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ORIGINAL ARTICLE

The San Diego Nathan Shock Center: tackling the heterogeneity of aging

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Abstract Understanding basic mechanisms of aging holds great promise for developing interventions that prevent or delay many age-related declines and diseases simultaneously to increase human healthspan. However, a major confounding factor in aging research is the heterogeneity of the aging process itself. At the organismal level, it is clear that chronological age does not always predict biological age or susceptibility to frailty or pathology. While genetics and environment are major factors driving variable rates of aging, additional complexity arises because different organs, tissues, and cell types are intrinsically heterogeneous and exhibit different aging trajectories normally or in response to the stresses of the aging process (e.g., damage accumulation). Tackling the heterogeneity of aging requires new and specialized tools (e.g., single-cell analyses, mass spectrometry-based approaches, and advanced imaging) to identify novel signatures of aging across scales. Cutting-edge computational approaches are then needed to integrate these disparate datasets and elucidate network interactions between known aging hallmarks. There is also a need for improved, human cell-based models of aging to ensure that basic research findings are relevant to human aging and healthspan interventions. The San Diego Nathan Shock Center (SD-NSC) provides access to cutting-edge scientific resources to facilitate the study of the heterogeneity of aging in general and to promote the use of novel human cell models of aging. The center also has a robust Research Development Core that funds pilot projects on the heterogeneity of aging and organizes innovative training activities, including workshops and a personalized mentoring program, to help investigators new to the aging field succeed. Finally, the SD-NSC participates in outreach activities to educate the general community about the importance of aging research and promote the need for basic biology of aging research in particular.

Keywords Aging · Heterogeneity · Organoids · Human cohort · Single-cell analysis · Machine learning

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Introduction

"*One key to understanding aging and particularly to taking action that might extend the human life span can be found in the differences in the rate of aging observed in different individuals. These differences indicate that many factors play a role in aging. When we know why some people age less rapidly than others, we may be able to create conditions that will minimize the loss of functioning cells and tissues, thereby enabling many more people to live as long as those who live longest today*" [1]. This quote from Nathan Shock himself embodies our basic philosophy at the San Diego Nathan Shock Center (SD-NSC), which is to understand the heterogeneity of aging, with the ultimate goal of increasing the number of healthy and productive years of life (i.e., increase healthspan). Since individuals age at different rates and are differentially susceptible to age-related declines and pathology, detailed knowledge about what underpins the heterogeneity of aging is needed to allow the development of more personalized interventions based on targeting aging and longevity pathways.

The heterogeneity in the rates and phenotypes of human aging is no doubt driven by a combination of genetic and environmental factors unique to each individual [2]. However, even when using model organisms that are as close to genetically identical as possible and carefully controlling the lab environment, there is significant variability in cell and organismal phenotypes that leads to different rates of aging of individuals [3–7]. The same is true even of single-cell organisms and individual cells in cultured cell models of aging. Thus, there are intrinsic heterogeneities at the molecular, cellular, and tissue/organ levels that change over time and conspire with genetic and environmental factors to determine an individual's aging trajectory (Fig. 1) [2, 7, 8]. For example, there is significant heterogeneity in muscle stem cells that affects how this tissue maintains homeostasis and function [9] and hence changes in the stem cell niche over time is likely a major contributor to the variability observed in muscle aging between individuals. This is not unique to muscle, with virtually all cell types and tissues exhibiting significant intrinsic heterogeneity that changes with age due to damage accumulation and other factors [10, 11]. Conversely, loss of heterogeneity in the hematopoietic system (e.g., restriction of myeloid cell diversity) with age can

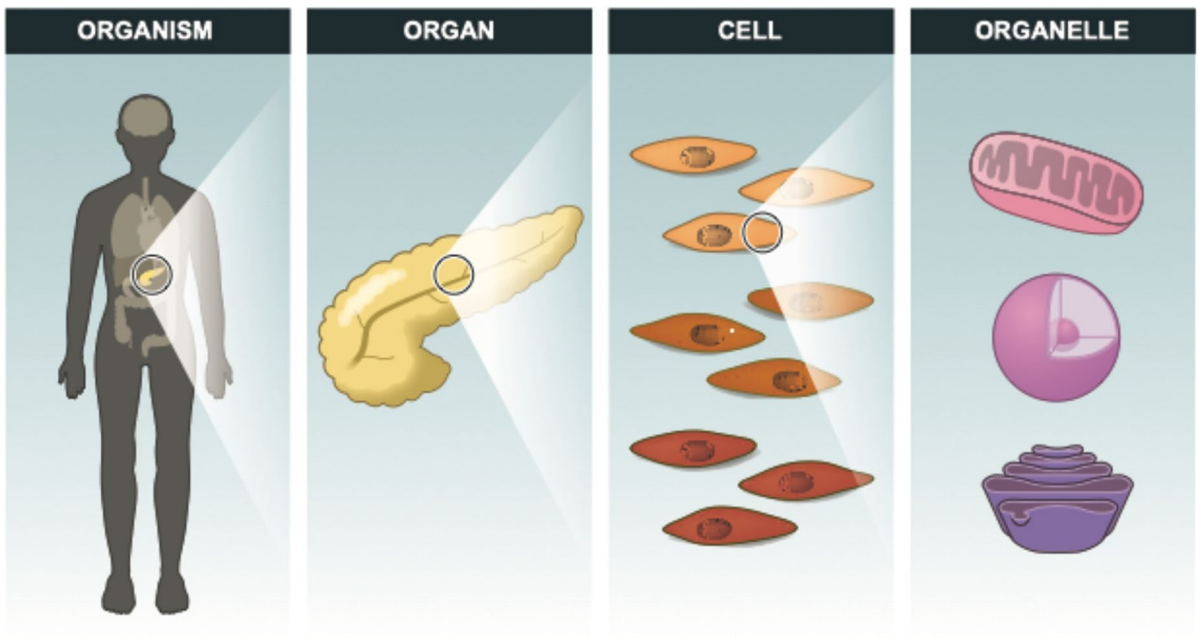

Fig. 1 The study of aging is complicated by the intrinsic heterogeneity of the process. This is manifest between individuals and within an organism across multiple scales down to the sub-cellular organelles and even at the molecular level

have detrimental consequences that promote age-related pathology [12]. However, despite the clear need to understand the role of heterogeneity in all facets of aging, there remains a dearth of attention to this matter in the field [13], in part due to technical barriers to measuring and studying heterogeneity. Therefore, we created the SD-NSC around the theme of the heterogeneity of aging to help fill this void for basic biology of aging researchers.

The SD-NSC, directed by Dr. Gerald Shadel, is facilitating research into the heterogeneity of aging by providing the research community access to three research resource cores. The Human Cell Models of Aging Core is providing new and improved human cell and tissue models of aging (induced cell types and organoids). This includes collecting and banking skin and blood samples from a clinical cohort that spans the breadth of the human adult lifespan that has been assessed for indicators of biological age and cellular bioenergetics. The Heterogeneity of Aging Core provides access to cutting-edge technologies for performing single-cell multi-omics, mass spectrometry-based, and advanced imaging analyses of biological samples. Finally, the Integrative Models of Aging Core provides computational tools for integrating different kinds of aging datasets (e.g., scRNA-seq and scATAC-seq) and mathematical modeling approaches for illuminating network interactions between known and newly discovered aging hallmarks. In addition to providing high-level services, each core is innovating within their research space and is committed to making these advancements readily available to the basic biology of aging research community.

Research resource cores

Human cell models of aging core

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The Human Cell Models of Aging Core is focused on creating powerful new human cell-based models of aging to enable a wide range of studies into the molecular and cellular heterogeneities of the human aging process. Approaches for generating these new models draw largely from recent advances in creating induced cell types via the direct conversion of skin cell samples, in which many aging characteristics (e.g., gene expression and epigenomic signatures) are maintained [14–16]. Thus, an individual's fibroblasts can be directly converted into additional, relevant cell types that can then be interrogated to reveal aging signatures. To enhance these activities, we are recruiting human subjects ranging in age from 20 to 75+ years, with no upper age limit (the SD-NSC clinical cohort) thereby representing the full breadth of the healthy adult human age span. These participants will be assessed for key clinical and physiological features of biological aging that are meaningful and relevant across all age groups, and blood and skin samples will be collected. Primary dermal fibroblasts derived from these skin samples will be used to create both

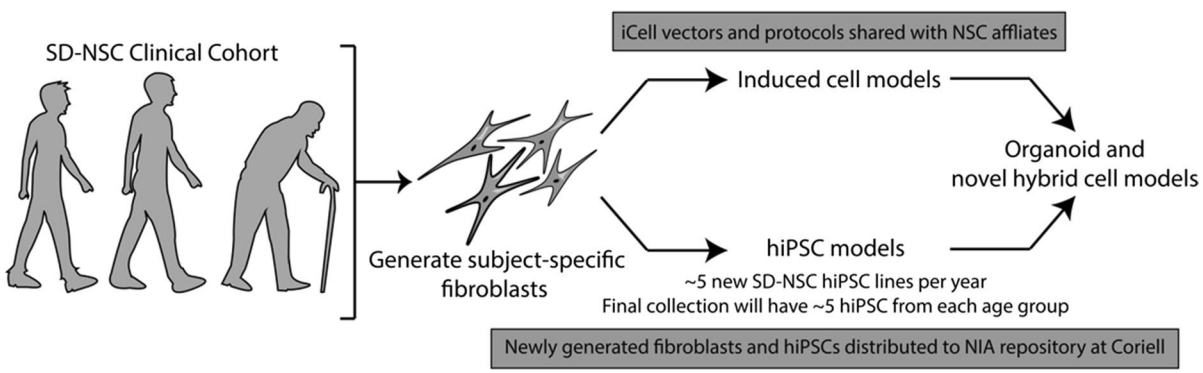

Fig. 2 Subject-specific fibroblasts and iPSCs will be generated from the SD-NSC clinical cohort that has been assessed for metrics of biological age and cellular bioenergetics. New cells lines will power models of aging through direct conversion and organoid technologies

reprogrammed stem cells and directly induced cell types, which will be banked (Fig. 2). The core will create standard operating procedures for generating a variety of induced cell types (e.g., neurons, vascular endothelial cells, and skeletal muscle) that aging researchers can use for downstream analysis, as well as pioneer efforts to induce cell types not yet achieved (e.g., hepatic, pancreatic, and cardiac), thereby expanding this important experimental toolkit.

For human cell models to have an even greater impact on basic biology of aging research, there is a profound need to move beyond traditional two-dimensional cell culture systems. The core will help drive technology development to create novel organoid and hybrid cell models that maintain age-related and tissue-specific heterogeneity. The use of human organoids in basic biological research is rapidly expanding, but to date, most organoids are derived from induced pluripotent stem cells (iPSCs), or from difficult to obtain primary tissue. Hence, the core will initially implement established iPSC-based organoid protocols [17–30] and then begin to introduce directly induced cell types. We will seek to gradually increase the proportion and diversity of induced cell types used to generate organoids, with the ultimate goal of developing robust protocols for creating tissue-specific organoids entirely from induced cell types that maintain hallmarks of aging (i.e., aged organoids). The core will also endeavor to develop hybrid organoid models consisting of both induced and iPSC-derived cell types (i.e., old and young cells). Altogether these represent new and robust systems for determining which cell types and cell-cell interactions drive human aging processes.

Heterogeneity of aging core

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The ability to systematically track the molecular and cellular processes that drive age-related functional decline within different cells and tissues is a critical step in understanding the heterogeneity of aging. The Heterogeneity of Aging Core brings together an array of powerful research technologies, methodologies, and expertise to support basic biology of aging research projects aimed at understanding heterogeneity across multiple scales. Services include single-cell RNA-seq and ATAC-seq, spatial transcriptomics, proteomics, metabolomics, and advanced imaging modalities (e.g., artificial intelligence-based image processing algorithms). Using these technologies in combination and potentially applying them to novel human cell-based models of aging being generated through the Human Cell Models of Aging Core provides investigators the ability to gain valuable insights into the spatiotemporal dynamics of molecular, cellular, and physiological processes that go awry during aging to drive tissue and organ dysfunction and pathology.

Single-cell approaches

The Heterogeneity of Aging Core provides services and training to enable single-cell sequencing approaches for investigating transcriptional and epigenetic heterogeneity of aging. The core can process human samples, as well as those from common model systems used in basic biology of aging research (e.g., yeast, *Caenorhabditis elegans*, *Drosophila*, rodents, and non-human primates). The team is experienced in generating single-cell suspensions from various tissues (e.g., liver, pancreas, bladder, bone marrow, lung, brain, adipose, mammary) and they are continuously developing methodologies to include new cell types. Quality control of raw data is accomplished via an analysis pipeline developed at the Salk Institute. Unsupervised clustering analysis (see "Integrative models of aging core" section) is then used to reveal cell types based on clusters in t-distributed stochastic neighbor embedding (tSNE) maps. An example of this is illustrated in Fig. 3, showing transcriptional differences in different cell types between young and old pancreas. The core can also perform trajectory inference analyses of scRNA-seq data to enable the mapping of dynamic changes in gene expression during aging [31–35]. In this same vein, the core also provides access to single-cell assays for transposase-accessible chromatin using sequencing (scATAC-seq) to allow interrogation of variations in chromatin accessibility of individual cells [36]. Combining scRNA-seq and scATAC-seq datasets or, preferably, performing 10X Genomics single-cell multiome assays to generate single-nucleus RNA-seq (snRNA-seq) and snATAC-seq datasets on the same cells provides a powerful means to define cellular heterogeneity in a wide range of cells and tissues to address the

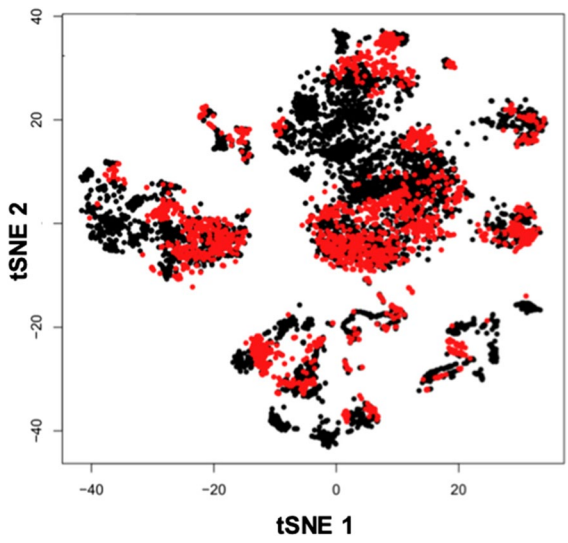

Fig. 3 tSNE plot of single-cell data from pancreas (young—black dots, old—red dots). Clusters represent the different cell types captured by this approach

heterogeneity of aging. Finally, the core is employing new technologies that enable spatial transcriptomics, which can be used to perform RNA-seq analysis or in situ hybridization on sectioned tissues or organoid cell models at single-cell or close to single-cell resolution. This approach allows analysis of heterogeneity within different regions of the same organ or organoid and can be integrated with scATAC-seq (e.g., to gain insight into spatiotemporal changes in enhancer activity) [37].

Mass spectrometry approaches

The core provides expert consultation concerning the design and implementation of a wide range of experiments using high-end mass spectrometry techniques. This includes protein identification, quantification, and post-translational modifications, secreted protein/peptide analysis, targeted and untargeted lipidomics (including bile acids), and targeted metabolomics, amino acid quantitation, and fatty acid analysis. They then provide analysis services, including custom developed proteomic and metabolomic analysis methods, including isotopic chemical labeling with tandem mass tags (TMT) and stable-isotope labeling with amino acids in cell culture (SILAC) [38]. The core is also continuously working to implement new analytic techniques in this rapidly evolving field. For example, the core has implemented a new hybrid technique for proteome quantification from cells and organoids in which cultures are first subjected to metabolic pulse-chase SILAC labeling, in which the normal culture media is exchanged with media containing heavy amino acids at different time points [39, 40]. All cells are then harvested together on the same day, providing samples with different levels of heavy amino acid incorporation. Cell organelles or compartments are fractionated prior to proteomic analysis, providing sub-cellular localization information on protein lifetimes. These SILAC labeled samples are then further labeled by TMT, which provides quantitative information on protein changes between samples and over time. Thus, a wealth of information on protein heterogeneity can be gained in a single experiment.

Advanced imaging approaches

Imaging is critical for probing the heterogeneity of aging, as it reveals the spatiotemporal dynamics of structural, morphological, and subcellular localization changes in aged cells at the tissue, cellular, subcellular, and molecular levels. Thus, the core provides high-end microscopes and imaging resources, including multiple high-end fluorescence and electron microscopes with live-imaging, high-throughput, and cryo capabilities. The core has several workstations for deep learning-based image processing and analysis and provides hands-on consultation and training for advanced imaging experiments, including sample preparation (i.e., probe selection, staining protocols, cell and tissue mounting techniques for optimal imaging conditions), carefully controlled image acquisition protocols, and quantitative image processing, segmentation, analyses, and visualization. The core staff has developed in-house deep learning-based models (Point-Scanning Super-Resolution, "PSSR") for live-cell imaging with higher speed and lower phototoxicity [41]. Training is available on how this resource can be used to acquire training data and implement pre-trained models in this workflow for increased throughput and spatiotemporal resolution while avoiding unwanted phototoxic cellular stress. The core has also developed advanced sample preparation protocols, such as tissue clearing, expansion microscopy, FISH, and correlative light and electron microscopy workflows, for lightsheet-to-Airyscan

imaging, as well as Airyscan timelapse imaging to electron microscopy. Finally, the core both develops and provides access and training on utilizing aging-relevant fluorescence probes in imaging assays for quantifying ROS, cAMP, cGMP, fatty acids, cholesterol, organelle movement and fission [42], mitophagy, autophagy, and DNA damage.

Integrative models of aging core

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Understanding the heterogeneity of aging requires researchers to tackle the aging process from multiple perspectives and to therefore generate, curate, analyze, and integrate an array of different types of data (e.g., from gene expression to cell morphology to organoid functionality). However, these data pipelines require domain-specific knowledge and must be implemented in a high-performance computing environment, raising significant barriers for researchers in the basic biology of aging field who do not have access to high-performance computing clusters and trained bioinformaticians. The Integrative Models of Aging Core seeks to lower these barriers by providing access to trained bioinformaticians and tools for the synthesis of diverse high-throughput datasets using integrative computational models [43–46], effective management of data [47], and assay-specific implementation of state-of-the-art analysis pipelines.

Data infrastructure

High-throughput datasets provide invaluable system-wide information about the activity of genes, proteins, and cells across aging phenotypes, but are typically noisy and include technology-specific challenges for pre-processing and normalization before the data can be analyzed. Therefore, standardized state-of-the-art pipelines must be implemented for downstream analysis and integration. Once processed, data must be stored and indexed so that downstream analyses and modeling can quickly identify sets of data associated with key variables (data type, age, tissue type, disease state, treatment, etc.) and extract the relevant underlying information. This is important for accurate model building, which must still be manually optimized over numerous iterations of data access, model training, model testing, and validation. To this end, the core maintains hardware and software resources required to pre-process and store sequencing, proteomic, metabolomic, and imaging data.

Integrating single-cell datasets

The core offers diverse tools for integrative analysis and clustering of single-cell data. Single-cell data pose specific challenges for interpretation and modeling. Different single-cell platforms can produce orthologous datasets for a particular sample (e.g., scRNA-seq, scATAC-seq, STARmap, flow cytometry, and mass spectrometry), but each approach has different sources of variability and experimental limitations [48]. Therefore, mathematical methods are needed to integrate data across these diverse datasets, and recent advances in integrative approaches now make this possible. These models create maps between clusters of cells in each dataset, which show similar correlative structures, thereby providing a way to systematically correct for technique-specific technical limitations and biases. For example, this method was used to integrate STARmap single-cell imaging data with scRNA-seq data to increase the resolution of the STARmap method [49].

Another powerful application of single-cell technologies is the reconstruction of specific biological processes by leveraging the heterogeneity of an evolving population [50]. These processes are revealed as patterns that are discovered using machine learning approaches, such as clustering and pseudotime ordering [51]. Individual clusters or "nodes" in this space represent specific biological snapshots of a biological state (e.g., long-lived cells), while connections between clusters reveal the dynamic transitions between these biological states (e.g., aging processes). By identifying both the specific states and how cells transition between them, these machine learning approaches can be used to generate specific hypotheses about these evolving cellular processes in the context of aging.

Predictive machine learning models

Statistical models, regression models, and classification models can elucidate relationships between data measured using orthologous approaches, multimodal

data, and variables of interest such as biological age, or age-associated disease states [52]. However, these models require both expertise in model design and implementation and a deep understanding of the modeled biology. To enable truly cutting-edge data integration services, the core is committed to working directly with teams of researchers to develop customized models to answer specific questions using well-validated modeling and machine learning libraries and toolkits, such as sci-kit learn [53] and caret [54]. A range of machine learning algorithms have been fit to bulk omic data to predict biological age [48–61]. The core aims to build and offer such machine learning models for predicting age-associated phenotypes from multimodal data. Importantly, due to the generality of machine learning models, collaborative efforts between biology of aging researchers can combine single-cell data with bulk measurements and shotgun proteomic and metabolomic measurements, as well as features from imaging datasets and other aging assays.

Methods for identifying global network geometry

The requirement for robustness under perturbation in biology implies significant redundancy between genes, proteins, and pathways in cell-biological networks. Thus, activity of any single node can be compensated for by the activity of "similar" nodes. This notion of "similarity" between nodes indicates the existence of hidden smooth geometrical structure. The existence of such hidden geometry, and its usefulness for network stability and communication within the network, has been demonstrated for such diverse networks as the internet [62] and plant metabolic products [63]. The core has developed tools for identifying global network geometry from multimodal aging datasets. These methods are based on mathematical techniques from algebraic topology that are unaffected by linear or nonlinear monotonic transformations of inputs [64], which is key for diverse data integration. The knowledge of identified hidden geometries can be incorporated into the clustering methods described earlier as well as the more standard embedding methods [65]. By providing a topological modeling service at the core, biology of aging researchers will be able to better visualize, cluster, and compare their multimodal datasets.

Widespread access to integrative modeling tools for studying the biology of aging

Integrative models promise to enable a systems-level understanding of aging biology phenomena, but are of limited use if the wider biology of aging community cannot understand them or apply them to their own specific datasets. Therefore, to maximize the use of the developed mathematical models and data, computational tools must be developed for applying models to specific datasets. Key for wide-spread adoptability and impact is the construction of interactive online interfaces for these models and sharing software via open source licensing [66]. A central initiative of the core is thus to develop interactive tools for applying the models, to distribute software via open source licensing, and to develop workshops and online resources to describe the limitations, interpretability, and use of these models to the basic biology of aging community.

Training and career development

Research development core

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The SD-NSC Research Development Core provides support for career development of junior researchers entering the basic biology of aging field, as well as established investigators in San Diego and beyond who wish to join the field. To this end, the SD-NSC Research Development Core awards six $15,000 pilot grants per year to help basic biology of aging investigators take advantage of the SD-NSC research resource cores to investigate the heterogeneity of aging in their model system. Award recipients receive fully subsidized access to the research resource cores, are provided training opportunities to visit the cores, and are paired with a senior biology of aging investigator to help ensure project success. For junior investigators, this pairing also constitutes a formal mentoring relationship to discuss project and career development. The core offers a yearly 1-day workshop at the Salk Institute the day after the annual La Jolla Aging Meeting (LJAM), a 1-day symposium featuring local research in San Diego on the biology of aging and a prominent keynote speaker from the aging research field. This symposium has been

organized since 2017 by Drs. Jan Karlseder (professor at the Salk Institute and member of the SD-NSC executive team), Peter Adams, and Malene Hansen with support from the Glenn Foundation for Medical Research. This workshop features an overview of the Research Development Core and the Pilot Grant Program, training modules for the three research resource cores (e.g., equipment overview, experimental workflows, and data analysis capabilities) with visits to the core facilities themselves, and a module to provide attendees with professional tools on how to write compelling grant applications. In addition to the LJAM-associated workshop, the scientific cores provide small group, hands-on training sessions, thus facilitating the uptake of these cutting-edge tools by the aging research community. Finally, the core makes available on the SD-NSC public website the presentation slides as well as video recordings of the 1-day workshop, to further disseminate details on center service offerings and increase the visibility of the SD-NSC.

Acknowledgements Dr. Malene Hansen was the original director of the SD-NSC Research Development Core and responsible for formulating its overall structure, innovative approaches, and integration with the annual LJAM meeting. Her tireless efforts were also instrumental in getting the entire SD-NSC off the ground successfully and transitioning seamlessly to the new Research Development Core Director Dr. Alessandra Sacco. The authors also wish to acknowledge Lara Avila at the Salk Institute for her steadfast and excellent coordination of SD-NSC workshops and other center activities and events. The authors wish to thank Rafael Arrojo E Drigo and Galena Erikson for allowing the use of preliminary data and other help to generate Fig. 3. This work is supported by the National Institute of Aging of the National Institutes of Health award number P30AG068635. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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