National Institutes of Health

WORKSHOP PROGRAM

National Institute on Aging

AGENDA

Virtual Workshop: Measures of Somatic Mutation-related Clonal Hematopoiesis in Humans: Enhancing Contributions to Clinical, Epidemiologic, and Genetic Aging Studies

Sponsored by NIA's Division of Geriatrics and Clinical Gerontology and Division of Aging Biology

March 24, 2021: 11am-4pm, ET
March 25, 2021: 11am-2:45pm, ET
Register for the workshop

Day 1

Time	Topic	Presenter
11:00 – 11:10 am	Welcome and Introductions	NIA
11:10 – 11:20 am	Workshop Goals	Dr. Evan Hadley NIA DGCG
11:20 – 11:50 am	Keynote Presentation: Somatic Mutations, CHIP, and Aging	Dr. Ken Walsh University of Virginia

Session I: CH Mutations and Association to Aging Phenotypes

Time	Topic	Presenter
11:50–12:10 pm	Age Related Clonal Hematopoiesisand Adverse Effects	Dr. Siddhartha Jaiswal Stanford University
12:10–12:30 pm	Influences on CH Outcomes:Time and Mutation Type	Dr. Margaret Goodell Baylor College of Medicine
12:30–12:50 pm	CVD and CHIP	Dr. Pradeep Natarajan Massachusetts General Hospital
12:50 –1:15 pm	Question and Answer Discussion	All
1:15 – 2:00 pm	Lunch Break	

Session II. Sequencing Technologies and Clinical Specimens

Time	Topic	Presenter
2:00 – 2:20 pm	Error–Corrected Sequencing to Characterize Clonal Hematopoiesisin Humans from Birth to Death	Dr. Todd Druley Washington University
2:20 – 2:40 pm	Computational Framework to Identify Mosaic/Somatic Chromosomal Alterations fromGenetic Data	Dr. Giulio Genovese Broad Institute of Harvardand MIT

Time	Topic	Presenter
2:40 – 3:00 pm	Single-cell Whole Genome Sequencing and the Pathogenic Mechanisms of Somatic Mutationsin Aging	Dr. Jan Vijg Albert Einstein College of Medicine
3:00 – 3:20 pm	Mapping the Prevalence of Clonal Hematopoiesis in Cell-free DNA using Ultra-deep Sequencing	Dr. Oliver Venn GRAIL, Inc Discussant – Dr. Steve Cummings University of California
3:20 – 4:00 pm	Question and Answers/Discussion	
4:00 pm	Adjourn	

Day 2

Time	Topic	Presenter
11:00-11:10 am	Welcome and Introductions	NIA

Session III: Clinical Applications of CHIP Analyses in Longevity Studies andTranslational Geroscience

Time	Topic	Presenter
11:10-11:30 am	Prognostic Relevance of CHIP	Dr. Jaroslaw Maciejewski Cleveland Clinic
11:30-11:50 am	Dynamic Clonal Hematopoiesis and Functional T-cell Immunity in a Supercentenarian	Dr. Erik B. van den Akker Leiden University Medical Centre
11:50-12:10 pm	Age ExWAS and CHIP	Dr. Alan Shuldiner Regeneron Pharmaceuticals
12:10-12:30 pm	Interplay between Inherited Germline Genetic Factors and Acquired Somatic Mutations and Contributionto Age-related Diseases	Dr. Alexander Bick Vanderbilt University
12:30-12:40 pm	Break	
12:40 – 1:00 pm	Rhesus Macaques as Natural and Engineered Models for Clonal Hematopoiesis of Aging	Dr. Cynthia Dunbar National Heart, Lung, andBlood Institute, National Institutes of Health
1:00 – 1:20 pm	Mice as a Model to Study Mechanisms of Age-related Clonal Expansion	Dr. Anastasia V. Shindyapina Brigham and Women's Hospital, Harvard Medical School

Time	Topic	Presenter
1:20 – 2:00 pm	Lunch Break	
2:00 – 2:45 pm	Wrap Up Discussion & Recommendations to NIA	
2:45 pm	Adjourn	

Alexander Bick, M.D., Ph.D.

Interplay between Inherited Germline Genetic Factors and Acquired Somatic Mutations and Contribution to Age-related Diseases

Abstract: Although we frequently think of our genome as fixed at conception, in fact our genome is incredibly dynamic over the human lifespan. For example, leukocyte telomeres shorten, mutations arise in hematopoietic stem cells leading to clonal hematopoiesis of indeterminate potential (CHIP), and mitochondria heteroplasmy evolves. In this talk I will describe recent work simultaneously analyzing germline and somatic whole genome sequence data to identify root causes of CHIP. We analyze high-coverage whole genome sequences from 97,691 participants of diverse ancestries in the NHLBI TOPMed program and identify 4,229 individuals with CHIP. We identified associations with blood cell, lipid, and inflammatory traits specific to different CHIP genes. Association of a genome-wide set of germline genetic variants identified five genetic loci associated with CHIP status, including one locus at TET2 that was African ancestry specific. In silico-informed in-vitro evaluation of the TET2 germline locus identified a causal variant that disrupts a TET2 distal enhancer leading to increased hematopoietic stem cell proliferation. Overall, we observe that germline genetic variation altering hematopoietic stem cell function and the fidelity of DNA-damage repair increase the likelihood of somatic mutations leading to CHIP.

Biography: Dr. Bick is a physician/scientist working in the field of human genomics. His scientific observations have advanced our understanding of the genetic basis for hematologic malignancy, characterized molecular disease mechanisms and identified both the promise and limitations of translating genomic findings into routine medical practice. He has a particular interest in understanding how the interplay between inherited germline genetic factors and acquired somatic mutations contributes to disease. Recently Dr. Bick has developed algorithms to identify clonal hematopoiesis at scale in hundreds of thousands of human genomes and used these datasets to characterize the causes and consequences of clonal hematopoiesis. In ongoing work, Dr. Bick leads efforts within the NHLBI TOPMed Program, the VA Million Veteran Program, the UK Biobank and the Vanderbilt BioVU Biobank to characterize the causes and consequences of clonal hematopoiesis. In parallel with his research, Dr. Bick has a clinical specialization in preventative genomic medicine. Dr. Bick sees patients with clonal hematopoiesis as part of his practice at the Vanderbilt Genetics and Therapeutics Clinic, aiming to translate what is learned from his research studies back to the care of his patients.

Steven Cummings, M.D.

Biography: Dr. Cummings is Professor of Medicine, Epidemiology and Biostatistics (emeritus) at the University of California, San Francisco, and Director of the San Francisco Coordinating Center in the California in the Pacific Medical Center Research Institute. Dr. Cummings has over 600 peer-reviewed publications, was elected to the National Academy of Medicine (formerly the IOM) in the National Academy of Sciences and honored with several international awards for his clinical research in osteoporosis. He has been the PI of the Longevity Consortium that has been funded by NIA for 16 years to conduct translational collaborative studies in the genetic and molecular basis of animal and human aging. He has been a PI on several large (3-10,000 participant) prospective cohort studies of aging with 20 to 40-year follow-up, including: the Study

of Osteoporotic Fractures for older women and MrOS for older men, and Health ABC that have analyzed risk factors and biomarkers of aging outcomes. Dr. Cummings is the lead PI of the Study of the new longitudinal Muscle, Mobility and Aging (SOMMA) of the cellular basis of aging that includes collection and archiving muscle, adipose tissue and blood cells with extensive phenotyping of 875 older adults. He also leads other studies of biomarkers for cell senescence and clinical outcomes of aging. He is a PI of a large prospective study funded by GRAIL, to use cell-free nucleic acid (cfNA) for early detection of cancer. Dr. Cummings is also an internationally recognized expert on osteoporosis and prevention of fractures and led or co-led the large pivotal clinical trials that won FDA approval for most of the drug treatments for osteoporosis, including alendronate, denosumab and zoledronate.

Todd Druley, M.D., Ph.D.

Error-Corrected Sequencing to Characterize Clonal Hematopoiesis in Humans from Birth to Death

Abstract: The observation that a small percentage of otherwise healthy adults acquire leukemia-associated clonal hematopoietic mutations as a function of age, known as clonal hematopoiesis of indeterminate potential (CHIP), was striking but based on sequencing technology with relatively poor resolution for mutations below an allele frequency of 0.02. The Druley lab developed an error-corrected sequencing (ECS) protocol in 2015 to computationally eliminate sequencing errors and provide validated resolution for mutations with allele frequencies of 0.0001, more than 100-fold less than the functional definition of CHIP. Using this strategy, we have demonstrated that, by age 50, nearly 100% of healthy adults harbor clonal hematopoietic mutations and these mutations preferentially engraft in bone marrow transplant recipients. Furthermore, children and young adults harbor clonal hematopoiesis far more frequently than previously appreciated, but in genes different than canonical CHIP. These studies have revealed the spectrum of clonal hematopoiesis throughout the human lifespan. Not all clonal hematopoietic mutations are created equal. While the clinical consequences of these very low allele-frequency mutations are variable and unclear, consequences are dictated by effect size and a host of additional factors such as age, gender, germline variability, co-morbidities and more. The prime example being clonal hematopoietic mutations in TET2, which correlate with atherosclerosis and cardiac dysfunction due to an aberrant inflammatory response. With this physiologic baseline of lifelong clonal hematopoiesis, future work will explore integration multiple data streams to identify correlations between clonal hematopoiesis, clinical disease, therapeutic morbidities and utility of longitudinal surveillance.

Biography: Dr. Todd Druley is the Chief Medical Officer for Somatic Oncology at Invitae since October 2020 after serving as the Chief Medical Officer of ArcherDX for nearly two years. He is also a board-certified pediatric hematologist/oncologist and Associate Professor of Pediatrics, Developmental Biology and Genetics at Washington University School of Medicine. He obtained a Bachelor's in Cell and Structural Biology and a minor in Chemistry from the University of Illinois in 1994. He then completed the MD/PhD program at the University of Illinois where he studied mechanisms of chemotherapy resistance. In 2002, Dr. Druley joined Washington University as a pediatric resident, then completing his fellowship in Pediatric Hematology and Oncology and joining the faculty in 2008. He is a member of the Children's Oncology Group (COG) Myeloid Disease and Epidemiology Committees. Research in the Druley Lab is based on improving molecular diagnostics in pediatric AML. Clinically, Dr. Druley has focused on pediatric cancer predisposition and established the region's first Pediatric Cancer Predisposition Program at

Siteman Kids / St. Louis Children's Hospital at Washington University in 2014.

Giulio Genovese, Ph.D.

Computational Framework to Identify Mosaic/Somatic Chromosomal Alterations from Genetic Data

Abstract: Clonal expansions become detectable in blood as we age when shared alterations become prevalent enough to make it possible to infer their presence from the genetic data. Chromosomal alterations, in the form of gains, losses, and CN-LOH events, are a type of genetic alteration that can be readily detected at cell fractions as low as 1% from DNA microarray data when using a statistical framework that leverages haplotype information. Dr. Giulio Genovese will present the basic ideas and details of this framework, implemented in the freely available software package MoChA, together with a few applications in the UK biobank, a cohort of almost 500k individuals from the United Kingdom. Mosaic autosomal alterations of somatic origin, almost virtually undetectable in blood-derived DNA of individuals below the age of 45, are detectable in more than 20% of individuals older than 80. He will show how these events, often precursors to more malignant versions leading to blood cancer diagnoses, show significant interplay and associations with inherited germline variants, with both cis and trans effects. Finally, he will show how the available MoChA framework can be used to inform single cell-RNA sequencing experiments to enhance the ability to detect mosaic chromosomal alterations in single cells.

Biography: Dr. Giulio Genovese is a Senior Computational Biologist at Stanley Center for Psychiatric Research at Broad Institute and Harvard Medical School. He is a mathematician by training and has become a geneticist by applying linkage analysis mathematical modeling to medical genetics. As a post-doctoral fellow, he performed analytic work to identify APOL1 as a major kidney disease susceptibility gene in Martin Pollak's lab. As a computational biologist, he designed a novel computational model to identify clonal expansions in blood from exome sequencing data under the supervision of Steven McCarroll. He further developed, together with his collaborator Po-Ru Loh, a novel statistical method to detect mosaic chromosomal alterations integrating population genetics information. This new method has allowed to peek at how clonal expansions in blood evolve within the UK biobank. Giulio worked on a more generalized framework called MoChA that is being used to extend similar analyses to other cohorts and other biobanks. He has a considerable experience in computer languages and statistical languages such as C, R, and python. He is experienced in the analysis of genome-wide genotype array data and next-generation DNA sequencing data. Dr. Genovese main scientific goal is to develop statistical models to allow him and other researchers to analyze large datasets generated in medical genetics, from complex diseases to cancer.

Margaret Goodell, Ph.D.

Influences on CH Outcomes: Time and Mutation Type

Abstract: DNMT3A is the most frequently mutated gene in clonal hematopoiesis. While the R882 hotspot mutation dominates in AML, other mutations spread throughout the protein are collectively more common in CH suggesting different potency or mechanisms of action in different diseases. By profiling over 250 mutations, we can classify some types of mutation that may have prognostic and therapeutic value long-term for patients. Collective data from the field also suggests that the timing of the original mutation may have a role in the likelihood of CH manifestation at different ages. These recent findings and their implications will be discussed.

Biography: Margaret ("Peggy") Goodell is Professor and Chair of the Department of Molecular and Cellular Biology, and Director of the Stem Cells and Regenerative Medicine Center, at Baylor College of Medicine, in Houston, Texas. Goodell's research is focused on the mechanisms that regulate hematopoietic stem cells, and their dysregulation in malignancies, particularly DNA Methyltransferase 3A (DNMT3A). Goodell is a former president of the International Society for Experimental Hematology (2013). She is a recipient of the Damashek Prize from the American Society of Hematology (2012), the Edith and Peter O'Donnell Award in Medicine from TAMEST (2011) and the Tobias Award from the International Society for Stem Cell Research (2020). Goodell is a member of the National Academy of Medicine, has received She is Chair of the Scientific Advisory Board of the Keystone Symposia and is a member of their Board of Directors. She has served on the editorial board of PLoS Biology, and as an Associated Editor of Blood, and currently serves on the editorial boards of Cell Stem Cell and Cancer Cell. Goodell directs a laboratory of about 15 trainees.

Evan Hadley, M.D.

Biography: Dr. Hadley is a Director of the National Institute on Aging's Division of Geriatrics and Clinical Gerontology, which supports clinical and translational research on aging throughout the U.S. He received his M.D. from the University of Pennsylvania and completed a research fellowship in NIA's Gerontology Research Center in 1980. Since then, he has been responsible for developing NIA research programs on a variety of topics including physical frailty, comorbidity, prevention, and treatment of diseases in older persons, clinical trials of interventions against disabling conditions, factors promoting healthy aging over the life span, and strategies to translate genomic and physiologic findings on age-related outcomes into interventions that delay adverse aging changes.

Siddhartha Jaiswal, M.D., Ph.D.

Age Related Clonal Hematopoiesis and Adverse Effects

Abstract: Diseases of aging such as heart disease and stroke are usually thought to occur due to a combination of hereditary and environmental influences. Recently, we discovered that somatic mutations (DNA alterations acquired after birth) in blood cells may be another factor that contributes to these diseases. Approximately 15-20% of people age 70 or older carry a cancer-associated somatic mutation in a substantial proportion of their blood cells, even though the vast majority do not have cancer. This condition has been termed "clonal hematopoiesis of indeterminate potential", or CHIP. It most commonly arises due to loss-of-function mutations in regulators of DNA methylation. CHIP carriers develop blood cancers at a higher rate than the general population, which is expected because it represents the "first-hit" on the path to cancer. Surprisingly, CHIP is also associated with increased all-cause mortality and higher risk of developing non-neoplastic diseases, like atherosclerotic cardiovascular disease. While the association between CHIP and early mortality and heart disease is robust, this information has not yet entered clinical practice. There are three major roadblocks to translating this evidence to patient care: 1) lack of CHIP cohorts for clinical trials, 2) lack of biomarkers for improving risk discrimination in CHIP, and 3) lack of mechanistic understanding of the factors underlying clonal expansion and risk of heart disease. Here, I will describe our studies using "epigenetic clocks" as a tool to provide risk discrimination for adverse outcomes in CHIP.

Biography: Dr. Siddhartha Jaiswal is an Assistant Professor of Pathology and the Director of the Rapid Autopsy Program at the Department of Pathology, Stanford University School of Medicine. His lab focuses on understanding the biology of the aging hematopoietic system. As a post-doctoral fellow, Dr. Jaiswal identified a common, pre-malignant state for blood cancers by reanalysis of large sequencing datasets. This condition, termed "clonal hematopoiesis", is characterized by the presence of stem cell clones harboring certain somatic mutations, primarily in genes involved in epigenetic regulation of hematopoiesis. Clonal hematopoiesis is prevalent in the aging population and increases the risk of not only blood cancer, but also cardiovascular disease and overall mortality. Understanding the biology of these mutations and how they contribute to the development of cancer and other age-related diseases is the current focus of Dr. Jaiswal's work in his lab. The studies at his lab utilize genetic and clinical information from large population-based cohorts to understand the impact of clonal hematopoiesis in humans. His lab also studies the effect of the mutations causing clonal hematopoiesis in human and mouse tissues through a combination of genomic profiling, functional assays, and mouse models of disease.

Candace Kerr, Ph.D.

Biography: Candace Kerr, Ph.D. is the Program Officer for the Cardiovascular Biology Program in the Aging Physiology Branch of the Division of Aging Biology (DAB) in the National Institute on Aging (NIA). NIA's Cardiovascular Biology Program supports research on age-related changes in hematopoiesis, age-dependent changes in cardiac and vascular structure and function. She is also the Program Officer for DAB's Stem Cell Program which has supported major findings on the genetics regulating stem cell lifespan and genomic stability, the relationships between stem cell survival and aged health, and the discovery of molecules that facilitate stem cell depletion and cellular senescence. These programs also support research investigating the role of stem cells in cardiac and vascular maintenance and renewal.

Jaroslaw Maciejewski, M.D., Ph.D.

Prognostic Relevance of CHIP

Abstract: The prevalence of clonal hematopoiesis of undetermined potential (CHIP) increases with age, a process likely exaggerated by the presence of hereditary cancer traits and/or chemotherapy. Consequently, CHIP may be more frequent in patients affected by cancer or multiple cancers and it has a strong age–related component justifying in a proper setting a term of "age-related clonal hematopoiesis" (ARCH). CHIP is initiated by a spectrum of somatic genetic hits constituting the prototypic leukemia-initiating lesions. They also serve as ancestral events for CHIP-derived myelodysplastic syndrome (MDS) and secondary acute myelogenous leukemia (AML). Since CHIP-initiating lesions have a low "driver" potential, the prognostic value of their presence is confounded by a slow progression and thus long disease anticipation with regard to the development of a myeloid neoplasia. The implications of these observations may be affected by: i) the perceived impact of chronologic (time passed) vs. biologic aging ("healthspan"); ii) toxic/genotoxic and immunosuppressive exposures facilitating the pace progression; iii) competing mortality (steaming from cardiovascular CHIP-related risks or unrelated diseases and accidents) increasing with the duration; and finally iv) the resultant incomplete penetrance. To date, most estimates of MDS/AML risk conveyed by CHIP are unlikely to be precise as they rely on cumulative frequencies. While most calculations are based on only a few serially recorded cases, stringent analysis should be based on the confirmation that the CHIP-associated hit was also present in the corresponding myeloid neoplasm. In addition, it is possible that the presence of

CHIP reflects a general risk not only for CHIP-derived, but also for other myeloid neoplasms (de novo vs. CHIP-derived MDS) or other cancers. Consequently, the prognostic relevance of CHIP may be multifactorial and thus much greater than that attributed to the increased risk of CHIP-derived neoplasms. In addition, the type of the initiating lesion (e.g., TET2, DNMT3A, JAK2 mutations, etc.), clonal burden, their on/off rate in serial studies, and the clinical phenotype of CHIP–derived neoplasms may further confound the prognostic assessment. However, understanding of these complex relationships may be essential for the diagnosis, monitoring, preventive and therapeutic strategies to be applied in a rational fashion. This presentation discusses these issues with a particular emphasis on myeloid neoplasia.

Biography: Dr. Jaroslaw Maciejewski's specific interests have evolved from molecular and immune mechanisms of bone marrow failure syndromes, including myelodysplastic syndrome (MDS) and related diseases, aplastic anemia (AA), paroxysmal nocturnal hemoglobinuria (PNH), large granular lymphocyte leukemia (LGL) and pure red cell aplasia (PRCA) to drug discovery for these conditions. His research in leukemia genetics is highly translational and directed towards identification of molecular lesions to be applied in diagnostics or serving as targets for rational drug discovery and clinical trial applications. His original research training, followed by clinical training, was at the Hematology Branch of NHLBI, and his career path as a physician-scientist began during his fellowship at the NIH where he investigated dysfunction of hematopoietic stem cells under the mentorship of Dr. Neal Young. This research contributed to the better understanding of immune-mediated attack and the resultant hematopoietic stem cell (HSC) damage in AA and PNH. In this application he also studied HSC biology, including HSC compartment damage and changes in disease and aging, respectively and HSC regeneration and expansion. Dr. Maciejewski's laboratory has made several important contributions to the understanding of hematologic disease documented by publications in Cancer Cell, Nature Genetics, Nature Medicine, NEJM, Nature, JCO, and Blood illustrating major achievements, including introduction of SNP arrays as a clinical karyotyping platform in MDS diagnostics and various applications of next generation sequencing in bone marrow failure. These studies led to discovery of various somatic and germ line mutations and their implications for the understating of the disease mechanisms as a consequence of these molecular lesions and clinical implications.

Pradeep Natarajan, M.D., MMSc.

CVD and CHIP

Abstract: Atherosclerotic cardiovascular disease, particularly complications of coronary artery disease (CAD), remains the leading cause of mortality worldwide, and age remains the dominant risk factor. The hematopoietic system is a critical contributor to vascular homeostasis and age-related dysfunction. At the helm of CAD is the disruption of atherosclerotic plaques whose stability depend on longstanding inflammation and regulation by multiple immune cells derived from hematopoietic progenitors. As hematopoietic stem cells (HSCs) age, they, like all stem cells, have increased propensity to acquire mutations as a result of high turnover, environmental exposures, and likely under-recognized genetic and epigenetic factors. Acquired mutations, age-related changes in DNA repair, and reduced regenerative potential with aging, provide an ideal opportunity for clonal selection and expansion. Large-scale whole exome sequencing (WES), from peripheral leukocyte-derived DNA of apparently healthy individuals permitted definitive detection of clonal hematopoiesis of indeterminate potential (CHIP), or clonal hematopoiesis with identifiable leukemogenic mutations (typically in DNMT3A, TET2, JAK2, and ASXL1) without overt dysplasia or neoplasia. More than 1 in 10 adults older than 70 years have CHIP. CHIP was

associated with an increased risk for CAD by 2-fold and premature MI by 4-fold in the general population independently of age or other traditional risk factors. CHIP activates an inflammatory cascade in infiltrating monocytes to promote atherosclerosis. Hypercholesterolemia-prone mice engrafted with Tet2-/- bone marrow (particularly with myeloid-specific Tet2 deficiency) developed larger atherosclerotic lesions compared to those receiving control bone marrow. Their leukocytes displayed enhanced induction of inflammatory mediators, such as IL6, IL8, and IL**1**β. Inhibition of NLRP3/IL**1**β mitigates atherosclerosis among hypercholesterolemic mice with Tet2- /- bone marrow. RNA sequencing of peripheral blood monocytes also indicates heightened inflammation, particularly the NLRP3 inflammasome pathway, for those with CHIP versus those without CHIP. Mendelian randomization studies indicate a likely causal relationship between IL6 and CAD risk in humans. CHIP represents a novel putative precision medicine approach to CAD event prediction and prevention.

Biography: Pradeep Natarajan is Director of Preventive Cardiology at Massachusetts General Hospital (MGH), Assistant Professor of Medicine at Harvard Medical School, and Associate Member of the Broad Institute of Harvard and MIT. He received his MD with Alpha Omega Alpha in 2008 from the University of California, San Francisco and MMSc in biomedical informatics in 2015 from Harvard Medical School. The primary goal of his research is to discover novel fundamental insights for atherosclerotic cardiovascular disease towards improved personalized risk prediction and reduction for premature atherosclerotic cardiovascular disease. Dr. Natarajan researches the germline and somatic genetic bases of human atherosclerosis using genetic epidemiology, large-scale sequencing studies, genotype-driven human investigation, and genetic testing implementation. His research program spans the MGH Cardiovascular Research Center, Broad Program in Medical and Population Genetics, and Broad Cardiovascular Disease Initiative. He is an investigator of several genomic consortia: Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium, Global Lipids Genetics Consortium, Myocardial Infarction Genetics, NIH/NHGRI Electronic Medical Records and Genomics (eMERGE) Network, NIH All of US Program, NIH/NHLBI Trans-Omics for Precision Medicine, NIH/NHGRI Centers for Common Disease Genomics, and Department of VA Million Veteran Program. He is Co-Lead of the largest human genetics consortia for plasma lipids – NIH/NHLBI TOPMed Lipids Working Group and Global Lipids Genetics Consortium. He is Lead of clonal hematopoiesis analyses in the NIH/NHLBI TOPMed Program.

Nalini Raghavachari, Ph.D.

Biography: Nalini Raghavachari, is a Program Officer in the Division of Geriatrics and Clinical Gerontology at the National Institute on Aging (NIA/NIH) in Bethesda, Maryland focusing primarily on the genetics of exceptional longevity, healthy aging, integrative multi-omic analyses and translational genomics. Nalini Raghavachari received her PhD in Biochemistry studying lipid metabolism in coronary heart disease from the University of Madras, India. She had her initial post-doctoral training in a Genetic Engineering Laboratory in Las Cruces, NM followed by Baylor College of Medicine and Texas A&M University in the field of genetics and genomics. She then applied her skills in genomics for drug discovery in a startup biopharmaceutical company, Procertus Biopharma to prevent chemotherapy induced alopecia and in Corning Life Technologies for the development of microarray based novel genomic and proteomic tools. She began her NIH career at the Clinical Center in 2003 as director of the Sickle Cell Genomics Program in the Critical Care Medicine Department. She then took up a dual appointment with the intramural division of the National Heart, Lung and Blood Institute in 2005 as director of the Genomics Core Facility and

served as an associate investigator in human genetic studies on vascular diseases, including the Framingham Heart Study, to identify disease mechanisms, predictive biomarkers, and therapeutic drug targets.

Anastasia Shindyapina, Ph.D.

Mice as a Model to Study Mechanisms of Age-related Clonal Expansion

Abstract: Like humans, mice spontaneously develop cancer with age, with standard laboratory strains predominantly dying from hematological malignancies at old age. In humans, clonal hematopoiesis of indeterminate potential (CHIP) is a premalignant state for blood cancers. We hypothesized that mice also develop it with age. Here, we find that mouse immune cells acquire somatic mutations with age, with some individual somatic clones of B cells expanding to represent over half of B cells. Mouse clonal B cells are increased in size and carry somatic mutations in genes often mutated in human lymphoma and CHIP, e.g. Trp53, Pim1, Tet2, Asxl1, and Dnmt3a. Multi-omics analyses of clonal B cells and genetic mouse models identified c-Myc as a master regulator of clonal expansions. The DNA methylome of clonal B cells suggested a role of epigenetic regulation of clonal selection similarly to CHIP in humans. Accumulation of clonal B cells was associated with poorer survival of mice and age-related myeloid bias. We demonstrate that an aging environment of the spleen triggers c-Myc expression in B cells in vitro, thereby contributing to clonal expansion. In addition, by reconstructing the Ig repertoire of the blood transcriptomes of GTEx subjects we find that human B cells clonally expand with CHIP. Similar to mice, there was a significant correlation between the size of top clones estimated from the Ig repertoire and VAF of somatic mutations, suggesting that B cell clones expand in the later stages of B cell maturation. Together, this study reveals many similarities between age-related clonal expansion in mice and humans, including somatic mutations in epigenetic regulators, aberrant DNA methylation, expansions of B cells after VDJ recombination, and increased mortality. It also shows that aged mice is a convenient model to characterize mechanisms of age-related clonal expansion in humans.

Biography: Dr. Anasthasia Shindyapina is currently a post-doctoral fellow at Brigham and Women's Hospital, Harvard Medical School. She earned her doctoral degree from the Department of Bioengineering and Bioinformatics, Moscow State University where she received training in computational and experimental science. During her doctorate training, she gained expertise in animal research and biomedical research involving human subjects and co-authored more than 10 peer-review papers. Her research was recognized at the highest level in Russia. She received two prestigious national awards for young scientists. As a guest lecturer, she taught seminars and contributed to teaching for a summer program at a remote White Sea Biological Station, while she also attended courses on aging and regenerative medicine. Together with her two fellow graduate students, she founded a startup in 2015 with the goal to develop new therapies and diagnostic tools for age-related vascular calcification. After completing her doctorate degree, Dr. Shindyapina joined the Gladyshev lab at Brigham and Women's Hospital, Harvard Medical School as a postdoctoral researcher where she has focused solely on aging research, with the idea to identify and target core drivers of aging thereby delaying the onset of multiple age-related diseases. Recent discovery of clonal hematopoiesis in humans revealed a new age-related phenotype which increases risk of two seemingly unrelated diseases of aging: heart diseases and cancer. Using aged mice as a model, Dr. Shindyapina and Dr. Jose Castro, also a postdoctoral researcher, demonstrated that mice develop clonal expansions with age as well, and dissected the role of DNA methylation and aging microenvironment in this process. They further proved causal role of

oncogene c-Myc in age-related cellular competition and characterized mutational processes associated with that. Their work for the first time demonstrated relevance of aging mice for studying mechanisms of clonal expansions in the context of aging.

Alan Shuldiner, M.D.

Age ExWAS and CHIP

Abstract: Aging is characterized by degeneration in cellular and organismal functions leading to increased disease susceptibility and death. Although our understanding of aging biology in model systems has increased dramatically, large-scale sequencing studies to understand human aging are now just beginning. We applied exome sequencing and association analyses (ExWAS) to identify age-related variants on 58,470 participants of the DiscovEHR cohort. Linear Mixed Model regression analyses of age at last encounter revealed variants in genes known to be linked with clonal hematopoiesis of indeterminate potential, which are associated with myelodysplastic syndromes, as top signals in our analysis, suggestive of age-related somatic mutation accumulation in hematopoietic cells despite patients lacking clinical diagnoses. In addition to APOE, we identified rare DISP2 rs183775254 (p = 7.4E-10) and ZYG11A rs74227999 (p = 2.5E-08) variants that were negatively associated with age in either both sexes combined and females, respectively, which were replicated with directional consistency in two independent cohorts. Epigenetic mapping showed these variants are located within cell-type-specific enhancers, suggestive of important transcriptional regulatory functions. To discover variants associated with extreme age, we performed exome-sequencing on persons of Ashkenazi Jewish and German descent ascertained for extensive lifespans. Case-Control analyses in 525 Ashkenazi Jews and 1,294 German cases (Males 1 92 years, Females 1 95years) were compared to 482 and 2,149 controls, respectively. Our results showed APOE rs429358 as the sole variant which replicated across both cohorts. In addition, we found significant association to FOXO3A variants post-Bonferroni adjustment, and nominally associated population-specific variants. Interestingly, CHIP variants appeared to be less frequent in offspring of long-lived parents compared to age-matched controls suggesting inherited mechanisms that prevent or delay somatic mutations in long-lived families. Collectively, our Age-ExWAS, the largest performed to date, confirmed and identified previously unreported candidate variants associated with human age and provide hints regarding the role of somatic mutations in aging.

Biography: A leading national expert and researcher in personalized medicine, Dr. Shuldiner focuses on the genetics of age-related diseases, including of type 2 diabetes, obesity, osteoporosis, and cardiovascular disease. He is best known for his studies involving Old Order Amish, a homogeneous population ideal for genetic studies. His group reported the first null mutation in the APOC3 gene and its association with low blood triglyceride levels and cardioprotection, which validates treatment for hypertriglyceridemia. His group also identified a common gene variant that reduces the benefit of clopidogrel, which many cardiologists now use to individualize anti-platelet therapy. Dr. Shuldiner serves as vice president at the Regeneron Genetics Center, a program that focuses on early gene discovery and functional genomics and facilitates drug development. He is also the John Whitehurst endowed Professor of Medicine (part-time) at the University of Maryland School of Medicine where he directs the Program for Personalized and Genomic Medicine, a program that focuses on implementing genomics on several steering and advisory committees related to his expertise in complex disease genetics and the translation of genetic discoveries to the clinical setting.

Erik van den Akker, M.Sc.

Dynamic Clonal Hematopoiesis and Functional T-cell Immunity in a Supercentenarian

Abstract: The aging hematopoietic system is characterized by an increasingly compromised immune function and a progressively skewed clonal contribution to peripheral blood production, also known as clonal hematopoiesis of indeterminant potential (CHIP). While both these aspects are associated with an increased risk of aging-related disease, it is currently unknown to what extent they co-occur at the extreme end of human lifespan. Investigated blood cells of an immuno-hematological normal female who reached 111 years, sampled across a 9-year period at ages 103, 110 and 111 years. We applied genetic sequencing approaches to investigate clonality in peripheral blood samples and sorted cell subsets. Immuno-competence was characterized using flow cytometry, T-cell receptor excision circle (TREC) assays, and in vitro proliferation assays. Identified a highly dominant DNMT3A-mutated HSC clone, with a complex subclonal architecture, and observed ongoing subclonal dynamics within the 9-year timeframe of our sampling. The hematopoietic stem cell output was multipotent, myeloid biased, and contributed 22% of the CD4+ T-cells. Nevertheless, T-cells showed a robust proliferation when challenged in vitro, and were characterized by a surprisingly high TREC content, indicative of an ongoing generation of naive T-cells. Within the blood of an exceptionally old individual who we followed for a 9-year period we observed extreme CHIP, as well as a surprisingly functional T-cell immunity. While this exploration is limited to only a single individual, it does suggest an independence between the two studied aspects of the ageing hematopoietic system. The presented findings warrant future research of CHIP in large cohorts of aged healthy individuals in relation to phenotypic and functional parameters of adaptive immunity.

Biography: Throughout Dr. Erik van den Akker's scientific career, he has developed a keen interest in studying the ageing hematopoietic system and its effects on immuno-capacity and health. He performed most of his (post) doctoral training within (inter)national collaborative projects NCHA, IDEAL and BBMRI that aimed to elucidate the determinants contributing to healthy ageing. He undertook various 'omics' studies (genetics, transcriptomics, epigenetics, metabolomics) assayed in various human tissues and animal models, leading to a strong analytical background, a broad experience in cross-disciplinary research, and an extensive (international) network. During this period, he specialized on developing methods for the integrative analysis of omics datasets to further our understanding of the molecular aspects of ageing. This resulted in several (co-)authorships in Aging Cell, including a paper describing a novel framework for meta-analyzing networks of co-expressed genes that jointly change their expression with age in blood (Van den Akker et al. 2014). During this time, he started studying the relation between the ageing hematopoietic system, its age-related pre-conditioning to evolve to leukemia, i.e., clonal hematopoiesis of indeterminate potential, and its effect on the immune system in the elderly, as highlighted by papers in Blood (van den Akker et al. 2016) and Leukemia (van den Akker et al. 2020). In parallel, he initiated collaborations with wet lab-oriented groups that perform (pre-)clinical research in ageing and immunology and thus perfectly complement my computational profile and research interests. This strategy has given me ample opportunity to develop a computational research line on systems immunology, illustrated by various senior authorships (including Cordes et al. Bioinformatics 2019, Melsen et al. J. Immunol. 2020, Holstege et al. Am J Hum Genet. 2020), and personal and joint grants. He is currently funded by the Dutch Research Council on a prestigious early career personal grant (ZonMW-Veni), further supporting the development of an independent research line on systems immunology. Lastly, Dr. Erik van den Akker have recently joined the management team of the Leiden Center for Computational

Oncology to spearhead the development of novel computational tools that provide more insights into tumor heterogeneity and tumor evolution under therapeutic pressure in acute myeloid leukemia.

Oliver Venn, Ph.D.

Mapping the Prevalence of Clonal Hematopoiesis in Cell-free DNA using Ultra-deep Sequencing

Abstract: Clonal hematopoiesis (CH) is defined by the presence of age-dependent somatic mutations in hematopoietic progenitor cells, and the prevalence of mutations VAF $\gtrsim$1% has been reported to occur in up to ~30% of individuals 60-70 years of age. The biological mechanisms and clinical significance of CH are just now being studied and methods of measurement considered. Using an assay ~100X more sensitive than exome sequencing we determined the prevalence and features of CH across the VAF spectrum (0.1-100%) in a cohort of participants from the Circulating Cell-free Genome Atlas (CCGA; NCT02889978) study. We then applied population genetics approaches to investigate CH biology, and assessed the impact on the interpretation of cell-free DNA (cfDNA) somatic variants. To survey CH in cfDNA, blood was prospectively collected from 749 participants (pts) with no cancer (NC) at recruitment and 878 pts with newly- diagnosed untreated cancer (C, 20 tumor types). Both white blood cells (WBC) and cfDNA were isolated. Paired WBC and cfDNA targeted sequencing (507 genes, 60,000X median coverage) identified somatic variants. Unique molecular barcodes and a machine learning-based noise model achieved a specificity of 1 false positive variant call per Mb of genome targeted at a limit of detection of ~0.1% variant allele frequency (VAF). For each cfDNA variant we inferred potential clonal hematopoiesis origin from the matched in the participants' WBC sample. 1,412 samples were eligible and evaluable (576 NC, 836 C; 18 solid tumor types). Of somatic cfDNA variants matched in participants' WBC, 7% of individuals had CH with VAF >10%, 39% had CH with VAF >1%, and nearly all pts (92%) had a somatic variant with VAF >0.1%. The rate was similar between NC and C (median age 62, 60), increasing in prevalence by 160% per decade, such that we observed 2.5 variants/Mb at age 60. Genes impacted by CH included DNMT3A (40%), TET2 (27%), and TP53 (10%), consistent with previous reports, with each showing prevalence age-dependence. To investigate drivers of CH, we tested for dN/dS imbalance across the 507 targeted genes. We identified 21 genes with signals of positive genetic selection (corrected p-value < 0.05) and no statistically significant signals of negative selection. Though strong signals of positive selection were observed in the dataset, of CH variants identified 94% were unique to individual patients, most of which were present at low VAF. The very deep (~60,000X) sequencing assay detected abundant CH signal in WBC and cfDNA within NC and C at <1% frequency, which has important implications for the design of CH investigations. We broad VAF spectrum may motivate re-evaluation of the $\gtrsim$1% VAF definition for CH mutations. Genetic selection-based inference may provide a powerful approach to understand CH biology. Additionally, private CH variation necessitates paired WBC-sequencing for somatic variant-based cfDNA early cancer detection and tumor genotyping (liquid biopsy) applications.

Biography: Oliver Venn is a Director of Bioinformatics at GRAIL, Inc., a company seeking to detect cancer early when it can be cured from cell free nucleic acids. Oliver leads the computational biology team performing inference on cell free DNA and cancer biology. Their work informs the classifier development and is used to interrogate classifier behavior. Recent work includes estimating tumor shedding rate into cfDNA (tumor fraction), analyses of clonal hematopoiesis, and cfDNA cancer signal localization (tissue of origin). Oliver holds a DPhil in Genomic Medicine and Statistics from the University of Oxford. In academia he worked in the

field of statistical population genetics applied to genomics.

Jan Vijg, Ph.D.

Single-cell Whole Genome Sequencing and the Pathogenic Mechanisms of Somatic Mutations in Aging

Abstract: Age-related accumulation of postzygotic DNA mutations results in tissue genetic heterogeneity known as somatic mosaicism. Although implicated in aging as early as the 1950s, somatic mutations in normal tissue have been difficult to study because of their low allele fractions. I will briefly review recent progress in studying somatic mutations and genome mosaicism and then show how single-cell whole genome sequencing can give us some insight into the possible pathogenic mechanisms of somatic mutations and genome mosaicism in tissues during aging.

Biography: Jan Vijg, Ph.D., is Professor and Chairman of the Department of Genetics at the Albert Einstein College of Medicine in New York since July, 2008. He received his Ph.D. at the University of Leiden, The Netherlands, in 1987. From 1990 to 1993 he was founder and Scientific Director of Ingeny B.V., a Dutch Biotechnology company. In 1993 he moved to Boston, to take up a position as Associate Professor of Medicine at Harvard Medical School. In 1998 he accepted an offer from the University of Texas Health Science Center in San Antonio, Texas, to become a Professor in the Department of Physiology. From 2006 to 2008 he was a Professor at the Buck Institute for Age Research in Novato, California. With his research team he was the first to develop transgenic mouse models for studying mutagenesis in vivo (in 1989) and has used these models ever since in studying the relationship between damage to the genome and aging. Since 2007 he has developed and applied single-cell methods for studying somatic mutations and epimutations in relation to aging. He has published well over 300 scientific articles and three books and is inventor or co-inventor on 8 patents. Dr. Vijg is the recipient of the Schreuder Award of the Netherlands Society of Gerontology (1987), the Nathan Shock New Investigator Award of The Gerontological Society of America (1994) and the Irving Wright Award of Distinction of the American Federation for Aging Research (2012). He is a Fellow of the American Association for the Advancement of Science (AAAS) and has been Chairman of the Board of Scientific Counselors of the National Institute on Aging's Intramural Research Program from 2013 to 2015, and Chair of the NIH study section NIAB from 2018-2019. He has been Editor in Chief of the journal Mutation Research from 2015-2018. Dr. Vijg's research has been continuously funded by the NIH since 1993. He is a founder of several biotech companies as well as the new Center for Single-Cell Omics (CSCOmics) at Jiaotong University School of Medicine in Shanghai, China (2019).

Kenneth Walsh, Ph.D.

Somatic Mutations, CHIP, and Aging

Abstract: Advances in DNA sequencing methodology reveal that age-associated somatic mutation accumulation is remarkably prevalent, revealing a degree of cellular heterogeneity within tissues that was previously underappreciated. When somatic mutations occur in "driver" genes, these cells can undergo a clonal expansion in physiologically normal cell populations. In the hematopoietic system, this condition is referred to as clonal hematopoiesis of indeterminate potential (CHIP). Epidemiological studies show that CHIP is associated with increased mortality due in large part to increased cardiovascular disease risk. Work in experimental systems indicate that CHIP is a causal risk factor for cardio-metabolic diseases. Mechanistic studies show that CHIP mutations can activate pro-inflammatory signaling in myeloid cells, and some of these finding

have been corroborated in human study cohorts. Overall, these findings support the concept that CHIP represents a new mechanism of cardiovascular and other age-related diseases that shares features with hematologic malignancies. While interest in CHIP and other forms of clonal hematopoiesis is growing, a number of unresolved issues will be discussed by the participants in this workshop to further advance the field: 1. Can we develop of a consensus on the measurement of clonal events within the hematopoietic system? 2. What is a clinically relevant clone size? 3. What are the molecular events that contribute to clone growth? 4. Are different "driver" gene clones functionally different with regard to mechanism and clinical outcome? 5. Is our understanding limited by sequencing constraints and by focusing on known cancer "driver" genes?

Biography: Kenneth Walsh, Ph.D. is Professor of Medicine at the University of Virginia School of Medicine and he also directs the School of Medicine's Hematovascular Biology Center (HBC). His laboratory broadly examines the molecular events that drive cardiovascular cell growth, differentiation and cell death. A major focus has been to elucidate mechanisms of inter-tissue communication and understand how these systems contribute to physiological versus pathological tissue growth in the cardiovascular system, particularly as they relate to systemic metabolic dysfunction and cardiovascular disease (CVD). Recent studies have investigated how "clonal hematopoiesis" functions as a causal risk factor for cardio-metabolic diseases. Recent large exome sequencing studies in humans have shown that aging is frequently associated with the appearance of pre-leukemic, somatic mutations in the hematopoietic system that provide a competitive growth advantage to the mutant cell and allow its clonal expansion. These aberrant clonal events in the hematopoietic system have been found to be associated with greater risk of CVD. Using murine genetic models, our work suggests that there is a causal connection between clonal hematopoiesis and CVD, and we have elaborated aspects of the underlying mechanisms.