahead of print.
5. Peng XP, Wilson JL, Bogunovic D. The Unique Landscape of Genetically-Driven Blood and Immune Diseases: Paradigms and Challenges. *NEJM Evidence.* Invited review. In press.

SRILAKSHMI RAJ, Ph.D.

Human Population Genetics

Our work involves leveraging the genetic and environmental variation among humans to understand variation in phenotype, disease risk and outcome. We use genetics to understand population structure and history, and use this information to investigate variation in genetic architecture of diseases among populations.

Our research is centered around using human population genetics to understand the role of genetics in human health outcomes. We are strongly interested in leveraging population differences in the genetic architecture of rare and common disorders to serve community health.

This type of research involves understanding both the environmental and clinical contexts in which genetics contributes to phenotype in different populations. We employ three main strategies to carry out this research. The first is evolutionary genetic approaches to understanding why humans vary, where in the genome this may occur, and how this may affect disease risk. The second is using population genetic approaches to address epidemiological questions, such as predicting who might be at highest risk for a particular disease and understanding the genetic architecture underlying this. Lastly, we use anthropological genetics approaches to understand the genetic variation and environmental contexts which may predispose an individual to disease. This involves extensive fieldwork to understand the contribution of human genetic and local environmental variation to phenotypic variation among specific populations.

This multi-pronged approach enables us to apply population genetic principles and approaches broadly to understand the genetic contribution to community health outcomes, which can be multifaceted and vary among populations and disease contexts.

Website: https://srirajlab.com

Select Publications:

1. Isshiki M, Griffen A, Meissner P, Spencer P, Cabana MD, Klugman SD, Colon M, Maksumova Z, Suglia S, Isasi C, Greally JM, **Raj SM.** (2025). Genetic disease risks of under-represented populations in New York City. *PLoS Genetics*. 21(6):e1011755.
2. Yang, D, Griffen A, Isshiki M, Greally J, Rastogi D, **Raj SM** (2025). Identifying the Genetic Ancestry of the Obesity-related Asthma Variant (rs6494395) at RPS27L. *Pediatric Pulmonology (in press).*
3. Andrawus M, GB David, I Terziyska, L Sharvit, A Bergman, N Barzilai, **SM Raj**, DR Govindaraju, G Atzmon. (2024) Genome integrity as a potential index of longevity in Ashkenazi Centenarian's families. GeroScience.
4. Sosa EA, S Rosean, D O'Shea, **SM Raj**, C Seoighe, JM Greally. (2024) Regulatory Landscape Enrichment Analysis (RLEA): a computational toolkit for non-coding variant enrichment and cell type prioritization. BMC Bioinformatics 25:179.
5. Wattacheril JJ, **S Raj**, DA Knowles, JM Greally. Using epigenomics to understand cellular responses to environmental influences in diseases. 2023. PLoS Genetics. 19(1):e1010567.
6. Myer PA, JK Lee, R Madison, K Pradhan, G Frampton, J Newberg, J Ross, J Venstrom, AB Schrock, SJ Klempner, L Augenlicht, C Isasi, A Verma, JM Greally, **SM Raj**, S Goel, SM Ali. The Genomics of Colorectal Cancer in Populations with African and European Ancestry. 2022. Cancer Discovery 12 (5):1282-1293.
7. **Raj SM**, R Ekanayake, M Bhat, J Kadandale, P Pingali. Multiple risk factors contributing to stunting in Karnataka state, India. Current Science 121(3):360-64.
8. **Raj SM**, R Ekanayake, K Crowley, M Bhat, J Kadandale, P Pingali. Risk factors in childhood stunting in Karnataka state vary by geography. Current Science 121(4):502-10.
9. Yu F*, Lu J*, Liu X*, Gazave E, Chang D, **Raj S**, Hunter-Zinck H, Blekhman R, Arbiza L, Van Hout C, Morrison A, Johnson AD, Bis J, Cupples LA, Psaty BM, Muzny D, Yu J, Gibbs RA, Keinan A, Clark AG, Boerwinkle E. 2015. A population genetic analysis of 962 whole genome sequences reveals natural selection in non-coding regions of the human genome. *PLoS One 10, e0121644.*
10. Cardona, A., Pagani L, Antao T, Lawson DJ, Eichstadt CA, Yngvadottir B, Shwe MTT, Wee J, Tyler-Smith C, Gallego Romero I, **Raj S**, Metspalu M, Villems R, Nielsen R, Willerslev E, Malyarchuk BA, Derenko MV, Kivisild T. (2014) Genome-wide analysis of cold adaption in indigenous Siberian populations *PLoS One* 9:e98076.
11. **Raj SM,** Pagani L, Romero IG, Kivisild T, Amos W (2013). A general linear model based approach for inferring selection to climate. *BMC Genetics* 14: 87.
12. Ye K, Lu J, **Raj SM**, Gu Z (2013). Human expression QTLs are enriched in signals of environmental adaptation. *Genome Biol Evol* 5:1689-701.
13. **Raj SM**, Halebeedu P, Iliescu FM, Romero IG, Kadandale, J, Thangaraj, K, Chandra HS, Muniyappa, K, Kivisild, T. Patterns of variation at diabetes and obesity-associated loci mirror neutral patterns of human population diversity and diabetes prevalence in India. *Annals of Human Genetics* 77(5): 392-408.

MICHAEL G. ROSENFELD

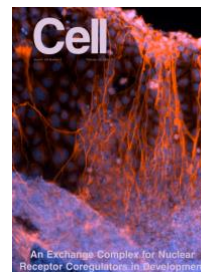**Investigator, Howard Hughes Medical Institute**
Professor, UCSD School of Medicine
National Academy of Sciences (1994)
Lab location: CMM-West, Room 353

Phone: 858.534.5858
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Website: investigators/rosenfeld_bio.html

Lab Composition and Activities: Two graduate students from different programs, a talented group of enthusiastic (and helpful) postdoctoral fellows and a full-time laboratory manager. We have one full laboratory meeting, one graduate student-only meeting, and one individual meeting each week. We also have joint lab meetings with another lab biweekly.

Research Interests: Our central laboratory focus is to:
1. Understand the molecular mechanisms of the transcriptional and chromosomal architectural programs that underlie development, regulation and disease, focusing on enhancer networks. We utilize diverse global genomic and single cell real time imaging approaches to uncover and investigate the "enhancer code" controlled by new, previously unappreciated pathways that integrate the genome-wide response to permit proper homeostasis and that also function in aging/senescence, in disorders of the CNS, including neurodegeneration, and in specific cancers.
2. These studies have led to identification of phase separation event in acute ligand/signal activation of enhancer-dependent transcriptional programs, the discovery of the role of RNA shape in mediating the functions of promoter antisense RNAs and enhancer RNAs, and the mechanisms by which enhancers "choose" their cognate promoters. These observations are rapidly altering our concepts of homeostasis and disease.
3. We are applying these principles and simultaneous single nuclei RNA-seq and ATAC-seq analyses to uncover the early events in Alzheimer's disease, distinguishing aging-dependent events in each CNS cell type and cellular senescence, and cancer metastasis, and studying events in learning and memory.

Current projects for potential rotations include:

- Using single nuclear RNA-seq, ATAC-seq, and ChIP-seq approaches to investigate the potential causative events leading to Alzheimer's disease. This will require delineating the trajectory trees and heat diffusion maps for all CNS cell types and identification of the transcription factors driving normal aging vs. disease-associated alterations and the regulatory enhancers during aging and in sporadic AD. Proof of hypotheses.

- Linking phase separation events of cell type-specific enhancers in determining chromosomal architecture and long distance cooperative enhancer interactions to an unexpected requirement for signal/ligand-dependent activation of topoisomerase 1 at enhancers, which is "read" by a specific component of the DNA damage repair machinery, but here functioning as an obligatory co-activator; identification of additional novel required factors and RNAs in regulated enhancer activation events .

- Developing new methods for real time, single cell imaging to explore regulation of enhancer networks, interactions with subnuclear structures, and enhancer bursting to understand the relation of enhancer/ subnuclear structural interactions in the 4D nucleome.

15 Representative Publications (2014-2021):

Yang F, Tanasa B, et al.Shape of promoter antisense RNAs regulates ligand-induced transcription activation. *Nature.* 2021 Jul;595(7867):444-449. doi: 10.1038/s41586-021-03589-x. Epub 2021 Jun 30.PMID: 34194047

Oh S, et al. Enhancer release and retargeting activates disease-susceptibility genes. *Nature.* 2021 May 26. doi: 10.1038/s41586-021-03577-1. Online ahead of print.PMID: 34040254

Alexanian M,et al. A transcriptional switch governs fibroblast activation in heart disease. *Nature.* 2021 Jul;595(7867):438-443. doi: 10.1038/s41586-021-03674-1. Epub 2021 Jun 23.PMID: 34163071

Nott A, et al. Brain cell type-specific enhancer-promoter interactome maps and disease-risk association. *Science.* 2019 Nov 29;366(6469):1134-1139. doi: 10.1126/science.aay0793. Epub 2019 Nov 14.PMID: 31727856

Nair SJ, et al. Phase separation of ligand-activated enhancers licenses cooperative chromosomal enhancer assembly. *Nature Struct Mol Biol*. 2019,26(3):193-203

Kim HS, et al. Pluripotency factors functionally premark cell-type-restricted enhancers in ES cells. *Nature*. 2018 Apr;556(7702):510-514.

Tan Y, et al. Dismissal of RNA Polymerase II Underlies a Large Ligand-Induced Enhancer Decommissioning Program. *Mol Cell.* 2018 Aug 16;71(4):526-539.e8. doi: 10.1016/j.molcel.2018.07.039.

Yang,F, et al. Glucocorticoid receptor: MegaTrans Switching Mediates the Repression of an ERα-Regulated transcriptional Program. *Mol Cell.* 2017 May4;66(3):321-331

Wang J, et al. LSD1n is an H4K20 demethylase regulating memory formation via transcriptional elongation control. *Nat Neurosci.* 2015 Sep;18(9):1256-64. doi: 10.1038/nn.4069. Epub 2015 Jul 27.

Telese F, et al. LRP8-Reelin-Regulated Neuronal Enhancer Signature Underlying Learning and Memory Formation. *Neuron*. 2015 May 6;86(3):696-710. PMID:2589230

Li W,et al. Condensin I and II Complexes License Full Estrogen Receptor α-Dependent Enhancer Activation. *Mol Cell*. 2015 Jul 16;59(2):188-202. PMID:26166704**.**

Puc J, et al. Ligand-dependent enhancer activation regulated by topoisomerase-I activity. *Cell*. 2015 Jan 29;160(3):367-80. PMID:25619691.

Skowronska-Krawczyk, D., et al. Required Interactions of Enhancers with Matrin-3 Nuclear Architecture for Transcriptional Activation by Homeodomain Factor. *Nature* 2014;514(7521):257-61. PMID:25119036.

Basnet H, et al. Tyrosine phosphorylation of histone H2A by CK2 regulates transcriptional elongation. *Nature*. 2014 Dec 11;516(7530):267-71. PMID:25252977.

Liu Z, et al. Enhancer activation requires trans-recruitment of a mega transcription factor complex. *Cell.* 2014 Oct 9;159(2):358-73. PMID:25303530.

Please feel free to email me or call@534-5858 for questions and if you would like to meet/Zoom and discuss projects. Thanks, Geoff

JAYANTA ROY-CHOWDHURY, MBBS, MRCP, AGAF, FAASLD

Our current focus is on developing cell and gene-based therapies for monogenic liver diseases, such as inherited hyperbilirubinemia (Crigler-Najjar syndrome, CN-1), a1 antitrypsin (AAT) deficiency, dyslipidemias and hemophilias A and B.

Subproject 1. Hepatocyte-based therapies for genetic liver diseases. To develop a minimally invasive alternative to liver transplantation, we are developing strategies to repopulate the liver by transplanted hepatocytes. To overcome the hurdles of inefficient hepatocyte engraftment and failure of transplanted hepatocytes to proliferate, we are evaluating targeted hepatic irradiation and mitotic stimulation of the transplanted hepatocytes. Regiospecific conformal hepatic irradiation (HIR) is being used to transiently disrupt the sinusoidal endothelial barrier, thereby enhancing initial engraftment. HIR makes the host hepatocytes in the irradiated region less mitotically competent. We are exploring different types of mitotic agents to stimulate the proliferation of the engrafted hepatocytes, which can competitively repopulate the host liver. We are also evaluating whether co-transplantation of liver sinusoidal endothelial cells can augment liver repopulation by hepatocytes. Our work was translated into the first successful hepatocyte transplantation in a CN-1 patient, and more recently in two patients with urea cycle disorders and one with phenyl ketonuria.

AAT deficiency (ATD) is one of the most common potentially lethal monogenic liver disorders in the West. In classic ATD, a mutant misfolded AAT (ATZ) tis secreted inefficiently and is retained within hepatocytes. Circulatory AAT deficiency leads to unrestrained neutrophil elastase activity in the lung, causing pulmonary emphysema, whereas ATZ accumulation within hepatocytes results liver disease. We showed that wildtype hepatocytes transplanted into transgenic mice expressing human ATZ competitively replace the host hepatocytes. Our current focus is to disrupt ATZ expression in a fraction of the hepatocyte mass by DNA break-enhanced homologous recombination in vivo, so that the gene-edited hepatocytes can repopulate the liver, thereby providing normal AAT and correcting the liver disease.

Subproject 2. Transplantation of endothelial cells to repopulate mutant liver endothelial cells (LSEC): LSECs are highly specialized endothelial cells that are important in maintaining liver architecture, as well as hepatocyte regeneration and function. In addition, these cells express coagulation factor VIII (the deficiency of which causes hemophilia A) and Von Willebrand factor. In ongoing work, we have found that intravenous infusion of LSECs following regional HIR results in engraftment of LSECs in the liver. Subsequent pharmacological stimulation of the LSECs results in regional liver repopulation by the transplanted cells. This procedure has cured the bleeding disorder in Factor VIII-deficient hemophilic mice.

In ongoing studies we are correcting the genetic lesions in mutant hepatocytes and LSECs, using CRISPR-cas or zinc finger nuclease-enhanced homologous recombination to generate phenotypically corrected for transplantation into animal models of inherited human liver diseases.

Recent Publications:

Ding J, Yannam GR, Roy-Chowdhury N, Hidvegi T, Basma H, Rennard SI, Wong RJ, Avsar Y, Guha C, Perlmutter DH, Fox IJ, Roy-Chowdhury J. Spontaneous hepatic repopulation in transgenic mice expressing mutant human alpha 1-antitrypsin by wildtype donor hepatocytes. J. Clin. Invest. 121:1930-4, 2011.

Chen Y, Li Y, Wang X, Zhang W, Sauer V, Chang CJ, Han B, Tchaikovskaya T, Avsar Y, Tafaleng E, Madhusudana Girija S, Tar K, Stephen S, Bouhassira E, Guha C, Fox IJ, Roy-Chowdhury J and Roy-Chowdhury N. Amelioration of hyperbilirubinemia in Gunn rats after transplantation of hepatocytes derived from human induced pluripotent stem cells. Stem Cell Reports 5:1-9, 2015.

Sauer V, Tchaikovskaya T, Wang X, Li Y, Zhang W Tar K, Polgar Z, Ding J, Guha C, Fox IJ, Roy-Chowdhury N, Roy-Chowdhury J. Human urinary epithelial cells as a source of engraftable hepatocyte-like cells using stem cell technology. Cell transplant, 2016, 25:2221-2243.

Peterson EA, Polgar Z, Devakanmalai GS, Li Y, Jaber FL, Zhang W, Wang X, Iqbal NJ, Murray JW, Roy-Chowdhury N, Quispe Tintaya W, Maslov AY, Tchaikovskaya TL, Sharma Y, Rogler LE, Gupta S, Zhu L, Roy-Chowdhury J, Shafritz DA. Genes and pathways promoting long-term liver repopulation by ex vivo hYAP-ERT2 transduced hepatcytes and treatment of jaundice Gunn rats. Hepatology Communications 2019; 3:129-146

Barahman M, Zhang W, Harris HY, Aiyer A, Kabarriti R, Kinkhabwala M, Roy-Chowdhury N, Beck AP, Scanlan TS, Roy-Chowdhury J, Asp P, Guha C. Radiation-primed hepatocyte transplantation in murine monogeneic dyslipidemia normalizes cholesterol and prevents atherosclerosis. J Hepatol. 2019 70:1170-1179.

Li Y, Guha C, Asp P, Wang X, Tchaikovskya TL, Kim K, Mendel M, Cost GJ, Perlmutter DH, Roy-Chowdhury N, Fox IJ, Conway A, Roy-Chowdhury J. Resolution of hepatic fibrosis after ZFN-mediated gene editing in the PiZ mouse model of human α1-antitrypsin deficiency. Hepatol Commun. 2023 Feb 27;7(3):e0070.

NAMITA ROY-CHOWDHURY, Ph.D., FAASLD

I. Inherited Disorders of Bilirubin Glucuronidation

UGT1A1 is a member of UDP-glucuronosyltransferases (UGT) family of enzymes, which is concentrated in the hepatic endoplasmic reticulum (ER). UGT1A1, which mediates the glucuronidation of bilirubin and estrogens. is required for biliary excretion of bilirubin. We showed that the genetic lesions in any one of the five exons encoding UGT1A1 can abolish or reduce bilirubin glucuronidation, causing potentially lethal Crigler-Najjar syndrome type I (CN-I), or its less severe variant, Crigler-Najjar syndrome type II (CN-II). We also showed that Gilbert syndrome, a milder form of inherited hyperbilirubinemia, is caused by a promoter polymorphism. We have been studying the regulation of UGT1A1 gene expression. Our current objective is to develop novel gene and cell-based therapies to cure this disease. Fibroblasts or renal tubular epithelial cells present in urine of CN1 patients will be reprogrammed to iPS cells, genetically corrected, differentiated into hepatocytes and transplanted into jaundice Gunn rat model of CN1.

II. Primary Hyperoxaluria Type 1 (PH1)

PH1 is an autosomal recessive disease caused by mutations in the alanine:glyoxylate aminotransferase gene (AGXT). In humans, insufficient AGXT activity in liver peroxisomes leads to increased oxalate production that causes calcium oxalate stones in the kidney and then in blood, heart, bones, etc. It is a lethal disease unless combined liver and kidney transplantation is performed. We have developed a mouse model of PH1. Our plan is to cure this disease by (a) gene therapy (b) transplantation of adult primary hepatocytes or (c) hepatocytes derived from human embryonic (hESC) or induced pluripotent stem cell (iPSC). For the latter, fibroblasts from the skin of normal volunteers or patients with PH1 are used to generate iPS. Initially we used viral vectors to generate the iPSCs, but now use non-DNA integrating approaches to generate iPS cells. The cells are differentiated to hepatocyte-like iHep cells for transplantation into our mouse model of PH1.

Publications:

Salido E, Li Xiao, Lu Y, Wang X., Santana A., Roy-Chowdhury N, Torres A, Shapiro L, Roy-Chowdhury J (2006) Alanine-glyoxylate aminotransferase deficient mice, a model for primary hyperoxaluria thatresponds to adenoviral gene transfer. Proc. Natl. Acad. Sci., USA, 103:18249-18254.

Jiang J, Salido EC, Guha C, Wang X, Moitra R, Liu L, Roy-Chowdhury J, Roy-Chowdhury N. (2008) Correction of Hyperoxaluria by liver repopulation with hepatocytes in a mouse model of primary hyperoxaluria type -1. Transplantation 85,1253-1260.

Ding J, Yannam GR, Roy-Chowdhury N, Hidvegi T, Basma H, Rennard SI, Wong RJ, Avsar Y, Guha C, Perlmutter DH, Fox IJ, Roy-Chowdhury J. (2011) Spontaneous hepatic repopulation in transgenic mice expressing mutant human alfa 1- antitrypsin by wild-type donor hepatocytes. J Clin Invest.121(5): 1930-1934.

Roy-Chowdhury N, Roy-Chowdhury J. (2015) Liver physiology and energy metabolism. In Sleisenger and Fordtran's Gastrointestinal and Liver Disease, 10th edition. Feldman M., Friedman LS, Brandt LJ, editors. Saunders-Elsevier, Philadelphia.Page 1223-1232.

Chen Y, Li Y, Wang X, Zhang W, Sauer V, Chang CJ, Han B, Tchaikovskaya T, Avsar Y, Tafaleng E, Madhusudana Girija S, Tar K, Stephen S, Bouhassira E, Guha C, Fox IJ, Roy-Chowdhury J, Roy-Chowdhury N. (2015) Amelioration of hyperbilirubinemia in Gunn rats after transplantation of hepatocytes derived from human induced pluripotent stem cells. Stem Cell Reports 5:1-9.

Peterson EA, Polgar Z, Devakanmalai GS, Li Y, Jaber FL, Zhang W, Wang X, Iqbal NJ, Murray JW, Roy-Chowdhury N, Quispe Tintaya W, Maslov AY, Tchaikovskaya TL, Sharma Y, Rogler LE, Gupta S, Zhu L, Roy-Chowdhury J, Shafritz DA. Genes and pathways promoting long-term liver repopulation by ex vivo hYAP-ERT2 transduced hepatcytes and treatment of jaundice Gunn rats. Hepatology Communications 2019; 3:129-146

Roy-Chowdhury N, Wang X, Roy-Chowdhury J. Bile pigment metabolism and its disorders. Emery and Rimoins's Pinciple and Practice of Medical Genetics, 7th edition, Rimoin DL, Connor JM, Pyeritz RE, Korf BR, editors. Churchill-Livingstone-Elsevier, Philadelphia. 2019

Roy Chowdhury N, Li Y, Roy Chowdhury J. Chapter 20. Disorders of bilirubin metabolism. In The Liver: Biology and Pathobiology, Sixth edition, Arias, IM, editor in chief. Wiley-Blackwell, Oxford, U.K. 2019

Roy-Chowdhury N, Guha C, Roy-Chowdhury N. Inherited disorders of bilirubin metabolism and biliary transport. In Physician's Guide to Diagnosis, Treatment and Follow-up, edited by Blau N, Dionisi-Vici C, Ferreira C, Vianey-Saban C, van Karnebeek C, editors. Springer Nature 2021

Li Y, Guha C, Asp P, Wang X, Tchaikovskya TL, Kim K, Mendel M, Cost GJ, Perlmutter DH, Roy-Chowdhury N, Fox IJ, Conway A, Roy-Chowdhury J. Resolution of hepatic fibrosis after ZFN-mediated gene editing in the PiZ mouse model of human α1-antitrypsin deficiency. Hepatol Commun. 2023 Feb 27;7(3):e0070.

JULIE SECOMBE, Ph.D.

Transcriptional Regulation by the KDM5 family of histone modifiers

In the Secombe lab, we are interested in understanding the transcriptional regulatory mechanisms that are important for development in addition to those that are needed for proper functioning of adults. In particular, we focus on the lysine demethylase 5 (KDM5) family of proteins that are able to regulate gene expression via their conical histone demethylase activity in addition to less characterized mechanisms that are independent of this activity. To do this, we combine analyses using the animal model *Drosophila melanogaster* that has an amazing array of genetic tools, in addition to human iPSC-cell models.

KDM5 functions during development: In *Drosophila*, the *Kdm5* gene is essential for viability, with animals that lack the gene dying before reaching adulthood. Interestingly, KDM5's famous histone demethylase activity is not essential for viability. What functions of KDM5 are critical for development? Which cell types are involved? What at the transcriptional programs KDM5 regulates in those tissues?

KDM5 function in neurons: Pathogenic variants in human *KDM5* family genes cause neurodevelopmental disorders that include intellectual disability, seizures, altered locomotion and disrupted sleep among other features. However, how loss of KDM5 family genes leads to these phenotypes remains unknown. We use *Drosophila* and human iPSC-derived neurons to answer a number of key questions. How do variants associated with cognitive impairment disrupt KDM5-regulated transcription? Which neuronal cell types require KDM5? Does KDM5 regulate distinct or similar transcriptional programs in different neuronal cell types? Using the *Drosophila* model, we are also examining the links between KDM5 and seizures, locomotion and circadian rhythm. Overall, we expect that this combining studies in *Drosophila* and human cells to dramatically enhance our understanding of human intellectual disability.

More information can be found at my website www.secombelab.org

Recent Publications:

Yheskel, M., Hatch, H.A.M., Pedrosa, E., Terry, B. K., Siebels, A. A., Zheng, X.Y., Blok, L.E.R., Fencková, M., Sidoli, S., Schenck, A., Zheng, D., Lachman, H.M., and J. Secombe (2024). KDM5-mediated transcriptional activation of ribosomal protein genes alters translational efficiency to regulate mitochondrial metabolism in neurons. Nucleic Acids Research, **52** (11), 6201-6219. PMID:38597673

Rogers, M.F., Marshall, O.J., and J. Secombe (2023). KDM5-mediated activation of genes required for mitochondrial biology is necessary for viability in *Drosophila*. *Development*, doi.org/10.1242/dev.202024 PMID:37800333Schneider, B.K., Sun, S., Lee, M., Li, W., Skvir, N., Neretti, N., Vijg, J., and **J. Secombe** (2023) Expression of transposons contributes to aging in *Drosophila. Genetics*, DOI: 10.1093/genetics/iyad073

Yheskel, M., Sidoli, S., and J. Secombe (2023). Proximity labeling reveals a new *in vivo* network of interactors for the histone demethylase KDM5. *Epigenetics & Chromatin* 16(8) doi.org/10.1186/s13072-023-00481-y. PMID:36803422.

Belalcazar, H.M., Hendricks, E.L., Zamurrad, S., Liebl, F.L.W., and Secombe J (2021) The histone demethylase KDM5 is required for synaptic structure and function at the *Drosophila* neuromuscular junction. *Cell Reports*, 34(7):108753. DOI:10.1016/j.celrep.2021.108753. PMID:33596422

Hatch, H.A.M., Belalcazar H.M., Marshall O.J., and Secombe J (2021) A KDM5-Prospero transcriptional axis functions during early neurodevelopment to regulate mushroom body formation. *eLife* doi.org/10.7554/elife.63886 PMID: 33729157

Hatch, H.A.M., O'Neill, M.H., Marion, R.W., Secombe, J.#., and **L.H. Shulman**# (2021) Caregiver-reported characteristics of children diagnosed with pathogenic variants in KDM5C. *American Journal of Medical Genetics - Part A*, doi:10.1002/amjg.a.62381 PMID:34089235
co-corresponding authors.

Drelon, C., Belalcazar, H.M. and J. Secombe (2019) The histone demethylase KDM5 controls developmental timing by promoting prothoracic gland endocycles. *Development,* 146:dev182568 doi: 10.1242/dev.182568. PMID:31862793

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Zamurrad, S., Hatch, H.A.M., Drelon, C., Belalcazar, H.M, and J. Secombe (2018) A *Drosophila* model of intellectual disability caused by mutations in the histone demethylase KDM5. *Cell Reports* 22, 2359-2369.

Drelon, C., Belalcazar, H.M. and J. Secombe (2018) The histone demethylase KDM5 is essential for larval growth in *Drosophila*. *Genetics*. pii: genetics.301004.2018. doi: 10.1534/genetics.118.301004.

Navarro-Costa, P., McCarthy, A., Prudêncio, P., Greer, C., Guilgur, L.G., Becker J., Secombe, J., Rangan, P and R. Martinho (2016). Early programming of the oocyte epigenome temporally controls late prophase I transcription and chromatin remodeling. *Nat. Comms*., 10;7:12331. PMCID:PMC4987523..

Liu, X., and Secombe, J# (2015) KDM5 recognizes chromatin context to activate genes essential for mitochondrial function. *Cell Reports*, 13, 2219-2231.

MILAN SEN, M.D., C.M.

Hip fractures represent a major cause of morbidity and mortality among the elderly population, with a 1-year mortality rate estimated to be 30%. This rate has remained stable in the literature over the past 40 years. To date, efforts to develop a biological measure of mortality risk in this population have not resulted in meaningful methods of risk assessment, nor have they elucidated novel areas of potential intervention aimed at mortality reduction.

The purpose of my current research with Professor Vijg is to establish a predictive relationship between pre-operative DNA methylation levels in the blood, and post-operative mortality in the geriatric hip fracture population. We hypothesize that in geriatric patients with an isolated acute hip fracture, elevated pre-operative DNA methylation levels will be associated with a higher risk of all-cause mortality at 1 year follow-up. If we are able to identify the high risk group using the DNA methylation clock, we hope to study a targeted pharmacological intervention in this select group.

<u>Selected Publications:</u>

- Z Sharfman, A Parsikia, TN Rocker, MD Kahn, S Sokol, ME Stone Jr., J McNelis, MK Sen, A Dimitroulias. Increased Morbidity and Mortality in Elderly Patients with Lower Extremity Trauma and Associated Injuries: A Review of 420,066 Patients from the National Trauma Database. Injury, 2021 Apr;52(4):757-766

- R Yang, D Murphy, A Goch, J Wang, V Charubhumi, J Fox, M Sen, B Hoang, D Geller. A Novel Tripod Percutaneous Reconstruction Technique in Peri-Acetabulum Lesion Caused by Metastatic Cancer. J Bone Joint Surg Am. 2020 Apr 1;102(7):592-599

- WB Cohen-Levy, J Liu, M Sen, SH Teperman, ME Stone. Prophylactic Inferior Vena Cava Filters for Operative Pelvic Fractures: A 12 Year Experience. Int Orthop. 2019 Aug 7.

- MK Sen, N Sama, M Raglan, C Bircher, M Bircher, DL Helfet. Treatment of Acetabular Fractures in Adolescents. Am J Orthop. 2015 Oct;44(10):465-70.

- JM Aho, MK Sen, M Saint-Cyr. Free and Pedicle Flaps in Lower-Extremity Trauma. Eur. J Plast Surg, June 2015, Vol. 38, No. 3:171-182.

- MR Brinker, BD Hanus, M Sen, DP O'Connor. The Devastating Effects of Tibial Nonunion on Health-Related Quality of Life. J Bone Joint Surg Am. 2013 Dec 18;95(24):2170-6.

- Kang Y, Scully A, Young DA, Kim S, Tsao H, Sen M, Yang Y. Enhanced mechanical performance and biological evaluation of a PLGA coated β-TCP composite scaffold for load-bearing applications. Eur Polym J. 2011 Aug 1;47(8):1569-1577.

FRANK SOLDNER, M.D.

Novel approaches to investigate the genetic, cellular, and molecular basis of complex neurological disorders

Our laboratory focuses on modeling human brain development and function in a cell culture dish to understand the molecular and cellular basis of complex disorders such as Parkinson's and Alzheimer's disease. A significant challenge of studying complex human diseases is the lack of relevant model systems that combine know genetic elements with disease-associated phenotypic readouts. This is particularly problematic for sporadic neurodegenerative diseases that have no well-defined genetic etiology and do not follow Mendelian inheritance patterns. Epidemiology and population genetics suggest that such diseases result from a complex interaction between multiple risk factors, both genetic and non-genetic (lifestyle and environmental). Although genome wide association studies (GWAS) have identified genomic variations, such as single nucleotide polymorphisms (SNPs), deletions, and insertions associated with a higher risk to develop specific neurological disorders, the vast majority of such sequence variants have no established biological relevance to disease or clinical utility to prognosis or treatment.

Three major recent innovations have fundamentally changed our ability to study human neurological disorders in a cell culture dish: (i) Reprogramming of somatic cells into human induced pluripotent stem cells (hiPSCs) to generate patient-derived disease-relevant neuronal cells, (ii) the development of genome engineering technologies such as the CRISPR/Cas9 system to modify the genome in human cells, and (iii) the availability of tissue-type and disease-specific genome-scale genetic and epigenetic information. Our previous work demonstrated that an interdisciplinary approach, integrating these technologies, enables us to study neurological disorders in a genetically controlled and systematic manner in human neuronal cells. Using these previously unavailable molecular and cellular tools, we were able to dissect the functional role of diseases-associated sequence variations in non-coding regulatory elements such as distal enhancer sequences in the pathogenesis of Parkinson's disease. My lab is extending this novel experimental framework in human pluripotent stem cell (hPSC)-derived two-dimensional (2D) monolayer and three-dimensional (3D) organoid neuronal culture systems to systematically investigate the genetic, cellular, and molecular basis of neurodegenerative disorders. We are establishing robust disease-relevant phenotypic readouts to perform unbiased compound and CRISPR/Cas9-based genome-scale genetic screens and will exploit these approaches to understand how genetic, epigenetic, and environmental factors contribute to the development and progression of neurological diseases.

Selected publications

Soldner, F. & Jaenisch, R. Stem Cells, Genome Editing, and the Path to Translational Medicine. *Cell*, 175, 615-632 (2018)

Soldner, F., Stelzer, Y., Shivalila, C. S., Abraham, B. J., Latourelle, J. C., Barrasa, M. I., Goldmann, J., Myers, R. H., Young, R. A. & Jaenisch, R. Parkinson-associated risk variant in distal enhancer of α-synuclein modulates target gene expression. *Nature* 533, 95–99 (2016).

Soldner, F., Laganière, J., Cheng, A. W., Hockemeyer, D., Gao, Q., Alagappan, R., Khurana, V., Golbe, L. I., Myers, R. H., Lindquist, S., Zhang, L., Guschin, D., Fong, L. K., Vu, B. J., Meng, X., Urnov, F. D., Rebar, E. J., Gregory, P. D., Zhang, H. S. & Jaenisch, R. Generation of Isogenic Pluripotent Stem Cells Differing Exclusively at Two Early Onset Parkinson Point Mutations. *Cell* 146, 318–331 (2011).

Soldner, F.*, Hockemeyer, D.*, Beard, C., Gao, Q., Bell, G. W., Cook, E. G., Hargus, G., Blak, A., Cooper, O., Mitalipova, M., Isacson, O. & Jaenisch, R. Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. *Cell* 136, 964–977 (2009).

Hockemeyer, D.*, Soldner, F.*, Beard, C., Gao, Q., Mitalipova, M., Dekelver, R. C., Katibah, G. E., Amora, R., Boydston, E. A., Zeitler, B., Meng, X., Miller, J. C., Zhang, L., Rebar, E. J., Gregory, P. D., Urnov, F. D. & Jaenisch, R. Efficient targeting of expressed and silent genes in human ESCs and iPSCs using zinc-finger nucleases. *Nat Biotechnol* 27, 851–857 (2009).

Hockemeyer, D.*, Soldner, F.*, Cook, E. G., Gao, Q., Mitalipova, M. & Jaenisch, R. A drug-inducible system for direct reprogramming of human somatic cells to pluripotency. *Cell Stem Cell* 3, 346–353 (2008).

(* Equally contributing authors)

SIMON SPIVACK, M.D., M.P.H.

Epigenetic Variability and Functional Impact on Gene Regulation in the Lung

The goal of the Spivack laboratory is to understand inter-individual differences in gene regulation in the lung, using genetic and epigenetic techniques. The mechanistic goal is to understand the subtleties of how specific high-resolution patterns of DNA methylation and microRNA expression regulate gene expression. We have developed several new functional genetic technologies to examine epigenetic function. We have recently completed initial genome-wide searches of the transcriptome, methylome, and microRNAome of lung cancers, and recently of epithelial progenitor cells that give rise to lung cancers. The translational goal is to use these functionally sifted epigenetic, genetic (and metabolomic and proteomic) features, and detect them non-invasively, to identify individuals at particularly high risk for lung cancer and other common lung disorders (asthma/COPD), to enhance prevention and early detection efforts for each disorder.

Mechanistically, the role of promoter sequence and epigenetic variation in the regulatory region of carcinogenesis and oxidant pathway genes is being explored *in vitro.* We've developed techniques in the lab, such as human genomic methyl-DNA reporter constructs, and CAS9-based methylome writing, in addition to studying native human gene regulation models in chromatin. Use of methylome-wide and transcriptome-wide interrogations of lung progenitor cells are ongoing. Other unique technologies include the laboratory's microRNA:mRNA binding assay.

Translationally, epigenetic and other biomarkers are being established in laser capture microdissected human lung and several unique, non-invasively collected surrogate specimens developed in the laboratory, such as mRNA expression signatures from brush-exfoliated buccal mucosa cells microRNA and other analyses from exhaled breath condensate, and cough capture, which are first reports for new airway biomarker classes. These airway- derived specimens continue to accrue from our sampling (currently n>1100) of a lung cancer case-control study. The specimens are being studied for quantitative gene expression, DNA mutation by collaboration (Vijg-Maslov), methylome shifts, and their regulatory substrates listed above, in multiple pathways. These expression, genetic, and epigenetic data are being linked with envionrmental exposures such as tobacco smoking to put a substantive metric to gene-environment interaction in humans.

<u>**Selected Recen Genetics Publications/Manuscripts**</u>**:**

Batbayar Khulan, Kenny Ye, Spencer Waldman, Ava Marsh, Miao Kevin Shi, Taha Siddiqui, Will Liao (NYGC), Masako Suzuki, Aditi Desai, Dhruv Patel, Jaiminkumar Patel, Jay Dobkin, Ali Sadoughi, Chirag Shah, Jan Vijg, Yakov Peter, Simon D. Spivack. Bronchial field progenitor basal cells show methylome-wide signatures reflective of smoking status of the donor. *[submitted summer 2024].*

Zefi O, Waldman S, Marsh A, Shi MK, Sonbolian Y, Khulan B, Siddiqui T, Desai A, Patel D, Okorozo A, Khader S, Dobkin J, Sadoughi A, Shah C, Spivack SD*, Peter Y*. Distinctive Field Effects of Smoking and Lung Cancer Case-Control Status on Bronchial Basal Cell Growth and Signaling; Accepted, *Respiratory Res.,* 2024 (*co-senior authors).

Mitchell MI, Ben-Dov Iddo Z, Ye K, Liu C, Shi M, Sadoughi A, Shah C, Siddiqui T, Okorozo A, Gutierrez M, Unawane R, Biamonte L, Parihk K, Spivack SD, Loudig O. Exhaled breath condensate contains extracellular vesicles (EVs) that carry miRNA cargos of lung tissue origin that can be selectively purified and analyzed. *J Extracell Vesicles, 2024 Apr;13(4):e12440. doi: 10.1002/jev2.12440.* PMID: 38659349, PMCID: <u>PMC11043690</u>,DOI: <u>10.1002/jev2.12440</u>.

Shi M, Han W, Loudig O, Shah C, Dobkin J, Keller S, Sadoughi A, Patel D, Desai A, Gombar S, Suh Y, Fernandez MK, DeLaRosa L, Wang T, Hosgood D, Pradhan K, Ye K, Spivack SD. (2023) An exhaled microRNA panel interrogated for lung cancer case-control discrimination. [*Scientific Rep*. 2023 Apr 24;13(1):6620. doi: 10.1038/s41598-023-33698-8. PMID: 37095155; PMCID: PMC10126132.].

Zhenqiu Huang, Alex Maslov, Xiao Dong, Shixiang Sun, Moonsook Lee, Chirag Shah, Ali Sadoughi, Aditi Desai, Dhruv Patel, Taha Siddiqui, Jaiminkumar Patel, Miao Shi, Spencer Waldman, Ava Marsh, Yakov Peter, *Simon D Spivack, *Jan Vijg (*co-senior authors).Somatic Mutations At Single Base Resolution in Single Bronchial Progenitor Cells Collected From Human Lung. *[Nature Genetics, 2022 Apr;54(4):492-498. doi: 10.1038/s41588-022-01035-w. Epub 2022 Apr 11].*

Dong X, Shi M, Lee M, Toro R, Gravina S, Han W, Yasuda S, Vijg J, Suh Y, **Spivack SD.** Global, integrated analysis of methylomes and transcriptomes from laser capture microdissected bronchial and alveolar cells in human lung. *[Epigenetics,* 10.1080/15592294.2018.1441650 *2018].*

Han W, Shi M, Spivack SD. Site-specific methylated reporter constructs for functional analysis of DNA Methylation. *Epigenetics.* 4;8(11). PMID: 24004978, 2013.

Shi M, Han W, Spivack SD. A quantitative method to identify microRNAs that target a messenger RNA using a 3'UTR RNA affinity technique. *Anal. Biochem.* 1;443(1):1-12. PMID: 23938772, 2013.

YOUSIN SUH, Ph.D.

Functional Genomics of Aging

We have been taking human genetics and functional genomics approaches to understand the fundamental mechanisms of aging in humans. Aging is the single largest risk-factor for most chronic diseases such as Alzheimer's disease, cancer, cardiovascular disease, and type 2 diabetes and is emerging as a major component in basic, translational and clinical research. Our long-term research goals are to investigate the (epi)genetic component that underlies the interface of intrinsic aging and disease. To gain insight into the (epi)genetic link between aging and disease, we focus on the identification of functional (epi)genetic variation in human populations and the assessment of their potential functional impact on aging and disease. We take an integrated approach to study the connection between disease and aging at different levels, i.e., from (epi)genetic determinants in the form of (epi)genetic variants, through cell type- and tissue-specific regulated gene expression, to molecular and cellular endpoints in the tissues, leading to new targets for interventions as well as (epi)genetic markers for aging and its associated diseases. To achieve translation of genetic association into clinical benefits, it is critical to assess the functional impact of associated (epi)genetic variants. Such functionalization of observed associations is now the main knowledge gap in human disease genetics.

To discover functional (epi)genetic variants associated with aging or diseases of aging, we have been conducting systematic multidisciplinary studies of human genetic data from whole genome sequencing (WGS), whole exome sequencing (WES), and genome wide association studies (GWAS), which are prioritized through integrative analyses of human genetic, functional genomic, and epigenomic data. We then use the paradigm of CRISPR/Cas9-mediated genome engineering of human pluripotent stem cells followed by differentiation of the genome-edited stem cells into multiple cell lineages in order to elucidate cell type-specific and combinatorial effects of functional variants. For top candidate functional variants, we generate mouse models to understand their in vivo roles in aging and aging-related diseases. Our approach is unique and important because it ascertains the biological significance and the causality of human genetic association data, uncovering the fundamental mechanisms underlying human aging and potential targets for intervention against aging.

We have relocated to Columbia University in the Departments of OB/GYN and Genetics & Development as of October 1, 2019. Dr. Suh leads a new program on Reproductive Aging and Women's Health with the goal of bridging basic aging biology and clinical medicine. While the female reproductive system is the first to age in the human body, very little is known about the basic biology of reproductive aging. We are leveraging our expertise in human genetics, functional genomics, molecular and cellular biology, and stem cell engineering to address key unanswered questions on reproductive aging in women. We focus on how the fundamental biology of aging influences reproductive aging and its sequelae in women and if geroprotectors can delay their onset and progression.

Selected Publications:

Suh, Y., Atzmon, G., Cho, M.-O., Hwang, D., Liu, B., Barzilai, N., Cohen, P. Functional insulin-like growth factor-I receptor mutations in centenarians. *Proc. Natl. Acad. Sci*. 105: 3438-3442. 2008.

G. Atzmon, M. Cho, R.M. Cawthon, T.. Budagov, M. Katz, X. Yang, G. Siegel, A. Bergman, D.M. Huffman, C.B. Schechter,W.E. Wright, J.W. Shay, N. Barzilai, D.R. Govindaraju, and **Y. Suh**. Genetic variation in human telomerase is associated with telomere length in Ashkenazi centenarians. *Proc.Natl. Acad. Sci*. 107 Suppl(1):1710-1717. 2010

Tazearslan, C., Huang, J., Barzilai, N., and **Suh,Y**. Impaired IGF1R signaling in cells expressing longevity associated human IGF1R alleles. *Aging Cell*. 10(3):551-4. 2011

Suh, Y. and Kennedy, B.K. Dialing down SUN1 for laminopathies. *Cell*. 149(3):509-10. 2012

Park, C., **Suh, Y***., and Cuervo*, A.C. Regulated degradation of Chk1 by chaperone-mediated autophagy in response to DNA damage. *Nature Communications*. 6:6823. 2015.

Johnson, S.C., Dong, X., Vijg, J., **Suh, Y.** Genetic evidence for common pathways in human age-related diseases. *Aging Cell*. 4(5):809-17. 2015

Lau CH, **Suh Y**. In vivo epigenome editing and transcriptional modulation using CRISPR technology. *Transgenic Research*. PMID: 30284145. 2018

Ryu, S., Han, J., Norden-Krichmar, T., Zhang, Q., Lee, S., Zhang, Z., Atzmon, G., Niedernhofer, L.J., Robbins, P.D., Barzilai, N., Schork, N.J., **Suh, Y**. Genetic signature of human longevity in PKC and NF-кB signaling. *Aging Cell* e13362. 2021

Jin, C, Wang, X., Hudgins, AD, Gamliel, A, Pei, M, Kim, S, Contreras, D, Hoeijmakers, J, Campisi, J, Lobo, RA, Williams, SZ, Rosenfeld, MG, **Suh, Y**. The regulatory landscapes of human ovarian ageing. 2022. https://www.biorxiv.org/content/10.1101/2022.05.18.492547v1 (Preprint)

MASAKO SUZUKI, D.V.M., Ph.D.

Long-term memory of adverse prenatal micronutrient environment of offspring

The long-term research goal of the Suzuki lab is to identify mechanisms of how the offspring memorize their environmental exposure status throughout life. The health conditions of the mother during pregnancy critically contribute to the pregnancy outcome as well as the health of the baby. It has been reported that adverse intrauterine environment exposure is associated with susceptibility to many diseases such as cardiovascular diseases, asthma, diabetes, and obesity. Therefore, health throughout the life of the baby would be attributed to the health of the mother during pregnancy. However, the mechanisms of how the adverse prenatal environment causes long-term effects of offspring, which increases the risk of disease later in life, is not elucidated.

Our research focuses on the effects of in utero micronutrient deficiencies on cell subtype proportions and cell memory in offspring, and the association with developing diseases later in life. We hypothesize that this adverse prenatal exposure changes the repertoire of cell subtypes that comprise the mature organs of offspring that confers much of the risk of developing the disease phenotype. We believe this will open a new paradigm for epigenetic studies of the Developmental Origins of Health and Diseases (DOHaD).

In our community, we have many understudied populations prone to these micronutrient deficiencies. For instance, vitamin D deficiency/insufficiency is much higher in Hispanic (23%) and Black (non-Hispanic) populations (46%) than White (non-Hispanic) subjects (6.6%). Moreover, while the current national prevalence rate of vitamin A deficiency in the United States is reported to be very low (<1%), our recent study in the Bronx showed close to 60% of Hispanic pregnant females we studied were vitamin A deficient (Suzuki et al., Nutrients 2021).

We are currently studying the effects of fat-soluble micronutrients, vitamins A and D, deficiency during development on offspring as this may be informative for our community. The prenatal vitamin A deficiency project focuses on identifying the molecular mechanisms of how prenatal vitamin A deficiency status alters the pulmonary disease risks in adulthood (R01, PI Suzuki, NHLBI). The prenatal vitamin D deficiency project focuses on the molecular mechanism of how prenatal deficiency conditions affect the hematopoiesis of the offspring in adulthood. In addition, we are expanding our research interest to macro and micronutrient imbalance (hidden hunger).

<u>**Select Publications:**</u>

Lundy K, Greally JF, Essilfie-Bondzie G, Olivier JB, Doña-Termine R, Greally JM, **Suzuki M***. Vitamin D deficiency during development permanently alters liver cell composition and function. Frontiers Endocrinology 2022 May 12; 13:860286. PMID: 35634491

Suzuki M*, Tomita M. Genetic Variations of Vitamin A-Absorption and Storage-Related Genes, and Their Potential Contribution to Vitamin A Deficiency Risks Among Different Ethnic Groups. Front Nutr. 2022; 9:861619. Review. PMID: 35571879

Cwiek A, **Suzuki M**, deRonde K, Conaway M, Bennett KM, El Dahr S, Reidy K, Charlton JR. Premature differentiation of nephron progenitors and dysregulation of gene pathways critical to kidney development in a model of preterm birth. Scientific Reports. 2021 Nov 4;11(1):21667. DOI: 10.1038/s41598-021-00489-y. PMID: 34737344

Suzuki M*, Wang T, Garretto D, Isasi CR, Cardoso WV, Greally JM, Quadro L. Disproportionate Vitamin A Deficiency in Women of Specific Ethnicities Linked to Differences in Allele Frequencies of Vitamin A-Related Polymorphisms. Nutrients. 2021; 13(6):1743.

Kong Y, Rastogi D, Seoighe C, Greally JM, **Suzuki M***. Insights from deconvolution of cell subtype proportions enhance the interpretation of functional genomic data. PLOS ONE. 2019 14: e0215987. PMID: 31022271.

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ANNE VAN ARSDALE, M.D./Ph.D.

Pathogenic role of Human Papillomavirus (HPV) DNA integration in HPV-associated dysplasias and carcinomas

Infection with high-risk Human Papillomavirus (hrHPV) is a necessary, key event in cervical carcinoma. A major advance in understanding cervical cancer was the recognition that HPV DNA is integrated into the human genome in almost all advanced cervical tumors. Human genome integration of HPV DNA 1) stably associates the viral oncogenes with a host cell, 2) potentially drives expression of host oncogenes that flank the sites of HPV DNA insertions, and 3) also causes human genome rearrangements. Cervical cancer develops through a series of progressive, dysplastic lesions termed cervical intraepithelial neoplasia (CIN1 through CIN3) that occur within the epithelial cells at the surface of the cervix. Interestingly, premalignant lesions in the vast majority of HPV infected women, including most high-grade lesions, do not progress to fully invasive carcinomas. Current clinical management of cervical cancer relies on early detection of premalignant lesions when treatment can be highly effective at preventing cancer, but entails high morbidity risks, causes significant anxiety, and incurs substantial financial costs. In collaborations with Dr. Cristina Montagna (CINJ), Dr. Jack Lenz (Albert Einstein) and Dr. Brian Haas (The Broad Institute/MIT), we have developed a research program to detect and map HPV-human DNA junctions at single nucleotide resolution using a hybridization capture and next generation sequencing approach along with a comprehensive bioinformatics pipeline that is now generalizable to any virus that causes oncogenesis. Current research efforts and key issues being addressed include: harnessing the unique molecular signatures of individual integration sites as personalized biomarkers in dysplasia and carcinoma, evaluation of integration sites in patient-derived histologic samples, and the evaluation of molecular, functional and spatio-temporal consequences of HPV integration on clonal expansion.

References:
Van Arsdale A, Patterson N, Maggi E, Agoni L, Van Doorslaer K, Harmon B, Nevadunsky N, Kuo DSY, Einstein MH, Lenz J and Montagna C. Insertional oncogenesis by HPV70 revealed by multiple genomic analyses in a clinically HPV-negative cervical cancer. Genes Chromosomes Cancer. 2020 Feb; 59(2): 84–95.

VLADISLAV VERKHUSHA, Ph.D.

Engineering Near-Infra Red Fluorescent Proteins, Biosensors and Optogenetic Tools

Non-invasive optical imaging, monitoring and manipulation of metabolic processes in living mammals is more feasible within the near-infrared (NIR) optical transparency window (650-900 nm) where hemoglobin and melanin absorbance significantly decreases, and water absorbance is still low. The most red-shifted fluorescent proteins (FPs) of the GFP-like family have excitation and emission spectra outside of the NIR region and suffer from low brightness and modest photostability. Natural bacterial phytochrome photoreceptors (BphPs) utilize an enzymatic product of heme, low-molecular-weight biliverdin, as a chromophore.

BphPs provide many advantages over other natural chromophore-containing proteins. Unlike the chromophores of non-bacterial phytochromes, biliverdin is ubiquitous in mammals. This makes BphP applications in mammalian cells, tissues and whole mammals as easy as conventional GFP-like FPs, without supplying chromophore through an external solution. BphPs exhibit NIR absorbance and fluorescence, which are red-shifted relative to that of any other phytochromes, and lie within the NIR optical window. This makes BphPs spectrally complementary to other existing optical probes and optogenetic tools based on the GFP, flavoprotein and rhodopsin-like protein families. Independent domain architecture and pronounced conformational changes upon biliverdin photoisomerization make BphPs attractive templates to design various photocontrollable genetically-encoded probes.

In our laboratory, we engineer new BphP-based FPs, biosensors and optogenetic tools. These include bright and spectrally resolvable permanently fluorescent NIR FPs, photoactivatable with non-phototoxic NIR light FPs, and reversibly photoswitchable FPs. We also focus on designing NIR reporters for protein interactions and biosensors for intracellular ions and metabolites. Lastly, we engineer BphPs into optogenetic elements allowing us to noninvasively regulate intracellular processes in vivo with NIR light.

We apply various directed protein evolution approaches based on rational structure-based design and random mutagenesis of template BphPs, high-throughput flow cytometry and multiwell plate spectroscopy. These conventional techniques allow screening for standard properties of genetically encoded probes, such as excitation and emission wavelengths, brightness, photostability, pH stability and folding efficiency. We also develop new protein engineering and high-throughput approaches to specifically optimize BphP-based constructs. These include time-resolved fluorescence lifetime measurements, expression in bacterial periplasmic space, screening of mutant libraries in yeast and mammalian cells using shuttle vectors and inducible somatic hypermutations.

The resulting NIR probes and molecular tools are tested in mouse models and applied to various *in vivo* studies. These NIR constructs extend optical methods to multicolor deep-tissue imaging, cell and tissue labeling, photoactivation and tracking, and detection of enzymatic activities and protein interactions in cells, tissues and whole mammals. The NIR optogenetic tools allow light-manipulations of cellular processes directly through the skin of living animals.

Selected Publications (for the past 5 years)

- Kasatkina L.A., Ma C., Sheng H., Lowerison M., Menozzi L., Baloban M., Tang Y., Xu Y., Humayun L., Humayun L., Vu T., Song P., Yao J. and Verkhusha V.V. Deep-tissue high-sensitivity multimodal imaging and optogenetic manipulation enabled by biliverdin reductase knockout. ***Nature Communications*** **2025**, 16: in press.
- Leopold A.V. and Verkhusha V.V. Engineering signaling pathways in mammalian cells. ***Nature Biomedical Engineering*** **2024,** 8: 1523-1539.
- Barykina N.V., Carey E., Oliinyk O.S., Nimmerjahn A. and Verkhusha V.V. Destabilized near-infrared fluorescent nanobodies enable background-free targeting of GFP-based biosensors for imaging and manipulation. ***Nature Communications*** **2024,** 15: 7788.
- Oliinyk OS, Ma C, Pletnev S, Baloban M, Taboada C, Sheng H, Yao J, Verkhusha VV. Deep-tissue SWIR imaging using rationally designed small red-shifted near-infrared fluorescent protein. ***Nature Methods*** **2023,** 20: 70-74.
- Kasatkina L.A., Ma C., Matlashov M.E., Vu T., Li M., Kaberniuk A.A., Yao J., and Verkhusha V.V. Optogenetic manipulation and photoacoustic imaging using a near-infrared transgenic mouse model. ***Nature Communications*** **2022**, 13: 2813.
- Oliinyk O.S., Baloban M., Clark C.L., Carey E., Pletnev S., Nimmerjahn A., and Verkhusha V.V. Single-domain near-infrared protein provides a scaffold for antigen-dependent fluorescent nanobodies. ***Nature Methods*** **2022**, 19: 740-750.
- Leopold AV, Thankachan S, Yang C, Gerashchenko D, and Verkhusha VV. A general approach for engineering RTKs optically controlled with far-red light. ***Nature Methods*** **2022,** 19: 871-880.
- Shemetov A.A., Monakhov M.V., Zhang Q., Canton-Josh J.E., Kumar M., Chen M., Matlashov M.M., Li R., Yang W., Nie L., Shcherbakova D.M., Kozorovitskiy Ye., Yao J., Ji N., and Verkhusha V.V. A near-infrared genetically encoded calcium indicator for *in vivo* imaging. ***Nature Biotechnology*** **2021**, 39: 368-377.
- Kaberniuk A.A., Baloban M., Monakhov M.V., Shcherbakova D.M. and Verkhusha V.V. Single-component near-infrared optogenetic systems for gene transcription regulation. ***Nature Communications*** **2021**, 12: 3859.
- Manoilov K.Y., Verkhusha V.V., and Shcherbakova D.M. A guide to the optogenetic regulation of endogenous molecules. ***Nature Methods*** **2021**, 18: 1027–1037.
- Redchuk T.A., Karasev M.M., Donnelly S.K., Hülsemann M., Virtanen J., Moore H.M., Vartiainen M.K., Hodgson L. and Verkhusha V.V. Optogenetic regulation of endogenous proteins. ***Nature Communications*** **2020**, 11: 605.
- Matlashov M.E., Shcherbakova D.M., Alvelid J., Baloban M., Pennacchietti F., Shemetov A.A., Testa I. and Verkhusha V.V. A set of monomeric near-infrared fluorescent proteins for multicolor imaging across scales. ***Nature Communications*** **2020**, 11: 239.

JAN VIJG, Ph.D.

Genome Dynamics in Aging

Aging is a universal process that brings life to a close at a rate that is specific for the species. In humans, life span has a limit of about 115 years. One process that has been implicated as a causal factor in the aging process is genome instability. Exactly how loss of genome integrity in normal somatic cells may lead to tissue degeneration, functional decline and increased risk of diseases, such as cancer, remains unknown. The main challenge in this respect is the lack of technology to analyze various types of DNA mutations in normal somatic cells. In the past we developed transgenic reporter systems in mouse and fruit fly, which allowed us to determine tissue-specific frequencies of various forms of genome instability, e.g., point mutations, deletions, translocations, as a function of aging. More recently, we developed single-cell and single-molecule, whole genome sequencing methods to analyze these same types of mutations directly in normal cells and tissues. These and other methods, e.g., single-cell DNA methylomics and single-cell multi-omics, are now being used to comprehensively characterize the landscape of mutations and epimutations in relation to the aging process.

<u>**Selected Publications:**</u>

Bahar R, Hartmann CH, Rodriguez KA, Denny AD, Busuttil RA, Dollé MET, Calder RB, Chisholm GB, Pollock BH, Klein CA, Vijg J. Increased cell-to-cell variation in gene expression in aging mouse heart. *Nature* 2006;441:1011-1014.

Vijg J, Campisi J. Puzzles, promises and a cure for ageing. *Nature* 2008;454: 1065.

White RR, Milholland B, de Bruin A, Curran S, Laberge RM, van Steeg H, Campisi J, Maslov AY, Vijg J. Controlled induction of DNA double-strand breaks in the mouse liver induces features of tissue ageing. Nat Commun. 2015;6:6790.

Gravina S, Dong X, Yu B, Vijg J. Single-cell genome-wide bisulfite sequencing uncovers extensive heterogeneity in the mouse liver methylome. Genome Biol. 2016;17:150.

Dong X, Milholland B, Vijg J. Evidence for a limit to human lifespan. Nature 2016; 538:257–259. PMID:27706136.

Dong X, Zhang L, Milholland B, Lee M, Maslov AY, Wang T, Vijg J. Accurate identification of single-nucleotide variants in whole-genome-amplified single cells. Nat Methods 2017;14:491-493. PMC5408311

Zhang L, Dong X, Lee M, Maslov AY, Wang T, Vijg J. Single-cell whole-genome sequencing reveals the functional landscape of somatic mutations in B lymphocytes across the human lifespan. Proc Natl Acad Sci USA 2019;116:9014-9019. PMC650011

Brazhnik K, Sun S, Alani O, Kinkhabwala M, Wolkoff AW, Maslov AY, Dong X, Vijg J. Single-cell analysis reveals different age-related somatic mutation profiles between stem and differentiated cells in human liver. Sci Adv. 2020;6:eaax2659. PMC6994209

Vijg J, Dong X. Pathogenic Mechanisms of Somatic Mutation and Genome Mosaicism in Aging. Cell 2020;18:12-23.

Zhang L, Dong X, Tian X, Lee M, Ablaeva J, Firsanov D, Lee S-G, Maslov AY, Gladyshev VN, Seluanov A, Gorbunova V, Vijg J. Maintenance of genome sequence integrity in long- and short-lived rodent species. Sci Adv 2021;7:eabj3284. PMC8550225

Huang Z, Sun S, Lee M, Maslov AY, Shi M, Waldman S, Marsh A, Siddiqui T, Dong X, Peter Y, Sadoughi A, Shah C, Ye K, Spivack SD, Vijg J. Single-cell analysis of somatic mutations in human bronchial epithelial cells in relation to aging and smoking. Nat. Genet. 2022;54:492-498.

TAO WANG, Ph.D.

Statistical Genetics and Genomics

The research field of my group is statistical genetics and genomics, with a strong focus on the analysis of genetic and genomic data from large-scale population based studies. Our research is focused on two highly related areas: the development of statistical genetics/genomics methodology and the application of statistical genetics/genomics methods to understand the complex genetic basis of common human diseases. Specifically, we are interested in developing statistical methods for multi-locus association analysis, multivariate genetic association analysis, family-based genetic association analysis, gene-gene and gene-environment analysis, genetic meta-analysis, and the estimation of genetic heritability and co-heritability between traits. Moreover, we have collaborated with scientists in many genetic/epigenetic studies of a variety of diseases, which include but are not limited to, congenital heart defects, aging, autism, cardiovascular diseases and cancers.

Recent publications:
Wang T, Lin CY, Rohan TE, Ye K. Resequencing of pooled DNA for detecting disease associations with rare variants. Genetic epidemiology. 2010; 34(5):492-501. NIHMSID: NIHMS587140 PubMed [journal] PMID: 20578089, PMCID: PMC4096227
Wang T, Pradhan K, Ye K, Wong LJ, Rohan TE. Estimating allele frequency from next-generation sequencing of pooled mitochondrial DNA samples. Frontiers in genetics. 2011; 2:51. PubMed [journal] PMID: 22303347, PMCID: PMC3268604
Wang T, Rohan TE, Gunter MJ, Xue X, Wactawski-Wende J, Rajpathak SN, Cushman M, Strickler HD, Kaplan RC, Wassertheil-Smoller S, Scherer PE, Ho GY. A prospective study of inflammation markers and endometrial cancer risk in postmenopausal hormone nonusers. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2011; 20(5):971-7. NIHMSID: NIHMS279677 PubMed [journal] PMID: 21415362, PMCID: PMC3096873
Ahn S, Wang T. A powerful statistical method for identifying differentially methylated markers in complex diseases. Pacific Symposium on Biocomputing. Pacific Symposium on Biocomputing. 2013; :69-79. NIHMSID: NIHMS441925 PubMed [journal] PMID: 23424113, PMCID: PMC3621641
Wang T, Zhou B, Guo T, Bidlingmaier M, Wallaschofski H, Teumer A, Vasan RS, Kaplan RC. A robust method for genome-wide association meta-analysis with the application to circulating insulin-like growth factor I concentrations. Genetic epidemiology. 2014; 38(2):162-71. NIHMSID: NIHMS592219 PubMed [journal] PMID:24446417, PMCID: PMC4049273
Guo T, Chung JH, Wang T, McDonald-McGinn DM, Kates WR, Hawuła W, Coleman K, Zackai E, Emanuel BS, Morrow BE. Histone Modifier Genes Alter Conotruncal Heart Phenotypes in 22q11.2 Deletion Syndrome. American journal of human genetics. 2015; 97(6):869-77. PubMed [journal] PMID: 26608785, PMCID: PMC4678435
Agalliu I, Wang T, Burk RD. β- and γ-Human Papillomavirus Types and Smoking in Head and Neck Cancer-Reply. JAMA oncology. 2016; 2(5):687-8. PubMed [journal] PMID: 27244681
Loudig O, Wang T, Ye K, Lin J, Wang Y, Ramnauth A, Liu C, Stark A, Chitale D, Greenlee R, Multerer D, Honda S, Daida Y, Spencer Feigelson H, Glass A, Couch FJ, Rohan T, Ben-Dov IZ. Evaluation and Adaptation of a Laboratory-Based cDNA Library Preparation Protocol for Retrospective Sequencing of Archived MicroRNAs from up to 35-Year-Old Clinical FFPE Specimens. International journal of molecular sciences. 2017; 18(3). PubMed [journal] PMID: 28335433, PMCID: PMC5372640
Dong X, Zhang L, Milholland B, Lee M, Maslov AY, Wang T, Vijg J. Accurate dentification of single-nucleotide variants in whole-genome-amplified single cells. Nature methods. 2017; 14(5):491-493. NIHMSID: NIHMS855609 PubMed [journal] PMID: 28319112, PMCID: PMC5408311

MELISSA WASSERSTEIN, Ph.D.

Clinical Research on Rare Genetic Disorders

As a clinical biochemical geneticist, my research focuses on optimizing the outcome of individuals with rare genetic disorders through expanded newborn screening, natural history studies, therapeutic clinical trials, and implementation of genomic diagnostics in diverse populations.

I am the Principal Investigator of ScreenPlus, the largest consented pilot newborn screening program in country. ScreenPlus will screen 175,000 consented infants for an additional 14 disorders on top of the routine newborn screening panel, utilizing a novel multi-tiered assay that may enhance accuracy, reduce false positives, and perhaps help with phenotypic severity prediction. ScreenPlus also includes an exploration of the ethical issues associated with newborn screening for complex conditions.

In addition, I have been studying the natural history of acid sphingomyelinase deficiency with a focus on defining endpoints for therapeutic clinical trials. We are now in clinical trial to assess the safety and effectiveness of recombinant human acid sphingomyelinase in adults with this rare disorder.

Another research interest is evaluating the implemention of genomic medicine in underserved populations through my role as an MPI in NYCKidSeq, a multi-site project in the CSER Consortium. In collaboration with partners at Mount Sinai and the New York Genome Center, NYCKidSeq is focused on implementing diagnostic whole genome sequencing in a diverse population of children with rare disorders, evaluating the utility of a novel educational tool for genetic counseling, and utilizing bioinformatic tools to allow improved diagnostic yield.

<u>**Selected Recent Publications**</u>

- **Wasserstein M,** Lachman R, Hollak C, Arash-Kaps L, Barbato A, Gallagher RC, Giugliani R, Guelbert NB, Ikezoe T, Lidove O, Mabe P, Mengel E, Scarpa M, Senates E, Tchan M, Villarrubia J, Chen Y, Furey S, Thurberg BL, Zaher A, Kumar M. A randomized, placebo-controlled clinical trial evaluating olipudase alfa enzyme replacement therapy for chronic acid sphingomyelinase (ASMD) in adults: One-year results. Genet Med. 2022 Jul;24(7):1425-1436.

- **Wasserstein MP**, Orsini JJ, Goldenberg A, Caggana M, Levy PA, Breilyn M, Gelb MH. The future of newborn screening for lysosomal disorders. Neurosci Lett. 2021 Jun 22;760:136080.

- Huizing M, Hackbarth ME, Adams DR, **Wasserstein M**, Patterson MC, Walkley SU, Gahl WA; FSASD Consortium, Adams DR, Dobrenis K, Foglio J, Gahl WA, Gasnier B, Hackbarth M, Huizing M, Lek M, Malicdan MCV, Paavola LE, Patterson MC, Reimer R, Walkley SU, Wasserstein M, Wang RY, Zoncu R. Mini-Review Free Sialic Acid Storage Disorder: Progress and Promise. Neurosci Lett. 2021 Apr 13:135896

- Pearson NM, Stolte C, Shi K, Beren F, Abul-Husn NS, Bertier G, Brown K, Diaz GA, Odgis JA, Suckiel SA, Horowitz CR, **Wasserstein M**, Gelb BD, Kenny EE, Gagnon C, Jobanputra V, Bloom T, Greally JM. GenomeDiver: a platform for phenotype-guided medical genomic diagnosis. Genet Med. 2021 Jun 10.

- **Wasserstein M**, Dionisi-Vici C, Giugliani R, Hwu WL, Lidove O, Lukacs Z, Mengel E, Mistry PK, Schuchman EH, McGovern M. Recommendations for clinical monitoring of patients with acid sphingomyelinase deficiency (ASMD). <u>Mol Genet Metab.</u> 2019 Feb;126(2):98-105

- Suckiel SA, Odgis JA, Gallagher KM, Rodriguez JE, Watnick D, Bertier G, Sebastin M, Yelton N, Maria E, Lopez J, Ramos M, Kelly N, Teitelman N, Beren F, Kaszemacher T, Davis K, Laguerre I, Richardson LD, Diaz GA, Pearson NM, Ellis SB, Stolte C, Robinson M, Kovatch P, Horowitz CR, Gelb BD, Greally JM, Bauman LJ, Zinberg RE, Abul-Husn NS, **Wasserstein MP**, Kenny EE. GUÍA: a digital platform to facilitate result disclosure in genetic counseling. Genet Med. 2021 Feb 2.

DANIEL A. WEISER, MD

Childhood Cancer Translational Research

The Weiser laboratory is focused on childhood cancer research with a goal of elucidating the underlying biology of the most aggressive malignancies. In such patients with typically incurable cancer, we are striving to identify new approaches to and types of treatment. We have multiple ongoing projects:

+ Identification of biologic drivers of neuroblastoma at ultra-high risk for treatment failure. Neuroblastoma is one of the most common and deadly childhood cancers. Despite intensive research, there are limited therapeutic strategies for patients with *de novo* chemotherapy resistance. We have been studying neuroblastoma since 2009 and are identifying additional biologic drivers of highly lethal tumors. We assess features (genetic, transcriptomic, proteomic, histologic) from patients with early death from tumor progression compared with tumor features from those with a maintained complete response. This guides our workup of potential oncogenic targets and discovery of novel therapies for patients, including selinexor, a pharmacologic Exportin-1 (XPO1) inhibitor that limits nuclear export of key regulatory proteins in cancer cells.

+ Evaluation of novel combinatorial treatment approaches in neuroblastoma. Our lab works with multiple international clinical and research consortium groups to perform preclinical studies that are part of the essential pipeline for opening of human clinical studies.

+ Repurposing of tenofovir, a reverse transcriptase inhibitor used in HIV, for treatment of neuroblastoma. We are exploring novel ways to target telomerase, the enzyme that maintains telomere length, for treatment of the most highly aggressive neuroblastoma.

+ Detection of circulating tumor DNA in fusion-negative sarcomas. With no reliable non-invasive approach for disease monitoring during and after treatment, we are applying next-generation sequencing and bioinformatics approaches to identify circulating tumor material with blood-based "liquid" biopsies. Our lab is the receiving and testing site for a nationwide clinical study to evaluate our methods and technology for integration into routine clinical care.

+ MYC transcript targeting in neuroblastoma and osteosarcoma. High *MYC* expression is associated with inferior outcome. We are developing a novel approach to pharmacologically target AU-rich elements in the 3'UTR to decrease and inactivate overabundant MYC transcript in cancer cells.

+ Prevention of cisplatin-induced ototoxicity in children with cancer. Sensorineural hearing loss, which can be severely debilitating, is one of many untoward effects of chemotherapy. We have developed a proprietary acetophenone compound to prevent cisplatin-induced ototoxicity without compromising anti-tumor activity. Pre-clinical testing is ongoing.

Lab website: https://sites.google.com/view/weiserlab/

<u>**Select publications:**</u>

- **Weiser DA**, Hayashi M, Applebaum MA. Breaking up isn't hard to do: isolating cell-free DNA fragments in osteosarcoma. *Clinical Cancer Research*. 2023 Jun 1;29(11):2017-2019. PMID 36976253.
- Geohagen B, Zeldin E, Reidy K, Wang T, Gavathiotis E, Fishman YI, LoPachin R, Loeb DM, **Weiser DA**. Acetophenone Protection Against Cisplatin-Induced End-Organ Damage. *Translational Oncology*. 2023 Jan. PMID 36477009.
- Galinski B, Luxemburg M, Landesman Y, Pawel B, Johnson KJ, Master SR, Freeman KW, Loeb DM, Hebert JM, **Weiser DA**. XPO1 inhibition with selinexor synergizes with proteasome inhibition in neuroblastoma by targeting nuclear export of IkB. *Translational Oncology*. 2021 May 8;14(8):101114. PMID 33975179.
- Rybinski B, Wolinsky T, Brohl A, Moerdler S, Reed DR, Ewart M, **Weiser DA.** Multifocal primary neuroblastoma tumor heterogeneity in siblings with co-occurring *PHOX2B* and *NF1* genetic aberrations. *Genes, Chromosomes and Cancer*. 2020 Feb;59(2):119-124. PMID 31515834.
- Geohagen B, **Weiser DA**, Loeb DM, Nordstroem LU, LoPachin RM. Enolate-forming compounds provide protection from platinum neurotoxicity. *Chemico-Biological Interactions*. 2020 Feb 1;317:108961. PMID 31978392.
- Rybinski B, Hosgood HD, Weiner SL, **Weiser DA**. Preclinical metrics correlate with drug activity in phase II trials of targeted therapies for non-small cell lung cancer. *Frontiers in Oncology*. 2020 Nov 5; 10:587377. PMID 33251146.
- Barris DM, Weiner SB, Dubin RA, Fremed M, Zhang X, Piperdi S, Zhang W, Maqbool S, Gill J, Roth M, Hoang B, Geller D, Gorlick R, **Weiser DA**. Detection of circulating tumor DNA in osteosarcoma. *Oncotarget*. 2018 Jan 18;9(16):12695. PMID 29560102.
- Niazi MKK, Chung JH, Heaton-Johnson KJ, Martinez D, Castellanos R, Irwin M, Master S, Pawel BR, Gurcan MN, **Weiser DA**. Advancing clinicopathologic diagnosis of high-risk neuroblastoma using computerized image analysis and proteomic profiling. *Pediatric and Developmental Pathology*. 2017 Sep-Oct;20(5):394. PMID 28420318.
- Bresler SC*, **Weiser DA***, Huwe PJ*, Park JH, Krytska K, Ryles H, Laudenslager M, Rappaport EF, Wood AC, McGrady PW, Hogarty MD, London WB, Radhakrishnan R, Lemmon MA, Mossé YP. ALK mutations confer differential oncogenic activation and sensitivity to ALK inhibition therapy in neuroblastoma. Cancer Cell. 2014 Nov 10;26(5):682-94. PMID: 25517749.

ZHENGDONG ZHANG, Ph.D.

Computational and Systems Biology of Cancer Metastasis and Human Aging

With recent resource and technology development, biology has entered a new data-driven phase in the 21st century. The research interest of my lab is computational biology and bioinformatics, focusing on algorithm development, data integration, and software implementation. With the advent of new DNA sequencing technologies, it is a particularly challenging and exciting time now to do such computational work, as more and more biological data are being generated at an ever-accelerating speed.

Gene expression in living cells is under strict spatial and temporal control, and its dysregulation is the direct cause of many human diseases. The primary focus of research in my lab is gene expression and its regulation, for which we take an integrated approach to study the following aspects on the whole genome scale:
- Gene expression profiles
- Transcriptional regulation of gene expression
- Epigenetic mechanisms and long range control of gene expression
- Gene copy number variation

The biological systems currently under investigation are breast cancer metastasis and human aging.
- <u>Breast cancer metastasis</u> is a complex multi-step process during which tumor cells spread from the primary tumor mass to distant organs. To study the genetic and biochemical determinations of this deadly aspect of cancer progression, we analyze various microarray and sequencing profiles to discover its regulatory sub-networks, DNA binding of key regulators, and copy number variations during the progression. This research project is supported by a grant from NIH/NLM.
- For reasons significant to individuals and the society as a whole, <u>human aging</u> is of great interest not only to the academic community but also to medicine and the public in general. However, despite much research progress made over the years, it still remains a poorly understood biological process. To gain novel insights, we use a systems-biology approach to analyze aging-related genes in the context of biological networks. This research project is supported by a New Scholar Award from the Ellison Medical Foundation.

Lab web site: www.zdzlab.org

Recent Publications:

MacArthur DG, Balasubramanian S, Frankish A, Huang N, Morris J, Walter K, Jostins L, Habegger L, Pickrell JK, Montgomery SB, Albers CA, Zhang ZD, et al (2012) A systematic survey of loss-of-function variants in human protein-coding genes. *Science*, **335**:806-807.

Zhang ZD, Du J, Lam H, Abyzov A, Urban A, Snyder M, Gerstein M (2011) Identification of genomic indels and structural variations using split reads. *BMC Genomics*, **12**:375.

Zhang ZD, Gerstein MB (2010) Detection of copy number variation from array intensity and sequencing read depth using a stepwise Bayesian model. *BMC Bioinformatics*, **11**:539

Zhang ZD, Frankish A, Hunt T, Harrow J, Gerstein M. (2010) Identification and analysis of unitary pseudogenes: historic and contemporary gene losses in humans and other primates. *Genome Biol.*, **11**, R26

Du J, Bjornson RD, Zhang ZD, Kong Y, Snyder M, Gerstein MB. (2009) Integrating Sequencing Technologies in Personal Genomics: Optimal Low Cost Reconstruction of Structural Variants. *PLoS Comput Biol.*, **5**, e1000432.

Zhang ZD, Nayar M, Ammons D, Rampersad J, Fox GE. (2009) Rapid in vivo exploration of a 5S rRNA neutral network. *J. Microbiological Methods*, **76**, 181-187.

Zhang ZD, Cayting P, Weinstock G, Gerstein M. (2008) Analysis of Nuclear Receptor Pseudogenes in Vertebrates: How the Silent Tell Their Stories. *Mol Biol Evol.*, **25**, 131-143.

Zhang ZD, Weinstock G, Gerstein M. (2008) Rapid Evolution by Positive Darwinian Selection in T-Cell Antigen CD4 in Primates. *J. Mol. Evol.*, **66**, 446-456.

Zhang ZD, Rozowsky J, Snyder M, Chang J, Gerstein M. (2008) Modeling ChIP Sequencing In Silico with Applications. *PLoS Comput Biol.*, **4**, e1000158.

DEYOU ZHENG, Ph.D.

Bioinformatics and Computational Genomics

The research field of my group is Computational Genomics and Bioinformatics, with a strong focus on mining large-scale experimental genomic data to decipher the function of the human genome and the genomes of other model organisms. We develop and apply computational techniques for integrating data of comparative genomics and functional genomics (and epigenomics) to decode the structure, function, and evolution of the human genome. More generally, we are interested in bioinformatic and statistical approaches for exploiting novel and biologically significant patterns in big genomic data. Recently, we have become more focused on the expression, regulation, and evolution of genes (both coding or non-coding) that play important roles in the development, specification, maturation, and maintenance of human neural system and heart. We also have a strong interest in understanding the genetic and epigenetic mechanisms contributing to cancer, including microenvironment. Working extensively with experimentalists and by deep sequencing of the transcriptomes in human neurons, mouse hearts, or various cancers at bulk and single cell levels, our bioinformatics studies have led to many interesting findings that contribute critical information to cancer, heart development, neuronal development, and brain disorders or diseases. Please visit this for more: https://einstein.pure.elsevier.com/en/persons/deyou-zheng

Selected publications:

Zheng, D*, Zhao, K and Mehler, M. (2009) Profiling RE1/REST-mediated histone modifications in the human genome. *Genome Biol* 10:R9. (*corresponding author)

Guo X, Zhang Z, Gerstein MB, **Zheng D**. (2009). Small RNAs originated from pseudogenes: cis- or trans-acting? *PLoS Comput Biol* 5(7): e1000449.

Goldberg AD, Banaszynski LA, Noh KM, Lewis PW, Elsaesser SJ, Stadler S, Dewell S, Law M, **Guo X,** Li X, Wen D, Chapgier A, DeKelver RC, Miller JC, Lee YL, Boydston EA, Holmes MC, Gregory PD, Greally JM, Rafii S, Yang C, Scambler PJ, Garrick D, Gibbons R, Higgs DR, Cristea IM, Urnov FD, **Zheng D***, Allis CD* (2010). Distinct factors control histone variant H3.3 localization at specific genomic regions. *Cell* 140:678-691. (*co-corresponding authors)

Lin M, Pedrosa E, Shah A, Hrabovsky A, Maqbool S, **Zheng D,** Lachman HM. (2011). RNA-Seq of human neurons derived from iPS cells reveals candidate long non-coding RNAs involved in neurogenesis and neuropsychiatric disorders. *PLoS ONE* 6: e23356.

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Adam RC, Yang H**, Rockowitz S,** Larsen SB, Nikolova M, Oristian DS, Polak L, Kadaja M, Asare A, **Zheng D,** Fuchs E**.** (2015). Pioneer factors govern super-enhancer dynamics in stem cell plasticity and lineage choice. *Nature* 521:366-370

Rockowitz S, Zheng D. (2015). Significant expansion of the REST/NRSF cistrome in human versus mouse embryonic stem cells: potential implications for neural development. *Nucleic Acids Res*. 43:5730-5743.

Wang P, Lin M, Pedrosa E, Hrabovsky A, Zhang Z, Guo W, Lachman HM***, Zheng D*.** (2015). CRISPR/Cas9-mediated heterozygous knockout of the autism gene CHD8 and characterization of its transcriptional networks in neurodevelopment. *Mol Autism* 6:55. (*co-corresponding authors)

Lin M, Lachman HM**, Zheng D.** (2016). Transcriptomics Analysis of iPSC-derived Neurons and Modeling of Neuropsychiatric Disorders. *Mol Cell Neurosci* 73:32-42

Zhao D, Lin M, Pedrosa E, Lachman HM**, Zheng D**. (2017). Characteristics of allelic gene expression in human brain cells from single cell RNA-seq data analysis. *BMC Genomics* 18:860

Wang P, Zhao D, Lachman HM**, Zheng D**. (2018). Enriched expression of genes associated with autism spectrum disorders in human inhibitory neurons. *Transl Psychiatry* 8:13.

Liu Y, Lu P, Wang Y, Morrow BE, Zhou B**, Zheng D.** (2019) Spatiotemporal Gene Coexpression and Regulation in Mouse Cardiomyocytes of Early Cardiac Morphogenesis. *J Am Heart Assoc.* 8(15):e012941.

Liu Y, Singh VK, Zheng D. (2020) Stereo3D: using stereo images to enrich 3D visualization. *Bioinformatics* 36:4189-4190.

Galbo PM, Zang X*, **Zheng D***. (2021) Molecular features of cancer-associated fibroblast subtypes and their implication on cancer pathogenesis, prognosis, and immunotherapy resistance. *Clin Cancer Res*. 27:2636-2647. (*co-corresponding authors)

Liu Y, Wang T, Zhou B**, Zheng D**. (2021) Robust integration of multiple single-cell RNA sequencing datasets using a single reference space. *Nat Biotechnol*. 39: 877-884.

Galbo PM, Madsen AT**, Liu Y,** Peng M, Wei Y, Ciesielski MJ, Fenstermaker RA, Graff S, Montagna C, Segall JE, Sidoli S, Zang X*, **Zheng D***. (2024) Functional Contribution and Clinical Implication of Cancer-Associated Fibroblasts in Glioblastoma. *Clin Cancer Res*. 30:865-876 (*co-corresponding authors)

Ferrena A, Wang J, Zhang R, Karadal-Ferrena B, Al-Hardan W, Singh S, Borjihan H, Schwartz EL, Zhao H, Oktay MH, Yang R, Geller DS, Hoang BH***, Zheng D***. (2024) SKP2 knockout in Rb1/p53 deficient mouse models of osteosarcoma induces immune infiltration and drives a transcriptional program with a favorable prognosis. *Mol Cancer Ther* 23:223-234. (*co-corresponding authors)

Astorkia M, Liu Y, Pedrosa EM, Lachman HM***, Zheng D***. (2024) Molecular and network disruptions in neurodevelopment uncovered by single cell transcriptomics analysis of *CHD8* heterozygous cerebral organoids. *Heliyon* 10:e34862 (co-corresponding authors)

Ferrena A, Zheng XY, Jackson K, Hoang B, Morrow BE, **Zheng D**. (2024) scDAPP: a comprehensive single-cell transcriptomics analysis pipeline optimized for cross-group comparison. *NAR Genom Bioinform*. 6:lqae134.

BIN ZHOU, M.D., Ph.D.

Heart Development, Aging, Disease, and Repair

We study factors and mechanisms that control heart development to understand the pathogenesis of congenital heart disease as well as to gain insights into potential repairing mechanisms to combat heart disease. We use mice, mouse and human stem cells as model systems in our research. We apply an integrated approach of genetics, developmental, molecular and systems biology and advanced single cell technologies and CRISPR gene editing to address three major questions: (1) How individual cardiac cells and lineages are specified, maintained, or diversified during cardiac development, disease, aging, or regeneration? (2) How cell-cell, or cell-environment communications are modulated to control cardiac functions under these conditions? (3) How fetal cardiac gene program is controlled during development and reactivated in the diseased heart?

For more details, please visit (https://einsteinmed.org/faculty/11217/bin-zhou).

Selected Publications:

Lu P, Wu B, Wang Y, Zhang J., Zhou B. A sandwiched ventricular explant assay to model mouse coronary angiogenesis ex vivo. STAR Protoc. 2023 Oct 27;4(4) doi: 10.1016/j.xpro.2023.102619

Wu B, Wu B, Benkaci S, Shi L, Lu L, Park T, Morrow BE, Wang Y, Zhou B. Crk and Crkl are required in the endocardial lineage for heart valve development. J Am Heart Assoc. 2023 Sep 19;12(18):e029683. doi: 10.1161/JAHA.123.029683

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Lu P, Wu B, Feng X, Cheng W, Kitsis RN, Zhou B. (2022) Cardiac Myosin Heavy Chain Reporter Mice to Study Heart Development and Disease. Circ Res https://doi.org/10.1161/CIRCRESAHA.122.321461 Circ Res 2022;0:10.1161/CIRCRESAHA.122.321461

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Wang Y, Lu P, Wu B, Riascos-Bernal DF, Sibinga NES, Valenta T, Basler K, Zhou B. (2018) Myocardial β-Catenin-BMP2 signaling promotes mesenchymal cell proliferation during endocardial cushion formation
J Mol Cell Cardiol 123:150-158

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Wu B, Wang Y, Xiao F, Butcher JT, Yutezy KE, Zhou B. (2017) Developmental mechanisms in aortic valve malformation and disease. Annu Rev Physiol 79:21-41

Zhang D, Wu B, Wang P, Wang Y, Nechiporuk T, Floss T, Greally JM, Zheng D, Zhou B. (2017) Non-CpG methylation by DNMT3B facilitates REST binding and gene silencing in developing mouse hearts. Nucleic Acids Res 45:3102-3115

Wang, Y, Wu B, Farrar E, Alfieri CM, Lui, W, Lu P, Zhang D, Mao K, Chu M, Yang D, Xu D, Rauchman M, Taylor V, Yutzey KE, Butcher JT, Zhou B. (2017) Notch-Tnf signaling is required for development and homeostasis of arterial valves. Eur Heart J 38:675-686

Wu B, Zhang Z, Lui W, Chen X, Moreno-Rodriquez RA, Wang Y, Chamberlain A, Markwald RA, O'Rourke B, Sharp DJ, Lenz J, Baldwin HS, Chang CP, Zhou B. (2012) Endocardial Cells Form the Coronary Arteries by Angiogenesis through Myocardial to Endocardial VEGF Signaling. Cell 151:1083-1096.

Wu B, Wang Y, Lui W, Langworthy M, Tompkins KL, Hatzopoulos AK, Baldwin, HS, Zhou B. (2011) Nfatc1 Coordinates valve endocardial cell lineage development required for heart valve formation. Circ Res 109:183-192.