National Institute on Aging (NIA)
Division of Neuroscience (DN)

Lipids in Brain Aging and AD/ADRD

APRIL 28-29, 2021

This meeting summary was prepared by Rose Li and Associates, Inc., under contract to the National Institute on Aging (NIA) Division of Neuroscience (DN). The views expressed in this document reflect both individual and collective opinions of the meeting participants and not necessarily those of NIA. Contributions to this summary by the following individuals are gratefully acknowledged: Caroline Sferrazza, Dana Carluccio, Amanda DiBattista, Elizabeth A. Finch, and Nancy Tuvesson.

Table of Contents

Acronyms

AD/ADRD	Alzheimer's disease and related dementias
ALS	amyotrophic lateral sclerosis
AP	action potential
ApoE	apolipoprotein E
APOE-TR	*APOE* targeted replacement (mouse line)
APP	amyloid precursor protein
CARS	coherent anti-stokes raman
CMA	chaperone-mediated autophagy
CNS	central nervous system
cPLA2	phospholipase A2
CSF	cerebrospinal fluid
CST	cerebroside sulfotransferase
DN	Division of Neuroscience
EM	electron microscopy
ER	endoplasmic reticulum
FAD	familial Alzheimer's disease
FTD	frontotemporal dementia
HD	Huntington's disease
HDL	high-density lipoprotein
IHC	immunohistochemistry
iPSC	induced pluripotent stem cell
KO	knockout
LAMP-2A	lysosome-associated membrane protein 2A
LD	lipid droplet
LDAM	lipid-droplet-accumulating microglia
LPL	lipoprotein lipase
Lrp10	LDL receptor related protein 10
MCS	membrane contact site
MCTP	multiple C2 domain-containing transmembrane protein
miRNA	microRNA
MP-IHC	multiplex immunohistochemistry
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NHP	nonhuman primate
NIA	National Institute on Aging
NMR	nuclear magnetic resonance spectroscopy
PD	Parkinson's disease
PIP_2	phosphoinositol biphosphate
pTau	phosphorylated tau
PTM	post-translational modification
ROS	reactive oxygen species

scRNA-seq	single cell RNA sequencing
scWGCNA	single cell weighted gene correlation network analysis
snATAC-seq	single nucleus assay for transposase-accessible chromatin using sequencing
snRNA-seq	single nucleus RNA sequencing
Synj1	synaptojanin 1
Tfam	transcription factor A, mitochondrial
TMAO	trimethylamine N-oxide
VLDL	very-low-density lipoprotein

Executive Summary

The Lipids in Brain Aging and AD/ADRD workshop was sponsored by the National Institute on Aging's (NIA) Division of Neuroscience (DN) to identify gaps and opportunities related to lipids in brain aging and Alzheimer's disease and its related dementias (AD/ADRD). Lipids are connected to many important biological mechanisms, including inflammation, autophagy, and metabolism. Moreover, known AD/ADRD risk genes, such as *APOE*, have apparent interactions with lipids. However, the precise role of lipids in neural processes and disease mechanisms remains unclear. The goal of the workshop was to gain insight about where the field of lipid biology in brain aging is headed and what research questions could be prioritized as a result.

The two-day workshop featured three sessions: (1) lipid droplets (LDs) in aging and AD/ADRD, (2) myelin in aging and AD/ADRD, and (3) *APOE* and lipid homeostasis in aging and AD/ADRD. A discussion of the key themes followed the presentations for each session, and each day concluded with an overarching discussion of the research gaps and opportunities that emerged throughout the day.

Session 1: Lipid Droplets in Brain Aging and AD/ADRD

Session 1 Chair, Dr. Lance Johnson, moderated presentations and discussion related to LDs in the context of aging and AD/ADRD. Dr. Johnson began the session with an overview of fundamental apolipoprotein E (apoE) lipid biology and the contribution of *APOE* genotype to risk for AD/ADRD, including the interaction between *APOE* genotype and its possible influence on LD formation and accumulation. This interaction raises many questions about how LD accumulation influences AD/ADRD pathogenesis, such as the possibility of cell type-specific LD accumulation that varies by *APOE* genotype. Dr. Maria S. Ioannou continued by outlining how fatty acids are transferred between cell types in the central nervous system (CNS) and discussed how this fundamental process is impacted by *APOE* genotype and pathological states (e.g., excitotoxicity). Dr. Sarah Cohen provided additional context about LD biogenesis and the coordination of lipid metabolism among organelles, highlighting the importance of membrane contact sites (MCSs) as sites of both lipid transport and accumulation of amyloid precursor protein (APP), α-synuclein, and apoE. Dr. Ana Maria Cuervo then discussed mechanisms of autophagy and how they are disrupted in the context of aging and disease, as well as the consequences of this disruption for lipid metabolism in the aging brain. Dr. Tony Wyss-Coray concluded the presentations by introducing LD-accumulating microglia, which may represent a pro-inflammatory and dysfunctional cellular state in the aging brain. Following the presentations, the speakers discussed how typical LD accumulation should be defined in the aging brain and the potential functional role of LDs, including whether that role is pathological or protective. The speakers highlighted key gaps and opportunities related to LDs, including but not limited to the following:

1. How do we define LDs in the brain?
2. Is the accumulation of LDs pathological or protective in brain aging and AD/ADRD?
3. What are LDs doing in the aged brain (e.g., function, dynamics, modulation)?

4. How do LD accumulation and LD composition vary by cell type, *APOE* genotype, or neuropathology?
5. What are the mechanisms of lipid transfer in the brain, and do they shift under different conditions?
6. How is LD biogenesis in the CNS similar or different from other tissues?
7. How do autophagy pathways compensate for each other in relation to lipid metabolism and aging, and how can these pathways be modified to restore normal lipid metabolism in the brain?
8. What is the relationship between apoE isoforms and glial function, and how might LDs impact this relationship?

Session 2: Myelin in Brain Aging and AD/ADRD

Session 2 Chair, Dr. Robbie Brinton, moderated presentations and discussion related to myelin in aging and AD/ADRD. Dr. Brinton began by introducing the emerging role of myelin as an auxiliary fuel source for the starving brain and suggesting that the bioenergetic transitions that occur during menopause may catalyze myelin breakdown, partly accounting for women's disproportionate risk of developing AD/ADRD. Dr. Douglas Rosene presented evidence from nonhuman primates (NHPs) on the factors that underlie age-related accumulation of myelin pathology and associated cognitive impairment, highlighting multiple potential mechanisms by which myelin integrity may deteriorate with age. Dr. Xianlin Han provided a closer examination of the sulfatide lipids that are present in the myelin sheath, and demonstrated how a novel sulfatide metabolism pathway interacts with many of the risk factors for AD/ADRD. Dr. Vivek Swarup then explored how single-cell multi-omics may contribute to the study of cell type-specific phenotypes in AD/ADRD. Dr. Kimberley D. Bruce continued to highlight the importance of cell type specificity with an introduction to lipoprotein lipase (LPL), which is predominantly expressed in microglia and considered to play a central role in neurodegenerative disease processes and demyelination. Following the presentations, the speakers discussed the role of myelin lipids in aging and AD/ADRD, the immune system's interaction with myelin, and observations across model systems. The speakers highlighted key gaps and opportunities related to myelin, including but not limited to the following:

1. What are the roles of myelin lipids and their signaling in aging and AD/ADRD?
2. How do these roles vary depending on context (e.g., brain region, model, interactions with other cell types)?
3. What is the role of the immune system, and how does it interact with myelin?
4. Does myelin catabolism occur in the human brain, and can therapeutic interventions be developed to protect against myelin catabolism or restore myelin integrity?
5. What are the relative contributions of different processes (e.g., direct damage, impaired repair mechanisms) to age-related myelin pathology, and what are potential interventions to reduce or mitigate these contributions?
6. How are myelin lipids (e.g., sulfatides) metabolized, and what are the effects of this metabolism?
7. How is gene expression differentially regulated in AD/ADRD versus normal aging as well as across cell types?

8. How does LPL interact with myelin and myelin-derived lipids, and does this interaction vary by cell type?

In the final session on Day 1, presenters from both Sessions 1 and 2 came together to discuss cross-cutting themes that had emerged. The speakers reviewed sex differences that have been observed related to lipid metabolism and recognized the knowledge gap concerning how these sex differences may influence aging processes, AD/ADRD pathology, and LD accumulation. The speakers also discussed the intersection of LD and myelin biology, including how the metabolism of myelin as an alternative fuel source could potentially impact LD accumulation. Numerous methodological gaps and opportunities were identified, such as the difficulty of measuring lipid flux in the brain, the potential contributions of spatial transcriptomics, and the need for multidisciplinary collaboration.

Session 3: APOE and Lipid Homeostasis in Brain Aging and AD/ADRD

Session 3 Chair, Dr. Guojun Bu, moderated presentations and discussion related to *APOE* and lipid homeostasis in aging and AD/ADRD. Dr. Bu presented an introduction of apoE function in brain lipid metabolism, reviewed several known apoE-related pathogenic pathways in AD, and emphasized the complexity of studying *APOE* as it is expressed by multiple brain and periphery cell types. He then focused on several lessons learned about the role of different *APOE* isoforms in brain lipid metabolism and AD/ADRD from animal and organoid models. Dr. Ana Valencia-Olvera then introduced the lipid transporter ABCA1, which plays an important role in the lipidation of apoE-containing lipoproteins, as a potential therapeutic target for AD. Dr. Valencia-Olvera discussed multiple ways of targeting ABCA1 activation, including direct induction of *ABCA1* gene expression by a retinoid X receptor agonist and modulation of ABCA1-dependent processes with apoE-derived peptides. Dr. Bill Rebeck expanded upon the recurring theme of cell type specificity in AD/ADRD-related lipid biology with an overview of cell-specific expression of apoE isoforms, including differential post-translational modification of apoE according to isoform as well as location in the CNS or periphery. Dr. Christopher Ramsden then introduced how multiplex immunohistochemistry can be leveraged to study *APOE*-related pathologies in AD/ADRD by enabling the labeling of up to 100 unique markers of cytoarchitecture and pathology in the same specimen. Dr. Dongming Cai examined the regulation of apoE-mediated lipid signaling on a single cell level, providing further context for ongoing discussions of sex differences and cell type specificity throughout the workshop. Dr. Cai's presentation also highlighted that multiple opportunities exist to develop novel therapies that target apoE-mediated signaling pathways, potentially enabling precision medicine-driven AD therapies that are tailored by sex and *APOE* genotype. Dr. Fei Yin continued to address the importance of cell type specificity in an overview on the disruptive role of apoE in brain fatty acid metabolism. Dr. Laila Abdullah then addressed carnitine/acylcarnitine-mediated lipid dysfunction in AD, underscoring the influence of *APOE* ε4 genetics and the comparison of measurements between the CNS and periphery. Dr. Hussein Yassine concluded by presenting opportunities to leverage lipid- or lipoprotein-based biomarkers in clinical trials, emphasizing gaps and opportunities related to the translation of lipoprotein and lipid studies from bench to bedside. Following the presentations, the speakers discussed the differential influences of *APOE* genotype, the importance of studying lipid composition, the interaction between peripheral and CNS lipids,

and how lipid biology could potentially be leveraged for the prevention of AD/ADRD. The speakers highlighted key gaps and opportunities related to apoE and lipid homeostasis, including but not limited to the following:

1. How does the peripheral lipid transport and metabolism by different *APOE* genotypes influence brain lipids?
2. How does the microbiome influence brain lipids in the context of different *APOE* genotypes?
3. How does brain lipid metabolism and composition affect brain aging and diseases of aging?
4. What are the differential effects of apoE isoforms on brain lipid metabolism, bioenergetics, and inflammation as they relate to brain aging and diseases of aging?
5. How do *APOE* and *APOE*-related risk factors mediate the effects of lipids on age-associated diseases?
6. Can lipids and lipoproteins act as useful biomarkers? Can we image brain lipids to guide clinical studies?
7. How can we translate insights into apoE-related pathologies from animal models to humans?
8. Is the dysfunction of apoE/ABCA1-mediated lipid efflux a major contributor to intracellular lipid and LD accumulation?
9. What are the effects of reducing apoE4 expression on brain lipid homeostasis, and is this a potential therapeutic strategy?
10. Can novel therapies be developed that target apoE-mediated lipid signaling pathways?
11. How can AD/ADRD therapeutics be tailored to sex and *APOE* genotype?

The workshop concluded with a discussion of additional cross-cutting themes that had emerged over the course of both days. Participants addressed the selective vulnerability of different cell types to LD accumulation and potential approaches to address knowledge gaps in this area. The speakers also recognized the importance of distinguishing typical aging processes from progression of age-related disorders when studying lipid metabolism. In addition, participants discussed ways to leverage the periphery to understand cognitive outcomes, acknowledging new techniques that can improve collective understanding of the gut-brain axis and directly inform studies of peripheral lipid metabolism and its influence on the brain.

Meeting Summary

Welcome and Introductions

Eliezer Masliah and Amanda DiBattista, National Institute on Aging (NIA) Division of Neuroscience (DN)

This workshop was sponsored by the National Institute on Aging's (NIA) Division of Neuroscience (DN) to identify gaps and opportunities related to lipids in brain aging, Alzheimer's disease, and Alzheimer's disease related dementias (AD/ADRD). Lipids are connected to many important biological mechanisms, including inflammation, autophagy, and metabolism. Moreover, known AD/ADRD-related risk genes such as *APOE* have apparent interactions with lipids. However, the role of lipids in neural processes and disease mechanisms remains unclear. The goal of the workshop was to gain insight about where the field of lipid biology and brain aging is headed and what research questions could be prioritized as a result.

Session 1: Lipid Droplets in Brain Aging and AD/ADRD

Session Chair: Lance Johnson, University of Kentucky

Lipid droplets (LDs) are lipid-rich organelles that regulate the storage and hydrolysis of neutral lipids. These organelles consist of a neutral lipid core that is primarily triglycerides and cholesterol esters, although this composition varies and may include various phospholipid species. LDs also have a phospholipid monolayer shell that contains a wide variety of surface proteins. The lipids that are stored in these droplets may be generated de novo or acquired from exogenous or endogenous sources. LDs may be up to 100 µm in size and can be visualized at the subcellular level by electron microscopy (EM) and at the cellular level by lipophilic fluorescent dyes (e.g., BODIPY) or by tagging common protein components.

Although LDs were initially considered to merely serve as storage for fat, these droplets are now recognized as dynamic organelles that play a role in modulating cellular metabolism and signaling. Moreover, LDs have been connected to inflammation and infection, the oxidation of fatty acids, the regulation of autophagy, and protection against potentially toxic lipids. Imbalance in lipid storage is linked with many peripheral diseases (e.g., cancer, diabetes, and cardiovascular disease) and neurodegenerative diseases, including AD/ADRD. Although recent evidence from postmortem human brain suggests that AD is associated with LD accumulation, most research on LD involvement in central nervous system (CNS) disorders has been conducted almost exclusively in animal models, underscoring a need for more thorough investigation of the role of LDs in human neurodegeneration.

Lipid Droplets in Alzheimer's Diseases: A Role for *APOE*?

Lance Johnson, University of Kentucky

Apolipoprotein E (apoE) is an important peripheral and CNS lipoprotein found on the surface of lipoprotein particles (primarily very-low-density lipoprotein [VLDL] in the periphery and high-density lipoprotein [HDL] in the CNS). In the CNS, apoE is primarily synthesized by astrocytes

and also by microglia upon activation. The majority of genetic risk for late-onset AD is attributed to *APOE* genotype: inheritance of one *APOE* ε4 allele increases AD risk 2- to 3-fold and inheritance of two *APOE* ε4 alleles can increase AD risk more than 12-fold. Conversely, the *APOE* ε2 allele appears to be protective against developing AD. *APOE* ε4 is associated with numerous metabolic abnormalities, including accumulation of intracellular lipids and impairment of lipid efflux by astrocytes. Evidence from immortalized astrocytes has shown that ε4-expressing astrocytes accumulate many more LDs than ε3-expressing astrocytes, which may be due to less oxidation of exogenous fatty acid in ε4-expressing astrocytes. It is not yet clear how ε4 genotype impacts LD formation in vivo. Important outstanding questions related to this topic include:

1. Whether ε4 brains (both animal model and human) have more LDs than brains with other *APOE* genotypes
2. Whether LDs accumulate specifically in astrocytes
3. Why LDs form preferentially in ε4 cells
4. Whether this preferential accumulation is pathological or protective
5. Whether ε4-associated changes in lipid storage are upstream of other metabolic abnormalities observed in ε4 carriers
6. How to target LD accumulation for AD/ADRD interventions, if LDs cause AD pathology and ε4 drives LD formation

Lipid Transport from Neurons to Glia in Health and Disease

Maria S. Ioannou, University of Alberta

During excitotoxicity, neurons generate a lot of reactive oxygen species (ROS), which may be a trigger for excess fatty acid production and LD formation. Although stored fatty acids can be transferred to mitochondria for consumption, neurons have a low capacity for fatty acid catabolism; excitotoxicity reduces this capacity further by promoting mitochondrial fragmentation. When mitochondria are fragmented, excess fatty acids can be released from the cell and transferred to other cells. In vitro evidence demonstrates that excitotoxicity induces excess fatty acid formation in neurons and furthermore that neurons can transfer fatty acids to co-cultured astrocytes, confirming that oxidative stress in neurons is sufficient to induce LD formation in neighboring glia. Knockout of *APOE* significantly reduces the transfer of fatty acids from neurons to glia; this effect is maintained even if the knockout is restricted to the donor neurons and astrocytes continue to express wildtype levels of *APOE.* Because neurons do not typically express *APOE* but rather upregulate *APOE* during cellular stress (including excitotoxicity), it is possible that *APOE* upregulation is a stress response that rids neurons of potentially toxic lipid accumulation, although the precise role of *APOE* in this process is unknown.

Excitotoxicity is the primary cause of neuronal death in stroke, and *APOE* ε4 accelerates the development of dementia after stroke. In a murine pial strip model of acute stroke, LDs accumulated in astrocytes relative to the analogous contralateral (i.e., unaffected) region, consistent with the possibility that excitotoxicity promotes fatty acid transfer to astrocytes.

Furthermore, *APOE* is upregulated in neurons within the ischemic penumbra of this model system, potentially to help with the clearance of toxic fatty acids. Outstanding research questions in this area include:

1. What are the different mechanisms of lipid transfer and do they shift under different conditions?
2. What is the composition of LDs in the brain and do they vary in different cell types or neuropathologies?
3. What are the function and physiological consequences of accumulating LDs in the brain?

Lipid Droplet Biogenesis and Interactions with Other Organelles

Sarah Cohen, University of North Carolina at Chapel Hill

LD biogenesis begins with the synthesis of triglycerides that accumulate within the endoplasmic reticulum (ER) membrane to form lens-like structures. Seipin proteins help to organize the lipids into nascent droplets prior to detachment from the ER and maturation. LDs specifically form at multiple C2 domain-containing transmembrane protein (MCTP) ER subdomains, which may promote LD biogenesis by inducing membrane curvature and supporting tethering of the ER to organelles. After LDs detach from the ER, they continue to grow via local lipid synthesis or coalescence with other LDs. While much of the work on LD biogenesis thus far has focused on the triglyceride synthesis pathway, much less is known about how the cholesterol ester synthesis pathway contributes to LD biogenesis, which may be important in the cholesterol-rich brain.

Lipid metabolism must be coordinated among organelles: the ER is a major site of lipid synthesis, LDs store and transport lipids, mitochondria and peroxisomes perform β-oxidation of lipids, and lysosomes play multiple roles in lipid hydrolysis and recycling. Although the LD's unique phospholipid monolayer prevents it from interacting with vesicular trafficking pathways that connect many other organelles, LDs can still interact with other organelles by forming close membrane contacts known as membrane contact sites (MCSs). Although membrane contacts are highly dynamic, the overall pattern of contacts between different organelles is stable over time. MCSs are the site of lipid transport between organelles and are also implicated in multiple types of neurodegeneration as sites where key proteins (e.g., amyloid precursor protein [APP] and presenilins in AD, α-synuclein in Parkinson's disease [PD]) localize. There is also evidence that apoE localizes to LD-ER MCSs, specifically by forming ring-like structures that encompass LDs during LD biogenesis and lipid synthesis, although the role of apoE in these processes is unknown. Further outstanding questions in this field include whether LD biogenesis in the brain uses the same machinery as in other tissues and why neurodegeneration-associated proteins localize to LD MCSs.

Selective Autophagy: When Quality Control Meets Lipid Metabolism

Ana Maria Cuervo, Albert Einstein College of Medicine

Autophagy is the mechanism by which intracellular components are degraded in lysosomes. Autophagy can occur by multiple pathways—including macroautophagy, microautophagy, and

chaperone-mediated autophagy (CMA)—that may differentially interact with LDs. The activity of autophagy pathways decreases with age and with the progression of metabolic and neurodegenerative disorders. Macroautophagy continuously facilitates the degradation of LDs in organs such as liver, by delivering LDs to lysosomes for breakdown by lipases in a process known as lipophagy. Lipophagy also occurs in the brain but differences in the regulation and in the contribution of lipophagy and conventional lipolysis to LD mobilization remains unknown. The complex process of macroautophagy can malfunction in aging and neurodegenerative diseases in multiple ways. In Huntington's disease (HD), autophagosomes fail to recognize the cargo that they typically deliver to lysosomes for degradation, which results in an accumulation of cellular and organellar debris (including LDs) in the cytosol. In AD, autophagosomes may encapsulate their cargo and traffic material properly to lysosomes but primary changes in lysosomes (i.e. lysosomal pH) prevent cargo degradation. The immediate consequences are intralysosomal accumulation of LDs and an energy deficiency. Image-based studies and lipidomics in animal model and human brain tissue have confirmed that giant autolysosomes that cannot undergo proper degradation accumulate LDs in AD.

Lipid toxicity and LD accumulation have an inhibitory effect on macroautophagy by hindering the ability of autophagosomes to fuse with lysosomes. Thus, LD accumulation creates a circular problem in which it inhibits the autophagic processes that are meant to protect against it. Lipid toxicity also impairs CMA, by decreasing levels of lysosome-associated membrane protein 2A (LAMP2A), the lysosomal receptor for CMA; reduced LAMP2A stability and altered LAMP2A dynamics are also observed with age and in conditions that produce proteotoxicity, including AD, PD, frontotemporal dementia (FTD), and amyotrophic lateral sclerosis (ALS). Loss of CMA in neurons produces neurodegenerative phenotypes and changes in protein homeostasis in animal models. Although CMA only targets proteins for degradation, CMA may act upstream of LDs by targeting LD surface proteins, which need to be removed from LD in order to initiate lipolysis and lipophagy, or by regulating levels of lipogenic enzymes through their lysosomal degradation. The consequences of convergent proteotoxicity and lipotoxicity for autophagy pathways are not fully understood. More research is also needed to understand how autophagy pathways may compensate for each other in relation to lipid metabolism and aging, as well as how these pathways may be modified to restore normal lipid metabolism in the brain. In addition, because autophagy is very nutrient-dependent, the interplay between autophagy and diet may have implications for the function of autophagy pathways in older age.

Lipid-droplet-accumulating Microglia in Mice and Alzheimer's Disease

Tony Wyss-Coray, Stanford University

The homeostatic functions of microglia decline with age. The microglia of aged mice have been shown to accumulate LDs that contain mostly glycerolipids and very few cholesterol esters. A CRISPR-Cas9 screen of microglia following induction of LD formation highlighted numerous genes that promote or inhibit LD formation, many of which have also been linked to autosomal dominant forms of neurodegeneration. Transcriptional and functional evidence suggest that lipid-droplet-accumulating microglia (LDAM) represent a pro-inflammatory and dysfunctional

cellular state in the aging brain associated with increased production of ROS, greater release of cytokines, and reduced levels of phagocytosis.

A combination of fluorescence microscopy and coherent anti-stokes raman (CARS) microscopy can be used to visualize dysfunctional LDAMs in human brain tissue as well as assess LD composition within LDAMs. Quantitative analysis of such images suggests that LDAMs accumulate lipid-rich myelin debris in AD brain tissue. Transcriptional profiling has revealed an LDAM signature that identifies LDAMs as a unique subset of microglia, and the relationship between LDAM and disease-associated microglia is not yet clear. Furthermore, LDAM genes are more differentially expressed in microglia homozygous for *APOE* ε4 compared to those expressing ε3, suggesting a preferential upregulation of the LDAM state in the *APOE* ε4 genotype. Knowledge gaps in the field include the composition, cellular source, and spatial location of LDs in normal human brain aging and in AD; the cellular and molecular relationship between apoE isoforms and microglial (or astroglial) function; and whether LDs in the brain are a potential therapeutic target.

Session Discussion

Prior to the workshop, some key knowledge gaps related to LDs in brain aging were identified by workshop organizers:

1. How do we define LDs in the brain?
2. Is accumulation of LDs pathological or protective in brain aging and AD/ADRD?
3. What are LDs doing in the aged brain (e.g., function, dynamics, modulation)?

Defining Typical LD Accumulation in the Aging Brain

It is not yet clear how much baseline LD accumulation should be expected to occur in the brain over the course of typical aging. LD turnover is likely a fast process resulting from the efficient balance of biogenesis and degradation. As a result, it is difficult to capture an accurate estimate of baseline LD accumulation from a single timepoint measurement outside of disease contexts. Technological advances, including more sophisticated immunohistochemistry (IHC) tools that target core LD proteins, systems that can elucidate dynamic turnover processes, and methods that can assess LD composition (e.g., CARS microscopy), will facilitate the determination of this baseline. It is also important to generate a set of consistent datasets and resources (e.g., EM datasets, induced pluripotent stem cell [iPSC] libraries) that can be used to facilitate comparisons of LD accumulation across contexts (e.g., different types of neurodegeneration, different sexes). To accurately assess LD accumulation in human postmortem tissue samples, the field must also establish postmortem interval effects on LD accumulation, which may be addressed by work in animal models.

LD Function in the Aging Brain

The role of LDs in the brain is also not yet clear, including whether their accumulation is pathological or protective or whether they can be used to detect brain pathologies. In light of evidence that suggests dietary challenges promote lipotoxicity and proteotoxicity, opportunities exist to study the interplay between proteins and lipids as it pertains to lifestyle (e.g., diet, exercise) and neuropathologies. One barrier to studying the role of LDs is the

inaccessibility of CNS samples in humans. Greater understanding of how peripheral samples can be used as a proxy for LD activity in the brain would greatly enhance the ability of the field to interrogate the role of LDs in humans. The identification of peripheral surrogate markers could also enable clinicians to administer future interventions for AD/ADRD that are rooted in LD biology. Magnetic resonance spectroscopy (MRS) may be a good candidate for non-invasive imaging of lipid species. New methods to extract LDs from brain tissues or cell lines are also needed to investigate LD function. While biochemical flotation is currently available for this purpose, this method does not have good cell-type resolution.

Session 2: Myelin in Brain Aging and AD/ADRD

Session Chair: Robbie Brinton, University of Arizona

Myelin as a Ketone Reservoir: Feeding a Starving Brain

Robbie Brinton, University of Arizona

Women are at higher risk of developing AD than men, which may be due in part to bioenergetic transitions that occur during menopause. Estrogen promotes the metabolism of glucose, which typically serves as the brain's primary source of fuel. As estrogen levels decline during menopause, the brain compensates for lower glucose availability with a stress starvation response that activates the utilization of auxiliary fuel sources, starting with amino acids, transitioning next to ketone bodies, and ultimately metabolizing fatty acids. These auxiliary fuels can be derived by catabolism of white matter. Evidence from animal models has demonstrated that LDs accumulate in parallel with myelin disintegration during perimenopause. The development of fuels derived from myelin can be tracked with lipidomics, which has confirmed that accumulation of ceramides (a product of myelin breakdown) occurs during perimenopause, and further that these ceramides are converted to fatty acids and then ketone bodies. Activation of the sphingomyelinase pathway, which is required for myelin breakdown, and fatty acid metabolic pathways have also been observed during this early aging time period. Early evidence from human brains supports preclinical observations of white matter loss and utilization of auxiliary or alternative fuels in the female brain across the same endocrine transition states that have been analyzed in animal models.

Astrocytes may play an important role in contributing to these metabolic processes as sites of β-oxidation of fatty acids. This role is supported by observed colocalization of astrocytes with markers of phospholipase A2 in white matter tracts after menopause. Debris from white matter catabolism is taken up by microglia. The functional capabilities (e.g., capacity for phagocytosis) of these microglia may differ by sex and *APOE* status, because functional markers are relatively depressed in female but not male *APOE* ε4 carriers. LDs also appear to accumulate most abundantly in astrocytes derived from female *APOE* ε4 carriers. This myelin breakdown pathway leads to parallel antigen presentation on microglia and infiltration of T lymphocytes into the brain, suggesting that the process for addressing a bioenergetic crisis can ultimately lead to potential autoimmunity in the brain. Many research gaps and opportunities are related to these processes, including confirmation that myelin catabolism occurs in the human brain

and identification of therapeutic interventions that could protect against myelin catabolism or restore myelin integrity.

Factors Underlying the Age-Related Accumulation of Myelin Pathology and Associated Cognitive Impairment in the Normal Aging Rhesus Monkey

Douglas Rosene, Boston University

Rhesus macaques display various cognitive impairments similar to aging humans. Despite the presence of amyloid plaques and hyperphosphorylated tau in older monkeys, no age-related change in the number of neurons or glia has been observed. However, magnetic resonance imaging (MRI) has revealed highly significant white matter atrophy as monkeys age that correlates with cognitive decline. Pathological nerve fiber profiles with degenerating myelin sheaths and degenerating axons increase with age, indicating disrupted interactions between oligodendrocytes and axons. One hypothesis posits that myelin damage results in action potential (AP) failures that reduce post-synaptic trophic feedback and lead to axonal loss, followed by synaptic and dendritic atrophy. This combination of myelin damage, axon loss, and synaptic atrophy ultimately results in cognitive decline.

A number of potential mechanisms exist that could cause myelin to deteriorate with age. Over time, oligodendrocytes may become less able to maintain myelin sheaths or repair damage. Oligodendrocytes in aging primates (NHPs) exhibit evidence of DNA damage, which may support this mechanism. It is also possible that age-related increases in oxidative damage and inflammation could actively damage myelin, which in combination with impaired oligodendrocyte function could result in myelin degradation that outpaces myelin repair. Many more activated and phagocytic microglia are observed specifically in the white matter of older NHPs. The neuroinflammatory environment of the aging brain also appears to promote T cell entry into white matter—but not gray matter—which may exacerbate myelin damage and the detrimental effects of inflammation in these regions. Many opportunities to investigate cognitive aging and myelin pathology in NHPs remain, including quantification of the relative contributions of different processes (e.g., direct damage, impaired repair) to myelin pathology and the identification of interventions that could reduce oxidative stress and inflammation, enhance myelination, or modulate T cell infiltration.

The Role of Myelin Lipids Played Beyond in Myelin Integrity: Lessons Learned from Studying Sulfatide Deficiency in Alzheimer's Disease

Xianlin Han, University of Texas Health Science Center at San Antonio

Sulfatide lipids are present in the myelin sheath. Mass spectrometry from human postmortem brains across different stages of AD has revealed a loss of sulfatide lipids in multiple brain regions compared to controls. Similar findings have been observed in animal models, including a reduction in sulfatide that parallels the development of Aβ pathology in APP transgenic mice. Sulfatide levels are lower in the brain but higher in cerebrospinal fluid (CSF) of *APOE* ε4 mice relative to both *APOE* ε3 and wildtype mice, suggesting that sulfatide levels are also apoE isoform dependent. These observations support the existence of a novel sulfatide metabolism

pathway in the CNS that contains many of the risk factors for AD. Astrocytes produce apoE-associated lipoproteins that acquire sulfatides from the myelin sheath, which can be detected in the CSF.

Constitutive and conditional knockouts of cerebroside sulfotransferase (CST)—the enzyme that produces sulfatides—have been generated to study the consequences of sulfatide loss in AD mouse models. In these mice, sulfatide deficiency induces marked glial activation and astrogliosis. Consistent with findings that sulfatide reduction occurs in parallel with Aβ deposition, sulfatide loss in AD mice leads to decreased levels of intracellular Aβ and increased levels of extracellular Aβ, highlighting a clear role for sulfatides in the facilitation of intracellular Aβ clearance and deposition. The loss of sulfatides also leads to increased levels of soluble or oligomeric Aβ, increased tau hyperphosphorylation, enlargement of the lateral ventricles, and severe neurogenic bladder in aged mice. The mechanisms of these effects of sulfatide deficiency are still unknown, and the metabolism of other myelin lipids should also be investigated.

Single-cell Multi-omics Identifies Oligodendrocyte Heterogeneity in Alzheimer's Disease

Vivek Swarup, University of California, Irvine

Although there are significant changes in gene expression as AD progresses, cell type–specific changes are difficult to resolve using bulk tissue analysis, in part because cell-type proportions change over the course of AD. Single cell omics enables the estimation of gene expression changes in a cell type–specific manner. Integrated analysis of single nucleus RNA-seq (snRNA-seq) and snATAC-seq enables the investigation of gene expression and regulation for a target cell type. This integrated analytic approach has been used to identify distinct neural clusters, including a large heterogenous population of oligodendrocytes. Moreover, these methods can resolve how the proportion of these oligodendrocyte subpopulations change throughout AD.

As AD progresses, there is an increase in mature and decrease in newly formed oligodendrocytes in the brain. The cell type–specific regulation of gene expression by cis-regulatory elements and transcription factors also appears to differ between AD and control tissue. Oligodendrocytes in AD tissue have more open chromatin at risk loci (e.g., *ADAM10*) than control tissue. This kind of single cell omics data can be used to construct cell type–specific transcription factor regulatory networks. In addition, a novel co-expression network analysis known as single cell weighted gene correlation network analysis (scWGCNA) can identify modules of co-expressed genes that are up- or down-regulated in AD; for example, *SREBF1*-targeted genes were shown to be downregulated in oligodendrocytes in early and late AD.

Lipoprotein Lipase in Neurodegenerative Disease

Kimberley D. Bruce, University of Colorado Anschutz Medical Campus

The role of lipoprotein lipase (LPL) in regulating lipid and lipoprotein metabolism is well established in the periphery. However, its role in the brain is less well understood. In addition to its canonical role in triglyceride and phospholipid hydrolysis, LPL can facilitate endocytosis of

lipoproteins. Recent evidence from the CNS of both mice and humans has shown that LPL is predominantly expressed in microglia, yet relatively little is known about the role of LPL in microglia function. The *LPL* gene is among the most upregulated genes in microglia during development, disease, and demyelination, suggesting a central role for microglial LPL in these processes. Data from experimental autoimmune encephalomyelitis (EAE) mouse models and ex vivo cerebellar slice cultures treated with lysolecithin show elevated levels of LPL activity during active demyelination and remyelination, supporting a role for LPL in regulating myelin dynamics. Additional evidence for this role has been collected in mouse models of microglia-specific LPL depletion, which recover more slowly from EAE than wildtype mice.

In microglia, the absence of LPL leads to polarization toward a pro-inflammatory state, as evidenced by a decrease in the expression of reparative genes and an increase in the expression of genes related to pro-inflammatory processes. Observations of increased citrulline, glycolysis, and cholesterol accumulation, as well as decreased fatty acid oxidation, monounsaturated fatty acids, and markers of phagocytosis all support the notion that LPL depletion induces this polarized state in microglia. These data suggest that LPL plays a key role in microglial metabolism and function. LD accumulation is also observed in LPL-depleted microglia, consistent with a pro-inflammatory phenotype. Microglia that lack LPL are less efficient at myelin and Aβ phagocytosis, highlighting the importance of LPL for the clearance of myelin debris and myelin-derived lipids that enables remyelination to occur. These data imply that neurodegenerative diseases such as multiple sclerosis (MS) and AD could be exacerbated by loss of LPL, and loss-of-function LPL mutations have been associated with an increased risk for AD. Many questions about the function of LPL in the brain and in microglia remain, including (1) how is myelin recognized by LPL, (2) does LPL hydrolyze myelin-derived lipids, (3) what happens to myelin-derived lipids after uptake by microglia, (4) which brain-derived lipoproteins are the preferred substrate for microglial LPL, and (5) how do LPL-derived lipids regulate the immunometabolic polarization of microglia?

Session Discussion

Prior to the workshop, some key knowledge gaps related to myelin pathology in brain aging were identified:

1. What are the roles of myelin lipids and their signaling in aging and AD/ADRD?
2. How do these roles vary depending on context (e.g., brain region, model, interactions with other cell types)?
3. What is the role of the immune system in brain aging and AD/ADRD, and how does it interact with myelin?

Interactions Between the Immune System and Myelin

There is clear evidence that immune activity in the brain impacts myelin, but the precise nature of this relationship is unclear. More research is needed to understand how microglia interact with myelin and whether these interactions are helpful or harmful. Although chronic activation of microglia may be detrimental over time, activated microglia are capable of clearing myelin-derived lipids to enable remyelination. This phagocytic activity also extends to AD/ADRD-

relevant pathology, including the clearance of Aβ. Other immune interactions with myelin occur when T cells infiltrate brain, which like microglial activation appears to occur almost exclusively in white matter, although the reason for this exclusivity is unknown.

The Role of Myelin Lipids

The influence of myelin lipids on AD/ADRD pathology is not yet understood. There is increased expression of myelin generation and lipid processing pathways during aging and disease progression, suggesting an effort by the brain to clear myelin debris and repair damaged myelin. In the event of a bioenergetic crisis, upregulation of these pathways could also represent an attempt to tap into auxiliary fuel sources. A greater understanding of these processes is needed in order to elucidate the role of myelin lipids in disease states. As these questions are pursued, it will be important to recognize that myelination changes are very dynamic and dependent on both brain region and cell type.

Observations Across Model Systems

There is ample opportunity to characterize comparability across model organisms related to myelin and LD biology in the context of brain aging. However, some phenomena may be more difficult to study depending on the selected model. For example, age-related changes in the myelin sheath observed in aging female NHPs have not been demonstrated in mice or rats. The timing of endocrinological transitions, which have implications for the use of myelin-derived lipids as a fuel source, may also vary across species. Compared to humans, menopause occurs much later in the life of NHPs, resulting in longer durations of lifetime estrogen exposure. Pre-, peri-, and postmenopausal phases can also be observed in mice, although these may be more difficult to characterize compared to other species. More rigorous development and characterization of model systems are needed in order to study lipid and lipoprotein metabolism, including the possibility of sex differences in these processes.

Day 1 Discussion of Research Gaps and Opportunities

Sex Differences

Sex differences related to lipid metabolism have been observed; for example, females respond to glucose challenges by utilizing lipids for fuel, while males utilize branched amino acids. There is also evidence of sex differences in brain metabolism more broadly, including differences in levels of glucose metabolism and protein turnover by autophagy. However, there is a knowledge gap concerning sex differences in lipid biology as it pertains to AD/ADRD and aging, such as whether there are sex differences in the accumulation of LDs. Data that are aggregated by diagnostic criteria may obfuscate sex differences in observational studies.

LDs and Myelin

It is reasonable to hypothesize that age-related demyelination contributes to AD/ADRD risk, particularly in light of imaging evidence that white matter is more atrophied than gray matter in the AD brain. Moreover, myelin's capacity to act as an alternative fuel source suggests a potential relationship between age-related demyelination and LD accumulation as pertains to the pathogenesis of AD/ADRD; however, the nature of this potential relationship is unclear. It

would be valuable to study the role of microglia in this relationship, because these cells should be able to respond to demyelination but can lose their capacity to do so. It is possible that LDs could serve as a marker for microglia that have reached a functional plateau, which may be indicative of AD or other neurodegenerative phenotypes. The development of surrogate markers would create an opportunity to study how LD accumulation and/or demyelination occur as a cause or consequence of AD/ADRD pathology; fractional anisotropy may be a candidate surrogate marker for white matter integrity. As these markers are developed, it is important to recognize that the baseline measurements may be less informative for the study of age-related disorders than the overall change that occurs within an individual over time. Thus, it would be useful to incorporate lipid biology into existing longitudinal studies of aging populations.

Methodological Gaps and Opportunities

Participants agreed that it is currently difficult to measure lipid flux in the brain. The optimization of a metabolomic method to trace lipid flux would enable the investigation of a range of outstanding questions (e.g., what is the role of LPL or other enzymes in lipid metabolism?). Methods that can label lipids based on cell type of origin would be similarly useful. Current tracing methods that label lipids with a fluorescent reporter are insufficient once the lipid is metabolized; click chemistry may offer another avenue by which lipids can be labeled to measure flux. Recent advances in spatial transcriptomics (e.g., scRNA-seq, snRNA-seq) represent another methodological opportunity to elucidate the lipid metabolic pathways that underlie aging processes and age-related disorders. Currently available imaging methods (e.g., MRS) can also likely be greater utilized to study lipid metabolism and turnover provided the instruments are optimized for the task. Finally, participants broadly agreed that the questions of lipid biology in AD/ADRD and brain aging require a multidisciplinary approach to put findings into the proper context. Multidisciplinary collaborations will benefit from more workshops of this kind that feature investigators with expertise in different disorders, model organisms, disciplines, and techniques.

Session 3: *APOE* and Lipid Homeostasis in Brain Aging and AD/ADRD

Session Chair: Guojun Bu, Mayo Clinic

APOE is the strongest genetic risk factor for AD; inheritance of the ε4 allele increases AD risk, and inheritance of the ε2 allele is protective. The functional role of apoE as a mediator of cholesterol and lipid transport is well established in the periphery but is less understood in the brain. In the periphery, apoE is strongly upregulated after injury and facilitates phagocytosis of lipid debris by macrophages and redistribution of lipids for remyelination by Schwann cells. More research is needed to investigate whether microglia leverage apoE for similar repair functions in the brain during aging and AD/ADRD.

In the brain, the majority of apoE is produced by astrocytes. Lipid transporters (e.g., ABCA1) load lipids onto apoE to form nascent apoE-associated lipoprotein particles. These nascent particles acquire more lipids as they interact with other cells, such as oligodendrocytes, and are therefore more lipid-rich when measured in the CSF. Multiple pathogenic pathways are thought

to underlie the effects of the apoE4 isoform, including toxic gain-of-function and loss-of-function properties relative to other isoforms. Much of the research on the apoE4 isoform relates to its inhibition of Aβ clearance and promotion of Aβ aggregation, both of which represent a toxic gain-of-function that has been demonstrated in both humans and animal models.

The goals of Session 3 are to assess current knowledge of apoE-related brain lipid metabolism as it relates to aging and AD/ADRD, address apoE isoform-dependent effects, identify gaps and opportunities related to apoE, and suggest ways to translate model studies to humans to inform the role of lipids in pathogenic events and as targets for diagnosis and therapy.

ApoE in Brain Lipid Metabolism in Aging and AD: Lessons Learned from Animal and Organoid Models

Guojun Bu, Mayo Clinic

Investigation of the different functions of apoE isoforms are important for understanding the role of apoE in brain lipid metabolism in aging and AD/ADRD. *APOE* ε2 and ε4 protect against and accelerate age-related cognitive decline, respectively, in a gene dose-dependent manner. These cognitive effects can be replicated in aged human *APOE* targeted replacement mice (*APOE*-TR). Relative to *APOE3*-TR and *APOE4*-TR mice, *APOE2*-TR mice exhibit higher levels of apoE in the brain, which correlates with better memory performance and higher physical activity levels. Furthermore, lower cortical and higher CSF cholesterol levels in *APOE2*-TR mice correlate with higher apoE levels, better memory performance, and increased activity levels at older ages. These observations are consistent with the hypothesis that apoE2 is better at promoting lipid transport and efflux than other isoforms, which may underlie its protective mechanism in aging and AD/ADRD. Enhanced lipid efflux is also a characteristic of the *APOE* p.V236E variant of *APOE* ε3—also known as the Jacksonville variant—which itself has protective properties.

The effects of *APOE* genotype, age, and sex can be studied using multi-omics techniques in *APOE*-TR mice. Current evidence suggests that *APOE* genotype has a larger effect than age or sex on the blood metabolome, such that the *APOE* ε2 genotype exhibits differentially expressed metabolites compared to ε3 and ε4. For the brain, in contrast, increasing age has the largest effect on differentially expressed metabolites.

Differential effects of apoE isoforms have also been studied in iPSC-derived cerebral organoids. In these cultures, apoE-deficiency exacerbates α-synuclein accumulation. This accumulation is partially rescued by apoE2 and apoE3, but not apoE4. LDs also accumulate in apoE-deficient organoids, and altered transcriptional profiles implicate lipid metabolism-related pathways as differentially modulated by apoE deficiency. Altogether, these data suggest that apoE plays an important role in lipid efflux and metabolism in the brain.

Many research questions related to apoE isoforms in aging and AD/ADRD should be pursued, including:

1. Is the superior role of apoE2 (and the Jacksonville variant) on lipid efflux and brain lipid metabolism the driving mechanism underlying protective effects in AD/ADRD and aging-related cognitive decline?
2. Is the dysfunction of apoE/ABCA1-mediated lipid efflux a major contributor to intracellular lipid and LD accumulation?
3. What are the effects of reducing apoE4 expression on brain lipid homeostasis and is it a potential therapeutic strategy?
4. What are the roles of apoE-mediated lipid transport in brain repair?

ABCA1 Activation in the CNS as a Therapeutic Target for Alzheimer's Disease

Ana Valencia-Olvera, University of Illinois at Chicago

ApoE-containing lipoproteins vary in multiple ways, including relative levels of lipidation. Compared to apoE3-containing lipoproteins, apoE4-containing lipoproteins are less lipidated, less stable, present at lower levels in the CNS, more prone to aggregate, and less efficient at clearing oligomeric Aβ. The lipid transporter ABCA1 plays an important role in the lipidation of apoE-containing lipoproteins, and as such it has become a candidate therapeutic target for AD. One method of targeting ABCA1 is to directly induce expression of the *ABCA1* gene. Preclinical evidence in an AD mouse model has shown that a retinoid X receptor (RXR) agonist can induce *ABCA1* expression and promote lipidation of apoE while preserving synaptic integrity relative to untreated mice. However, RXR agonists activate multiple pathways and generate fatty acids, ultimately inducing systemic toxicity. Compounds that induce *ABCA1* expression with minimal induction of *SREBP1c* expression in hepatic cells can achieve these beneficial CNS effects while avoiding systemic toxicity and lipogenesis; two such compounds have been validated.

The activity of ABCA1 can also be targeted by apoE-derived peptides. The apoE-derived peptide CS6253 (CS) is a cholesterol acceptor that promotes cholesterol efflux in an ABCA1-dependent manner; this peptide also promotes the lipidation of pre-existing lipid particles. The therapeutic potential of this peptide was tested in the EFAD mouse model, which contains five familial AD (FAD) mutations and is homozygous for either *APOE* ε3 (E3FAD) or *APOE* ε4 (E4FAD). Mortality and AD-related pathology in these mice mirror *APOE*-mediated AD risk in humans (i.e., E3FAD male mice exhibit the lowest mortality and least AD-related pathology, while E4FAD female mice exhibit the greatest mortality and most AD-related pathology). When administered to EFAD mice, the CS peptide increased ABCA1 and lipidated apoE levels, reduced multiple readouts of AD pathology, and improved memory performance and synaptic integrity. However, the treatment effects on Aβ pathology were primarily observed in males and only when administered in a prevention paradigm but not in a reversal paradigm (i.e., after the onset of AD pathology). Moreover, engagement with ABCA1 and improvements in memory performance and synaptic integrity were only observed in E3FAD males. The observation that this treatment was only efficacious in a subpopulation underscores the need to treat AD with a personalized medicine approach.

Cell-specific Expression of apoE Isoforms

G. William (Bill) Rebeck, Georgetown University

Differences among the various isoforms of apoE have been recognized for decades. In human CSF, apoE4 is associated with smaller lipoproteins than apoE3 and apoE2. The differential lipidation of apoE isoforms may be due to variable post-translational modifications (PTMs), which also differ between the CNS and periphery. In general, apoE contains more and different PTMs in the CSF than in the periphery, which results in biochemically distinct species of apoE. ApoE is heavily glycosylated and sialylated in the CSF, with much of the glycosylation localized to the C-terminus. This glycosylation introduces large, charged moieties to the lipid binding domain and has the potential to change the way that apoE binds with HDLs in the CSF. This C-terminal glycosylation is largely not seen in the plasma.

In the CNS, most of the apoE secreted by astrocytes and microglia is glycosylated. Both astrocytes and microglia secrete apoE4 at lower levels than apoE3 and apoE2. PTM differences may play a role in the higher level of cholesterol efflux from astrocytes in the presence of apoE3 compared to apoE4, which suggests that apoE3 is more effective at clearing lipids from glia than apoE4. Outstanding questions in this area include:

1. How are these processes affected in non-AD conditions (e.g., Lewy Body Dementia, FTD, chemotherapy)?
2. How are processes of lipid metabolism affected by *APOE* genotype and apoE modifications?
3. How do other lipid-related genetic risk factors affect neurodegeneration?

Multiplex Immunohistochemistry to Investigate Apolipoprotein E-related Pathologies in Sporadic Alzheimer's Disease

Christopher Ramsden, Intramural Research Program, NIA

Histology has been used to study AD since the first patient was identified. Recent methodological advances greatly expand the depth of information that can be obtained from labeled brain tissue. Multiplex-IHC (MP-IHC) coupled with 10-color wide field fluorescence microscopy enables the visualization of up to 10 fluorescent markers simultaneously. Furthermore, tissue samples can be stripped and re-stained in multiple rounds to visualize more than 50—and in some cases up to 100—different labeled markers of cytoarchitecture and pathology in the same specimen. The iterative nature of MP-IHC therefore provides an opportunity to add tremendous spatial and morphological context to apoE and AD-related pathology.

The translation of MP-IHC from animal model tissue to human tissue is challenging because of higher levels of postmortem tissue degradation; more variable fixation methods; and the overall increase in size, complexity, and variability of tissue specimens. Nonetheless, it is important to study human tissue directly because animal models cannot always faithfully recapitulate pathways that mediate human age-associated disease or the complexity of the human brain. As MP-IHC becomes optimized for human tissue, it can be leveraged to study the

changes that occur across the clinicopathological spectrum of AD/ADRD. Currently, MP-IHC can be used to visualize classic AD pathologies, including amyloid plaques, hyperphosphorylated tau, peri-plaque astrocytosis, and synaptic loss in human tissue. This method can also be applied to visualize different markers for apoE and has been used to label apoE-enriched neuritic plaques in human entorhinal cortex and dentate gyrus, as well as more diffuse Aβ protein deposits that are devoid of apoE and neuritic pathologies. As the use of MP-IHC in human tissue continues to progress, this method will help to bridge gaps from animal models to humans; provide spatial and morphological context for apoE and AD-related pathologies throughout the disease spectrum; and enable insights into disease mechanisms, biomarkers, and new therapeutic targets for AD.

Regulation of apoE-mediated Lipid Signaling and the Interplay Between apoE and Sex on the Single Cell Level in AD

Dongming Cai, Icahn School of Medicine at Mount Sinai

Postmortem brain tissue from individuals with mild cognitive impairment (MCI) or early AD has revealed that *APOE* ε4 carriers exhibit a reduction in phosphoinositol biphosphate (PIP_2) levels accompanied by elevated expression of the PIP_2 degrading enzyme synaptojanin 1 (synj1). Human transcriptomic data have identified *SYNJ1* as a key driver of AD gene networks. Changes in PIP_2 and synj1 levels are recapitulated in the aged *APOE* ε4 mouse brain, and knockdown of *SYNJ1* can rescue cognitive deficits in these mice. Synj1 is highly enriched in neurons and microglia; synj1 protein expression is elevated in cultured *APOE* ε4 microglia, which exhibit reduced phagocytic activity and enlarged lysosomes compared to *APOE* ε3, both of which are rescued by synj1 knockdown. In E4FAD mice, synj1 reduction can also reduce amyloid plaque burden induced by chronic neuroinflammation.

The apoE-synj1-PIP_2 pathway is differentially regulated by the microRNA (miRNA) miR-195. In human postmortem brain tissue and antemortem CSF samples, levels of miR-195 are reduced in *APOE* ε4 carriers, which coincides with AD progression. Furthermore, CSF levels of MiR-195 positively correlate with cognitive function and negatively correlate with levels of phosphorylated tau (pTau). Overexpression of miR-195 can rescue cognitive deficits and reduce amyloid plaque burden in *APOE* ε4 mice and rescue endolysosomal defects in human iPSC-derived neurons. These observations lend support to the notion that opportunities exist to develop novel therapies targeted at apoE-mediated lipid signaling pathways.

While *SYNJ1* is a key driver gene of AD networks in males and females, many studies suggest that apoE has a stronger effect on AD risk in females than males. However, the molecular mechanisms underlying the interaction between apoE and sex are not well characterized. Human gene expression profiles have identified *LRP10* as a key driver of sex-biased AD networks. This gene codes for LDL receptor related protein 10 (Lrp10), which is a distant relative of more commonly studied apoE receptors. Postmortem brain tissue shows that Lrp10 colocalizes with neurons and microglia, but not astrocytes, suggesting cell type–specific expression. While *LRP10* mRNA levels are upregulated in AD brains (especially in female carriers of *APOE* ε4), protein levels are downregulated compared to controls. In EFAD mice, similar

LRP10 expression patterns have been observed, and overexpression of *LRP10* reduces cognitive deficits and AD pathology in E4FAD female mice. Furthermore, *LRP10* overexpression increases the recruitment of microglia and decreases amyloid plaque burden compared to controls in E4FAD female mice. More work is needed to investigate the sex- and apoE-specific network and molecular signatures with cell type specificity as well as to develop precision medicine–driven AD therapies that are tailored by sex and *APOE* genotype.

Brain Fatty Acid Metabolism and Neurodegeneration: A Disruptive Role of apoE4

Fei Yin, University of Arizona

Cell types differ in their metabolic needs and functions. In the brain, astrocytes are responsible for metabolizing fatty acids; however, apoE4 may disrupt this process. In *APOE*-TR mice expressing either apoE3 or apoE4, apoE4 was shown to induce an adaptive response in neurons in which mitochondrial function and fatty acid synthesis are reduced upon lipid toxicity caused by higher levels of free fatty acids. In astrocytes, apoE4 altered fatty acid catabolism in mitochondria, which appeared more fragmented than in apoE3 counterparts. Fatty acid catabolism is critical to regulating lipid homeostasis in astrocytes, and this disruption by apoE4 led to LD accumulation in these astrocytes. Furthermore, there is evidence that this homeostatic dysregulation may lead to a metabolic shift in which astrocytes expressing apoE4 metabolize more glucose. ApoE4 also impairs lipid clearance from neurons to astrocytes, which impairs astrocytic support of neuronal growth and energetic function.

Astrocyte-specific transcription factor A, mitochondrial (Tfam) knockout (KO) mice enable the study of the effects of astrocyte-specific metabolic disruption. These mice have viable astrocytes but reduced fatty acid metabolism. The loss of Tfam induces accumulation of lipids that colocalize with mitochondria in astrocytes, suggesting that the mitochondrial catabolism of fatty acids is impaired. Accumulation of LDs is also apparent in astrocytes of these mice. Functionally, this knockout and related mitochondrial dysfunction and lipid accumulation leads to an increase in proinflammatory cytokines and induces neurotrophic, synaptic, and cognitive deficits. These effects are exacerbated by apoE4, suggesting that apoE4 renders the brain more vulnerable to disrupted fatty acid metabolism. More research is needed to understand:

1. How lipid metabolism acts as a bidirectional link between bioenergetic decline and neuroinflammation
2. The effects of interactions between disrupted brain fatty acid metabolism and peripheral lipids
3. How brain lipid metabolism interacts with AD proteinopathies

The Effects of *APOE* ε4 on Carnitine/Acylcarnitine-mediated Lipid Dysfunction in Alzheimer's Disease

Laila Abdullah, Roskamp Institute

Carnitine is largely acquired from the diet (although some endogenous production occurs in the brain) and is ultimately converted to trimethylamine N-oxide (TMAO) in the liver. TMAO is notable for its association with cardiovascular disease, diabetes, and renal disorders. Carnitine

is an important component of fatty acid metabolism because it facilitates entry of long chain fatty acids into mitochondria for β-oxidation; long chain fatty acids cannot enter mitochondria directly but rather must first combine with carnitine to form acylcarnitine. Medium and short chain fatty acids can enter mitochondria directly, but their presence as acylcarnitines in plasma may be indicative of incomplete β-oxidation. Peroxisomes can supplement β-oxidation when demand on mitochondria is high, and also shorten very long chain fatty acids prior to metabolism by mitochondria.

APOE ε4 carrier status can increase reliance on fatty acid metabolism by impeding glucose metabolism, which can in turn contribute to oxidative stress and inflammation. Given the role of carnitine in lipid transport, analysis of carnitine and its metabolites in the periphery could provide insight into the functional contribution of apoE in lipid metabolism. In patients with preclinical MCI and early-stage AD, plasma TMAO and acylcarnitine levels were highest in *APOE* ε4 carriers. Moreover, acylcarnitine levels are associated most strongly with measures of AD pathology (e.g., Aβ42/40) in *APOE* ε4 carriers. In postmortem human brain tissue, levels of TMAO were higher in *APOE* ε4 carriers diagnosed with AD compared to controls and non-carriers. In non-carriers, brain and plasma TMAO levels correlate with amyloid and tau pathologies; however, in *APOE* ε4 carriers TMAO levels correlate with cerebrovascular pathologies. In the same non-carriers, brain acylcarnitine levels are associated with amyloid and tau pathologies and plasma acylcarnitine levels are associated with cerebrovascular pathologies, but the associations are much more convoluted for *APOE* ε4 carriers. Differential carnitine, TMAO, and acylcarnitine profiles between brain and plasma are also evident in EFAD mice. Many research gaps and opportunities related to these observations can be pursued. The differential blood and plasma profiles may reflect the local effects of brain *APOE* ε4 because carnitine can be produced endogenously in the brain. The observed TMAO changes parallel cerebrovascular abnormalities and AD pathologies in *APOE* ε4 carriers, although the impact of TMAO levels on AD is not yet known. Elevation of acylcarnitine in plasma could indicate incomplete β-oxidation in *APOE* ε4 carriers, and further study of fatty acid metabolism by peroxisomes and mitochondria is warranted. Finally, the influence of *APOE* genotype on peripheral and brain carnitine/acylcarnitine transport and metabolism as well as TMAO levels is not well understood and could have implications for brain bioenergetics and cerebral vasculature.

Translating Lipoprotein and Lipid Studies from the Bench into the Clinic: Gaps and Opportunities

Hussein Yassine, University of Southern California

Despite the importance of brain lipids and lipoproteins to AD/ADRD pathogenesis, very few lipid- or lipoprotein-based biomarkers are currently being used in the clinical management of AD/ADRD patients, selection of patients into clinical trials, or assessment of treatment response. Challenges to the development of lipoprotein-based brain biomarkers include the complexity of lipoprotein and lipid measurement in CSF, the unknown predictive capacity of these biomarkers for brain outcomes, and the high cost of development. Nonetheless, CSF-based biomarkers may be viable options to assess brain lipids in the clinic. Lipidation of apoE4

in the CSF, for example, may be indicative of ABCA1 activity in the brain. ApoE4 is prone to aggregation, which traps ABCA1 in endosomes and impairs ABCA1-mediated lipidation of apoE4, ultimately promoting further apoE4 aggregation. On the basis of basic research in this area and observations that people with MCI or early-stage AD have lower levels of ABCA1 activity in the CSF than cognitively normal counterparts, the hypothesis arose that induction of ABCA1 activity could enhance lipidation of apoE4 and reduce apoE4 aggregation. Lower ABCA1 activity correlates with hypolipidation in the CSF. However, cell-based assays are not easily translatable to the clinic, and the determinants of ABCA1 activity in CSF are not clear.

There has been little progress on the measurement of brain HDL particles. Ion mobility and nuclear magnetic resonance spectroscopy (NMR) have been successfully applied to study lipoprotein particles in plasma to assess cardiovascular risk; however, the relevance of CSF lipoprotein particle concentrations to AD is unknown. Small, but not large, HDL particle levels in plasma and CSF are positively correlated, suggesting that small HDL particles may be exchanged between the periphery and the brain. In CSF, small HDL particle concentrations correlate with Aβ42 levels and memory performance. Small HDL particles are promising AD biomarkers and can potentially act as biomarkers for cognitive outcomes following some interventions (e.g., HDL mimetic therapies that induce ABCA1 activity), although validation is needed and more specific subsets of HDL particles must be identified.

Imaging can also be leveraged to assess brain lipids and neuroinflammation. Imaging of Aβ and tau are currently used in the selection of participants and as treatment targets in AD/ADRD clinical trials. However, there is a gap in understanding brain lipid changes in the living human brain as they relate to AD/ADRD risk. Imaging brain lipids could offer spatial information on brain lipid uptake and turnover in a region-specific manner that relates to disease risk and other biomarkers. Moreover, imaging brain lipids can provide orthogonal information on pathways that are not currently represented by amyloid and tau imaging. In one example application, imaging of arachidonic acid uptake may be used to assess phospholipase A2 (cPLA2) activation in the living brain. Levels of cPLA2 phosphorylation differ by *APOE* genotype and disease stage. Activation of cPLA2 is induced by apoE4 and results in the production of inflammatory mediators, and inhibition of cPLA2 may be a promising therapeutic target for AD/ADRD.

Session Discussion

Prior to the workshop, some key knowledge gaps related to apoE and lipid homeostasis in brain aging were identified:

1. How does the peripheral lipid transport and metabolism by different *APOE* genotypes influence brain lipids?
2. How does the microbiome influence brain lipids in the context of different *APOE* genotypes?
3. How does brain lipid metabolism/composition affect brain aging and diseases of aging?
4. What are the differential effects of apoE isoforms on brain lipid metabolism, bioenergetics, and inflammation as they relate to brain aging and diseases of aging?

5. How do *APOE* and *APOE*-related risk factors mediate the effects of lipids on age-associated diseases?
6. Can lipids and lipoproteins act as useful biomarkers? Can we image brain lipids to guide clinical studies?
7. How can we translate what has been learned in animal models to humans?

Prevention of AD/ADRD

As knowledge of lipid biology in AD/ADRD and brain aging continues to grow, the ultimate goal will be to translate findings into the clinic for the prevention and treatment of AD/ADRD. For agents that demonstrate preventive potential preclinically (e.g., ABCA1 activation by apoE-derived peptides), clinical trials will need to include younger individuals who either have a genetic risk for AD/ADRD, a family history of AD/ADRD, or otherwise have a concern about their cognitive abilities without reaching the threshold of diagnosis for AD or MCI. Better definitions for these at-risk or prodromal populations must be developed in order to optimally design preventive clinical trials. Moreover, subpopulations of these at-risk groups will need to be defined in order to identify those individuals for whom a given treatment may or may not be efficacious. Biomarkers will help researchers define these populations, and HDL is one promising candidate for the early classification of individuals for high or low disease risk based on HDL particle counts. Furthermore, HDL lipidation profiles are sensitive to *APOE* genotype. If the biomarker is a readout of treatment efficacy, then a new biomarker may need to be selected once AD pathology develops; manipulation of HDL lipidation, for example, may no longer be effective once amyloidosis is present.

Interactions Between Peripheral and CNS Lipids

The relationship between peripheral and brain lipid metabolism is not yet clear, including how this relationship may change according to *APOE* genotype. Although some evidence exists (e.g., correlations between HDL in plasma and CSF) that small HDL particles may exchange between the periphery and the brain and that *APOE* ε4 genotype may enable this exchange, participants did not universally agree that this phenomenon occurs. The absence of this exchange, however, would not indicate that peripheral lipid transport and metabolism cannot affect the brain. Evidence from the cardiovascular field demonstrates that peripheral lipids can influence vascular function, which in turn could feasibly impact brain lipid metabolism. In addition, the extent to which *APOE* genotype and progressive neurodegeneration can influence the relative permeability of the blood–brain barrier is another knowledge gap that would inform the relationship between peripheral and CNS lipid biology.

Peripheral lipids could also serve as a biomarker in this field. Peripheral lipid changes correlate somewhat with known AD/ADRD biomarkers even when factors that influence peripheral lipids—including diet or medication use—are not controlled. Studies that investigate how peripheral lipids change as a result of diet, medication, or other external factors will help to provide insight into the utility of peripheral lipids as biomarkers.

Lipid Composition

In the context of AD/ADRD and brain aging, much biochemical research on lipids focuses on the size of a lipid particle. To move the field forward, more research on the composition of these particles should be generated to complement existing knowledge about the effects of particle size. A wide variety of lipids exist (e.g., sulfatides) that may be differentially associated with AD/ADRD-related health outcomes, and technology now exists to examine lipid composition more thoroughly. One understudied area that can impact lipid composition, transport, and metabolism is the microbiome. Importantly, the microbiome varies greatly across sexes and *APOE* genotypes in ways that may differentially impact AD/ADRD and other age-related outcomes. Another knowledge gap in this area relates to stoichiometric differences in the number of apoE molecules that are associated with individual lipoprotein particles and how these differences vary by apoE isoform.

Interrogating the Effects of APOE Genotype

It is difficult to dissect the differential effects of *APOE* genotype in part because *APOE* ε4 is such a strong driver of amyloid pathology that it can become challenging to tease out the effects of apoE isoforms that are specific to lipid biology. Conditional knockout mice targeting different enzymes involved in lipid synthesis or metabolism should continue to be leveraged in studies that seek to specifically address the influence of *APOE* genotype on lipids. Participants also agreed that care must be taken to select the appropriate endpoints for studies in this field, because traditional emphasis on amyloid or tau pathology may not always be appropriate and may limit the ability for researchers to address the role of *APOE* ε4 in other forms of neurodegeneration that do not have associated amyloid pathology. Some potential endpoints and biomarkers that are relevant to lipid metabolism and are amyloid-independent include cortical cholesterol or other lipids in the CSF, capacity for lipid efflux, and lipid distribution across different cell types.

Differential Effects of APOE Genotype

There is ample evidence that lipid metabolism and AD/ADRD pathology are differentially impacted by *APOE* genotype. In the realm of bioenergetics, fatty acids are the preferred alternative fuel for *APOE* ε4 carriers when glucose resources are lacking. In the periphery, *APOE* ε4 appears to promote inflammation, although whether the same is true in the brain is unclear. Glial functioning, including the phagocytic capacity of microglia, is also impacted by *APOE* genotype, and more research is needed to understand how this relationship changes as aging or disease development progresses. The differential impacts of *APOE* genotype are an especially important area of investigation because they affect a wide range of relevant processes, including lipid metabolism, neural repair mechanisms, and development of AD/ADRD pathology hallmarks (e.g., Aβ deposition). It is also important to recognize that these differential effects may additionally be cell type specific; microglia, for example, may behave differently with regard to lipid metabolism and neuroinflammation based on *APOE* genotype.

Concluding Discussion

Selective Vulnerability to LD Accumulation

Some neurons are more likely to accumulate LDs than others, and the mechanism that underlies this selective vulnerability is not yet understood. LDs are a known component of the cellular stress response and the injury repair process, but lingering LDs can be toxic. Thus, greater understanding of the balance of lipid metabolism and how that balance varies under different conditions (e.g., across disorders) is needed. One opportunity to dissect this vulnerability is to investigate the types of LDs that accumulate in neurons; while some LDs form as part of a stress response, others serve as a dedicated lipid storage compartment. Another aspect of this selective phenotype is the capacity for cells to clear lipids, as some cell types will have higher rates of autophagy and lipophagy than others. Spatial transcriptomics methods represent one opportunity to pursue these cell type-specific research questions. With further study, it is possible that autophagy levels could be used as a marker for vulnerability to LD accumulation among different types of neurons.

Targeting Lipid Metabolism in Typical Aging and Age-Related Disorders

As the study of lipid biology in brain aging and AD/ADRD progresses, it is important to distinguish typical aging processes from age-related disorder progression. The effects of known risk factors for AD/ADRD, such as *APOE* genotype, have broad influences on longevity and non-CNS disorders (e.g., cardiovascular disease) regardless of the presence of classic amyloid or tau pathologies. It is increasingly important to leverage models that do not have pathological amyloid or tau lesions in order to specifically investigate how lipid metabolism changes throughout the lifespan and in conditions of age-related disease. In general, participants broadly agreed that lipid metabolism is central to both healthy aging and age-related brain diseases.

Leveraging the Periphery to Understand Cognitive Outcomes

Peripheral lipid profiles correlate with some measures of AD/ADRD and cognition. In light of these observations, the use of plasma-based readouts (e.g., plasma PLP profile) to predict cognitive outcomes is a considerable opportunity in the field of lipid biology in AD/ADRD and brain aging. Techniques now exist to improve collective understanding of the gut-brain axis that could directly inform studies of peripheral lipid metabolism and its influence on the brain as well as the development of plasma-based biomarkers of lipid biomarkers. Peripheral lipid biomarkers could be correlated with markers of AD/ADRD pathology (e.g., pTau) to investigate the role of lipids in neuropathology and neurodegeneration.

Appendix A: Meeting Agenda

Day 1: April 28, 2021

10:00 am Welcome and Introductions – Eliezer Masliah and Amanda DiBattista, NIA DN

Session 1: Lipid Droplets in Brain Aging and AD/ADRD
Chair: Lance Johnson, University of Kentucky

10:20 am Lipid Droplets in Alzheimer's Disease: A Role of *APOE*? – Lance Johnson, University of Kentucky

10:40 am Lipid Transport from Neurons to Glia in Health and Disease – Maria S. Ioannou, Unveristy of Alberta

11:00 am Lipid Droplet Biogenesis and Interactions with Other Organelles – Sarah Cohen, University of North Carolina at Chapel Hill

11:20 am Selective Autophagy: When Quality Control Meets Lipid Metabolism – Ana Maria Cuervo, Albert Einstein College of Medicine

11:40 am Lipid-droplet-accumulating Microglia (LDAM) in Mice and Alzheimer's Disease – Tony Wyss-Coray, Stanford University

12:00 pm Session Discussion

12:20 pm Break

Session 2: Myelin in Brain Aging and AD/ADRD
Chair: Robbie Brinton, University of Arizona

12:40 pm Myelin as a Ketone Reservoir: Feeding a Starving Brain – Robbie Brinton, University of Arizona

1:00 pm Factors Underlying the Age-Related Accumulation of Myelin Pathology and Associated Cognitive Impairment in the Normal Aging Rhesus Monkey – Douglas Rosene, Boston University

1:20 pm The Role of Myelin Lipids Played Beyond in Myelin Integrity: Lessons Learned from Studying Sulfatide Deficiency in Alzheimer's Disease – Xianlin Han, University of Texas Health Science Center at San Antonio

1:40 pm Single-cell Multi-omics Identifies Oligodendrocyte Heterogeneity in Alzheimer's Disease – Vivek Swarup, University of California, Irvine

2:00 pm Lipoprotein Lipase in Neurodegenerative Disease – Kimberley D. Bruce, University of Colorado Anschutz Medical Campus

2:20 pm Session Discussion

2:40 pm Break

3:00 pm Overall Discussion of Research Gaps and Opportunities – Day 1

4:00 pm Adjourn for Day

Day 2: April 29, 2021

Session 3: APOE and Lipid Homeostasis in Brain Aging and AD/ADRD
Chair: Guojun Bu, Mayo Clinic

10:00 am ApoE in Brain Lipid Metabolism and Aging and AD: Lessons Learned from Animal and Organoid Models – Guojun Bu, Mayo Clinic

10:25 am ABCA1 Activation in the CNS as a Therapeutic Target for Alzheimer's Disease – Ana Valencia-Olvera, University of Illinois at Chicago

10:45 am Cell-specific Expression of apoE Isoforms – G. William (Bill) Rebeck, Georgetown University

11:05 am Multiplex Immunohistochemistry to Investigate Apolipoprotein E-related Pathologies in Sporadic Alzheimer's Disease – Christopher Ramsden, Intramural Research Program, NIA

11:25 am Regulation of apoE-mediated Lipid Signaling and the Interplay Between apoE and Sex on the Single Cell Level in AD – Dongming Cai, Icahn School of Medicine at Mount Sinai

11:45 am Break

12:00 pm Brain Fatty Acid Metabolism and Neurodegeneration: A Disruptive Role of apoE4 – Fei Yin, University of Arizona

12:20 pm The Effects of *APOE* ε4 on Carnitine/Acylcarnitine-mediated Lipid Dysfunction in Alzheimer's Disease – Laila Abdullah, Roskamp Institute

12:40 pm Translating Lipoprotein and Lipid Studies from the Bench into the Clinic: Gaps and Opportunities – Hussein Yassine, University of Southern California

1:00 pm Session Discussion

1:20 pm Overall Discussion of Research Gaps and Opportunities

2:00 pm Adjourn