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Special Issue: Heterogeneity and Successful Aging: Perspective

Workshop Report—Heterogeneity and Successful Aging Part II: Approaches to Investigate Heterogeneity in Aging Research

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Abstract

Heterogeneity in aging is a fundamental biological process arising from multifactorial etiologies, including genetic, lifestyle, and socioeconomic factors. Modeling this heterogeneity in animal systems is critical for elucidating the underlying mechanisms of aging and for leveraging these insights in translational research. Here we present part II, a summary of the model organism research presented at the *NIA Heterogeneity and Successful Aging* workshop, held in May 2023.

Keywords: Age-related pathology, Animal model, Anti-aging, Biodemography, Bioinformatics, Biology of aging, Frailty

Aging is characterized by a time-dependent increase in disease susceptibility and morbidity. As researchers, our primary goal is to elucidate the mechanisms of aging and to develop interventions that extend both health span and life span. Model organisms—including nematodes, flies, fish, rodents (mice, rats, naked mole rats), cats, dogs, and nonhuman primates—have been crucial for mechanistic and translational studies of human aging (1). Although average values of aging phenotypes have been extensively examined, the variance of aging phenotypes within and between age groups—commonly referred to as heterogeneity in aging (2–4)—has only recently received significant attention (5,6). In May 2023, the Division of Aging Biology at the National Institute on Aging (NIA) convened the virtual workshop "Heterogeneity and Successful Aging" to compile existing knowledge on heterogeneity in aging and to discuss how phenotypic heterogeneity can be harnessed to understand and predict factors that determine successful aging and prolonged health. We define heterogeneity in aging as the variability in complex trait outcomes associated with chronological age. A multidisciplinary panel consisting of aging experts, geneticists, AI/ML experts, ichthyologists, human and animal behaviorists, and more discussed strategies to better understand mechanisms promoting heterogeneity in aging and for translating heterogeneity of aging to improve therapeutic opportunities and clinical care of our aging population. The diverse panel of experts contributing to a rich discussion focused on the following themes: investigators currently studying heterogeneity in aging and the methods used to tackle this challenging area of research, the need to train multidisciplinary teams, future directions of research to advance our understanding of the biology of aging, and improve the healthspan and clinical care of our aging population. This report summarizes key insights from the workshop on using animal models to define, quantify, elucidate mechanisms, and ultimately exploit this heterogeneity to promote successful aging. A complete transcript of the conference proceedings can be found on the NIA website (7).

Genetically Diverse Mice

The C57BL/6J mouse strain is currently the most widely used and the reference mouse stain. The lack of genetic diversity in this inbred strain while useful in generating repeatable results,

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may hinder generalizable research in aging. As an alternative, Dr. Gary Churchill and colleagues developed a genetically diverse mouse stock from 8 different inbred founder strains, resulting in 52 million cataloged genetic variants called the Diversity Outbred Stock (DO) (8). Dr. Churchill described variability and heterogeneity of aging at the molecular and tissue levels in large cohorts of 960 DO mice across 5 groups (192 mice per group) under various dietary interventions (9,10). RNA and protein profiling revealed different aspects of aging: transcriptomic data pointed to immunity and inflammation senescence whereas changes in the proteome pointed to energy metabolism and proteostasis. The levels of *Cdkn2a* expression, a marker of cellular senescence, increased with age in kidney tissue but was highly variable across DO population. Dr. Churchill proposed the DO mouse stock as a novel resource for the aging community to better model genetic heterogeneity present in human populations. Furthermore, he underscored the importance of longitudinal studies for aging research despite the challenges they pose. In contrast, although cross-sectional studies are easier to complete, they may generate misleading results that risk generational confounding.

Dr. Robert W. Williams discussed how genetics and environment, and the heterogeneity in each of these parameters, may affect aging outcomes. Studying interactions between genotype and environment (GxE) is a crucial direction in the field of aging research. Dr. Williams defined successful aging broadly as maintaining function until a certain age, then experiencing a swift decline. Heterogeneous aging data are needed, as well as strategies for integrating these data into a cohesive body of knowledge. Factors that promote successful aging vary with environment, so there is a need for accurate healthcare tailored to an individual's environment and genome. He advocated for studies involving multiple genotypes and the use of genetically diverse mouse populations such as reciprocal F1 crosses (diallel panel) (11), recombinant inbred panels such as BXD (12) mice, derived from a cross between C57BL/6J mice (B6) and DBA/2J mice (D2), and outbred mice such as UM-HET3 (13,14). Williams lab has exploited the BXD recombinant inbred lines to carry out genetic mapping and GxE studies (15) and studied nearly 13 000 UM-HET3 mice for mapping age-related quantitative trait loci (QTLs) (16). QTL mapping in mice and functional validation in *C. elegans* confirmed the MRPS5 gene is associated with lifespan and aging rates (17). Dr. Williams' group also compared the lifespan outcome and changes in omics profiling after high-fat or normal-chow diet consumption in mice (GxE interaction) (15). Although mean lifespan was significantly improved in mice consuming a low-fat diet, some genotypes showed improved lifespan on the high-fat diet. These GxE studies reveal diversity in longevity outcomes.

Longitudinal High Content Live Data Acquisition in Animal Models

Longitudinal investigations of animal behavior and phenotypes represent a rapidly expanding field, driven by advancements in instrumentation, technology, and computational and statistical methods. The development of novel mathematical models, coupled with innovative phenotyping techniques, has the potential to open new frontiers in aging research. In this context, Drs. Vivek Kumar and J. Graham Ruby demonstrated their approaches to longitudinal data acquisition from video recordings of mouse behavior, whereas Dr. Claire N. Bedbrook presented her work on life-long monitoring of fish behavior.

According to Dr. Kumar, many behavioral paradigms are limited by their subjective methodologies, lack of standardization, temporal fragmentation, and, in some cases, validity. This is particularly a challenge in longitudinal interventional studies that can last several years. Changes in research personnel and other technical challenges may introduce unwanted variation in the data that can confound biologically important phenotypic variation. To address these problems, his group utilizes computer vision and machine learning for automated behavioral quantification in the laboratory mouse with the goal of increasing reproducibility, scalability, and replicability of frailty studies (18). Currently, frailty in rodents is measured using a subjective index-based approach that introduces technical heterogeneity in long-term aging studies (19). Kumar's lab has approached the assessment of frailty using advanced behavioral methods in several domains. His team generated one of the largest frailty datasets in mice with over 600 mice that were tested using traditional manual frailty indexing as well as machine learning-based behavior frailty assessment. The team characterized features such as gait, posture, flexibility, among others, to train an algorithm to predict frailty status (20). The new method, called visual frailty index, is sensitive, accurate, and highly scalable and has the potential to enable the investigation of heterogeneity and of new interventional studies for healthspan and lifespan in rodents (18).

Similarly, Dr. Ruby discussed capturing the heterogeneity of aging in mice through video monitoring of home cage behaviors (21) and highlighted the need for improved reproducibility and scalability in frailty measurements. He utilized Calico's custom home cage video monitoring system to assess frailty in mice, noting that outputs from this digital frailty index correlated well with both chronological age, age-related decline, and with scores from manual (ie, clinical) frailty assessments (19). Among frailty parameters determined from video images, wheel-running was a particularly rich source of age- and frailty-relevant data. Notably, correlation of both manual and video-based frailty with age was strong for C57BL/6J mice and weaker with DO mice, suggesting genetic diversity as a large source of heterogeneity, a discrepancy that is also reflected in manual FIs. Ruby highlighted the opportunities for improvement to video-based home cage frailty and emphasized that this tool is not explicitly an age predictor; rather, increased frailty reflects the physiological decline that accompanies age.

Dr. Bedbrook presented her research tracking whole-life behavior to model aging and predict remaining lifespan in the African turquoise killifish. Model lifespan of 4 to 7 months enables study of rapid aging (22,23). Lifelong tracking of behavioral profiles allows Bedbrook to characterize aging trajectories and variability in health endpoints (24). She further used these data to develop models that can predict aging trajectories and remaining life. For instance, using MoSeq (25), an unsupervised method for behavior segmentation, she extracted approximately 100 behavioral syllables for killifish and correlated behavioral trajectories over lifespan. Given the large amount of data that is collected—a million frames per day for several months per animal—Bedbrook highlighted the big data challenge.

Selected Cohorts and Successful Aging

A complementary approach to addressing heterogeneity in animal models is through selectively bred lines for a desired trait. Dr. Lauren G. Koch has developed rat models of high and low intrinsic exercise capacity, also adopted by Dr. John P. Thyfault for metabolic studies. They illustrated advances in understanding the biology of aging made possible by these selected cohorts of extreme phenotypes such as for discovering epistatic interactions, synergistic actions, and gene modifiers in aging.

Dr. Koch applied a two-way artificial selective breeding scheme to generate two rat populations with the same genetic background but with different intrinsic exercise capacity (26,27). Her goal was to explore the mechanistic basis of health and disease, which supports work linking exercise with longevity based on energy transfer principles. After 24 years and 48 generations of breeding, the 2 rat groups display significant divergence in exercise capacity and are known as high and low-capacity runners (HCR and LCR, respectively). Dr. Koch's data showed that HCR rats lived longer and had better sustained healthy function of the heart, muscles, liver, and brain (28–30). Based on her work with the HCR and LCR rats, Dr. Koch formulated the Energy Transfer Hypothesis of Aging (ETH) stating that "variation in capacity for aerobic energy transfer is the central mechanistic determinant of the divide between aging and longevity." Dr. Koch emphasized the importance of measuring the maximum rate of oxygen consumption attainable during exercise (VO_2 max) as clinical biomarker. She noted that VO_2 max reflects wide genetic heterogeneity (31), can predict all-cause morbidity and mortality (32) and is a biomarker of oxygen metabolism.

Dr. Thyfault's research focuses on how hyper-caloric diets and inactivity contribute to metabolic disease. His work shows that exercise improves metabolic health of skeletal muscles, liver, pancreas, heart, adipose tissue, and vasculature. Similar to findings from the Koch laboratory, the Thyfault group has shown that HCR rats have a lower risk of all-cause mortality than LCR. Dr. Thyfault provided compelling evidence connecting aerobic capacity and disease risk (eg, Alzheimer's disease, metabolic syndrome, and fatty liver disease). Furthermore, HCR rats are protected against metabolic dysfunction induced by a high fat diet (HFD) (33), and this metabolic protection appears driven by an enhancement of mitochondrial content and function in all metabolic tissues (34). In his studies on fatty liver, the presence of disease was inversely correlated with aerobic fitness (35) suggesting that the liver is highly responsive to physical activity interventions. Moreover, HCR rats were protected from HFD-(hyphen) induced steatosis. In short-term HFD intervention, HCR and LCR rats had different transcriptional responses. Despite consumption of HFD, liver from HCR rats showed heightened bile acid and cholesterol metabolism, and a more robust metabolic flexibility, including maintenance of glucose homeostasis. In contrast, chronic HFD-feeding led to mitochondria collapse in LCR rats (36). Thyfault argued that high fitness elevates mitochondrial content in the liver which is consistent with epidemiological findings demonstrating a strong association between midlife fitness, aerobic capacity, and later life disease incidence.

Longitudinal Studies in Human

Although not a model organism, deep phenotyping approaches advantaged by the use of wearable devices are more readily being used to quantify heterogeneity of aging in human populations. These devices enable monitoring of individual parameters over time and facilitate categorization and stratification of individuals in a study population. Dr. Megan Huisingh-Sheetz discussed the utility of wearable accelerometers as a high-resolution method for assessment and management of frailty in older adult clinical care, and showed how lower overall activity is correlated with increased frailty (37). Dr. Huisingh-Sheetz emphasized the need for computational method development to effectively leverage the richness of data obtained from wearables. For instance, the accelerometer dataset was used to generate 98 distinct measures, and machine learning-based analysis achieved 83% accuracy in the prediction of cognitive decline in older adults. Although physical activity is widely recognized as a highly effective intervention for disease prevention, Medicare coverage is a central impediment to implementation of frailty-focused interventions in aging adults. Dr. Huisingh-Sheetz believes that new technologies such as remote interactions and voice-activated home devices may overcome current challenges associated with care of frail individuals.

Future Perspectives

Box 1 highlights the most promising areas for advancing our understanding of aging heterogeneity and includes suggestions facilitating translation of healthy aging. The main themes can be summarized as: heterogeneity in animal models, longitudinal and lifelong health monitoring, generation and interpretation of large datasets, fostering of multidisciplinary teams and training of new generations of aging researchers, and translation of basic knowledge into geroprotective interventions.

This is a field with aging experts, geneticists, statisticians, computer scientists, human and animal behaviorists, and more. This is an incredibly wide range of expertise which is ultimately a strength of the field. The aging field should be commended for enabling a diverse and inclusive environment and must continue to foster a multidisciplinary approach, expanding opportunities for the inclusion of research sub-specialties underrepresented in aging research, and critical for driving technical on conceptual innovation. Strategies to encourage collaboration of multidisciplinary teams and approaches to study heterogeneity in aging were proposed. Specifically, in the era of big data science, the inclusion of mathematicians, engineers (eg, med devices and new instrumentation), computer scientists, and statisticians, together with experts able to introduce biological, physiologic, clinical and other perspectives to such analyses was viewed as critical to the advance of research in the field of aging. Computational methods such as machine learning and computer vision can be used to train models that quantify biological age and its heterogeneity in genetically diverse model organisms. Models can also be used to predict aging trajectories and generate testable hypotheses. Lifelong behavioral monitoring of short-lived species such as killifish are promising and should be extended to other model organisms such as mice and rats. Finally, although highly controlled studies to quantify aging traits in model organisms are useful, studies that examine aging in humans in nonexperimental conditions, that is, "in the wild," are also enabled by new technologies. Although panelists agreed that privacy issues of daily monitoring by wearable devices and electronic tracking systems need to be addressed. The panelists discussed the importance of cross-species translation, particularly novel strategies for hypotheses testing in higher order model

Future perspectives

- **Increase the interindividual diversity of animal models for research** (e.g., by using genetically diverse rather than exclusively inbred rodent strains).
- **Develop robust, sensitive, reproducible, and scalable assays** to standardize biomarkers of biological aging across the field and to establish critical endpoints for measuring successful aging.
- **Create and deploy novel sensors and ecosystems** for lifelong assessment of aging trajectories in both humans and model organisms.
- **Generate and share harmonized multimodal datasets** for quantifying aging trajectories (e.g., high-resolution behavioral data, spatial and single cell genomics, proteomics, etc).
- **Develop new methods leveraging machine learning and AI** to quantify heterogeneity in aging within complex, multidimensional datasets.
- **Assemble multidisciplinary teams** with expertise in the biology of aging, computational science, genetics, genomics, mathematics, and statistics to analyze multimodal and multidimensional data.
- **Train the next generation of researchers** skilled in handling large multidimensional and multimodal datasets, and in integrating biological insights with big data for hypothesis testing.
- **Incorporate clinical studies and electronic medical records** to gain a comprehensive understanding of individual and population changes across the lifespan.
- **Facilitate cross-species and translational applications** of discoveries from model organisms to humans.
- **Leverage integrated large datasets** to identify and prioritize novel therapeutic targets.

Box 1: Promising areas for advancing our understanding of aging heterogeneity.

organisms. Training of a workforce that can leverage ML/AI to interrogate complex, multidimensional, multimodal, and big data is also a high priority.

Lastly, the group discussed the promise of interventions to minimize disease burden, including studies of senolytics, time-restricted feeding, physical activity, and social connection. Caloric restriction was discussed as both a potential human intervention and as a research tool. Continued development of new technology is necessary to enable and build upon much of this work. This research can inform implementation strategies for community level interventions aimed at decreasing morbidity and mortality. Considerations included measures beyond the frailty index (eg, whole animal imaging, molecular indices, life course geropathology), comparable and translatable phenotypes, data integration within and between animal models and humans, machine learning/AI models and trait outcomes, cross-sectional versus longitudinal design, and data analysis, and statistical methods in animal models for applications in humans.

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Conflict of Interest

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH or the United States Government.

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Author Contributions

V.K. prepared the initial draft of the manuscript. A.L.H., G.A.K., J.G.R., P.S. substantively edited the initial draft. All authors contributed to the content, and reviewed or edited the manuscript.

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