

Infectious Etiology of Alzheimer's Disease: Is there a Causative Role for Infectious Agents in Alzheimer's Disease?

National Institute on Aging

Division of Neuroscience

October 5-6, 2021

Virtual Workshop

Revised November 5, 2021



This meeting summary was prepared by Rose Li and Associates, Inc., under contract to the National Institute on Aging (NIA). The views expressed in this document reflect both individual and collective opinions of the meeting participants and not necessarily those of NIA. Review of earlier versions of this meeting summary by the following individuals is gratefully acknowledged: Bethany Stokes, Kayla Harr, Beth Finch, Dana Carluccio

Acronym Definitions

A β	amyloid-beta
AD	Alzheimer's disease
ADRD	Alzheimer's disease and related dementias
AIDS	acquired immunodeficiency syndrome
AMP	antimicrobial peptide
APP	amyloid precursor protein
<i>B. fragilis</i>	<i>Bacteroides fragilis</i>
<i>C. pneumoniae</i>	<i>Chlamydia pneumoniae</i>
COVID-19	coronavirus disease 2019
CMV	cytomegalovirus
CNS	central nervous system
CSF	cerebrospinal fluid
EBV	Epstein-Barr virus
HAND	HIV-associated neurocognitive disorder
HHV-6	human herpesvirus 6
hiNSC	human induced neural stem cell
HIV	human immunodeficiency virus
HRS	Health and Retirement Study
HSV1	herpes simplex virus 1
JHBRC	Johns Hopkins Brain Resource Center
LOAD	late-onset Alzheimer's disease
MRI	magnetic resonance imaging
MS	mass spectrometry
NIA	National Institute on Aging
NINDS	National Institute of Neurological Disorders and Stroke
NSHDS	Northern Sweden Health and Disease Study
OE	olfactory epithelium
OPC	oligodendrocyte precursor protein
<i>P. gingivalis</i>	<i>Porphyromonas gingivalis</i>
PCR	polymerase chain reaction
RFA	Request for Application
RNASEq	ribonucleic acid sequencing
ROSMAP	Religious Orders Study/Memory and Aging Project
<i>S. sonnei</i>	<i>Shigella sonnei</i>
SVV	simian varicella virus
TCR	T cell receptor
<i>T. gondii</i>	<i>Toxoplasma gondii</i>
VZV	varicella zoster virus
WGS	whole genome sequencing

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Meeting Summary

Opening Remarks and Workshop Objectives

Eliezer Masliah and Mack Mackiewicz, NIA

In recent years, the National Institute on Aging (NIA) has received significant funding to help researchers advance understanding of the pathogenesis of Alzheimer's disease (AD) and AD-related dementias (ADRD). Many studies have shown that AD/ADRD pathology is not caused solely by proteinopathies, but also by genetic, environmental (including toxins and infectious agents), and general aging-related factors; each component must be understood to improve diagnostic strategies and identify therapeutics that can slow or prevent AD/ADRD progression.

The goal of this workshop is to review evidence of how infectious agents contribute to AD/ADRD and to identify current research gaps.

Keynote: AD Pathology—An Orchestrated Innate Immune Response of the Brain?

Rudolph Tanzi, Harvard University

Dr. Tanzi started off the meeting by presenting an overview of the antimicrobial protection hypothesis of AD, originally promoted by Dr. Rob Moir. The hypothesis has two key postulates: first, that AD pathology (plaques, tangles, neuroinflammation) evolved as an orchestrated innate immune response in combination with evolutionarily conserved AD susceptibility gene variants, which promoted AD pathology and protected the brain against microbial infections; and second, that microbial infections can also drive the development of AD. A major objective of this workshop is to understand the relative roles of genetics, infection, and other environmental exposure factors in driving AD etiology and pathogenesis.

Despite compelling genetic support for the Amyloid Hypothesis of AD, including the contributions of *APP*, *PSEN1*, *PSEN2*, and *APOE* to disease pathogenesis, many AD clinical trials targeting amyloid-beta (A β) have failed to improve cognition. This observation suggests that these treatments may target amyloid too late within the disease trajectory and that cheaper and safer therapies that reduce amyloid deposition *before* cognitive symptoms develop are needed. Another possibility is that therapeutics targeting hallmark AD pathological targets alone may not be sufficient and thus further investigations into infectious etiology are needed to develop additional therapeutic targets. Dr. Tanzi and colleagues created 3D cell culture models to help improve drug screening and repurposing for neurodegenerative disorders, leading to several late-stage trials. Using these models, Dr. Tanzi and colleagues have found that higher A β 42/40 ratios enhance tau tangle formation. They thus developed a γ -secretase modulator that selectively decreases A β 42/40 and tau pathology and that is currently involved in an ongoing Phase I clinical trial. Dr. Tanzi and colleagues have also used the 3D cellular models to identify therapeutics to target aberrant microglial activity.

A β is an antimicrobial peptide (AMP) that traps and kills microbes within A β deposits. In a mouse model of Salmonella infection, Dr. Tanzi and colleagues observed that the infection seeded A β plaque formation within 48 hours, which led to significant neuronal death. Similar observations have been made following infection with herpes simplex virus 1 (HSV1). Using data from multiple brain banks, Dr. Tanzi and colleagues have performed metagenomic analyses to identify microbes in AD brains, but thus far have identified no significant differences in levels of viruses between AD brains and controls; however, a small increase in levels of periodontal bacterial species were identified in AD brains, compared to controls. Dr. Tanzi suggested that these findings, together with others to be presented in the meeting, provide compelling evidence for a role of brain microbial infections in AD etiology and pathogenesis. Understanding the roles of specific microbes and the relative contributions of microbes versus genetic risk factors in driving age-related pathology are key future objectives.

Session I: Herpes Simplex and AD—the Epidemiological Perspective

Session Chair: Steven Jacobsen, NINDS

Herpes Virus Infections, Antiviral Treatment, and AD

Hugo Lövheim, Umeå University

The current HSV1-AD hypothesis postulates that HSV1 undergoes frequent reactivations, enters the brain, and causes A β to oligomerize as a chronic antimicrobial response, leading to an accumulation of AD pathology. In order to investigate the relationship between HSV1 infection and AD risk, Dr. Lövheim and collaborators initiated the Umeå Herpes Simplex and Alzheimer's Disease Project, which leverages longitudinal samples from the Betula Cohort Study and the Northern Sweden Health and Disease Study (NSHDS). Analyses of these cohorts indicate that HSV carriers in the NSHDS, but not the Betula, cohort had an increased risk of AD. However, analyses of the Betula study also identified an association between recent HSV reactivations (measured by anti-HSV immunoglobulin M antibodies in serum) and an increased risk of AD, with the onset of symptoms delayed by 8-10 years. Further analyses of both cohorts led to the observation that HSV carriers who are APOE4-positive or have significant genetic risk scores (based on nine other AD risk genes) are at increased risk of AD and cognitive decline, which indicates that the combination of HSV infection and genetic risk is highly predictive of overall AD risk. Dr. Lövheim and colleagues sought to assess whether antiviral treatment can impact dementia risk. Analyses of data from Swedish registries revealed that HSV carriers who did not undergo treatment were at higher risk of developing dementia, whereas those who underwent treatment were at lower risk. Further analysis of the Betula cohort indicated that antiviral treatment led to a 70 percent lower risk of developing AD and that this effect was most evident when treatment was received at the time of a HSV reactivation.

Association of Herpes Virus Diagnosis, Anti-Herpetic Medication, and Herpes Virus Vaccination with Dementia

Christian Schnier, University of Edinburgh

Dr. Schnier and colleagues analyzed national observational cohorts from Wales, Denmark, Scotland, and Germany to investigate the relationship between HSV subtypes, antiherpetic medications, and the development of dementia, but found no significant associations. However, these analyses may not have been powered to identify small but clinically significant associations within a heterogeneous population. Dr. Schnier and colleagues then investigated the relationship between shingles (herpes zoster) vaccination, AD risk, and vascular dementia risk using routinely collected health data in Wales during 2013-2020. Of the 336,000 individuals assessed (half of whom received the shingles vaccine), approximately 18,000 (≥ 70 years old) developed dementia. Overall, those who received the vaccine were less likely to develop AD and even less likely to develop vascular dementia. The shingles vaccine was also associated with lower risk of mortality, stroke, and myocardial infarction. Further evaluation of all underlying causes of mortality found only small, non-significant differences between unvaccinated and vaccinated individuals. Lower risk for AD and dementia has also been associated with other vaccines, including the flu vaccine, possibly indicating a non-specific vaccine effect. Overall, Dr. Schnier and colleagues did not find evidence that HSV infection causes dementia, although they did identify an association between the state of the immune system and incident dementia.

Understanding the Brain Microbiome in AD: Challenges and Opportunities

Benjamin Readhead, Arizona State University

Studies have implicated HSV infection in seeding A β aggregation and resulting in dementia and AD pathologies, as well as cognitive decline. One study by Dr. Jacobson used a computational approach to detect microbial sequences in two AD cohorts (Religious Orders Study/Memory and Aging Project [ROSMAP] and the Johns Hopkins Brain Resource Center [JHBRC]), leading to the detection of human herpes virus 6 (HHV-6) in multiple cohort subjects; however, HHV-6 was detected in fewer samples than expected and no association with AD was identified. Studies of the infectious etiology of AD face multiple challenges, including what biological component to measure (e.g., microbial sequences, peptides, or antibodies) and how to interpret the results of analysis (e.g., does the finding indicate the component is a cause, an accelerant, or an opportunist?). To mitigate these challenges, Dr. Readhead and his team have developed data resources using (1) microbial capture enrichment and shotgun metagenomics methods that can detect viruses, bacteria, and fungi with high sensitivity and (2) antibody epitope repertoire methods that can identify host and microbial targets within CSF. Dr. Readhead and his team leveraged these approaches to assess epitopes within specific proteins, such as HSV1's nuclear protein UL4, which has been found to exhibit an immunogenic signal associated with AD. Preliminary epitope analyses identified sequences associated with the U79/U80 protein, which is correlated with genes enriched during AD and related to neurofibrillary tangle pathology. Dr. Readhead and colleagues aim to integrate metagenomics and antibody repertoire data with other types of omics and clinical data to contextualize findings and address interpretive challenges.

HHV-6 and Neurologic Disease

Steven Jacobsen, NINDS

Studies have found that HHV-6 is the only herpesvirus known to integrate within chromosomes, specifically telomere regions, in order to establish latency. HHV-6A and HHV-6B were recently reclassified as distinct viral species based on their biological and epidemiological differences; however, the sequences of these variants are 90 percent identical. HHV-6 is a ubiquitous virus associated with roseola, encephalitis, epilepsy, multiple sclerosis, and AD. Several other viruses, including HSV1, varicella zoster virus (VZV), and cytomegalovirus (CMV), have also been associated with AD. Recent literature indicates that HHV-6 and HHV-7 are found at high levels within the AD brain and that these viruses can dramatically accelerate amyloidosis in AD mouse models and cell cultures. These findings suggest that viruses like HHV-6 can trigger A β 's antimicrobial processes and that virions likely stimulate A β aggregation and eventually lead to AD pathology. Dr. Jacobson and colleagues sought to analyze existing sequencing data generated from 901 brains across multiple AD cohorts using PathSeq. Of the 901 brains, only 6 brains were HHV-6-positive and most of these brains exhibited low levels of HHV-6. Further analyses found no significant difference in the detection frequency or viral load of HHV-6, or any other CNS-related virus, between AD and control brains. Dr. Jacobson and colleagues also analyzed whole genome sequencing (WGS) data from more than 8,000 subjects using PathSeq and identified that high PathSeq score are indicative of HHV-6 infection.

Moderated Discussion of Session I

Moderator: Steven Jacobsen, NINDS

Early Risk Factors

Participants emphasized the importance of identifying early risk factors for AD that can be targeted therapeutically to halt or mitigate disease progression. However, identifying these factors in postmortem brains can be challenging because a pathological insult or initiating factor may have occurred years to decades before a patient began to exhibit AD pathology and thus that early factor may no longer be present, or may be present at levels below detection, at the time of analysis. Further, once an individual exhibits AD pathology, therapeutics targeting early factors are likely unable to prevent or slow progression because the pathologies have become fully established over the intervening decades. Studies leveraging historical samples (i.e., tissue samples collected before the onset of key AD pathologies) to improve early detection may be the optimal approach to identifying risk factors before AD symptom onset.

Determining Causation

Unlike AIDS, which is caused solely by HIV, AD pathologies could be caused, seeded, or driven by a variety of microbes. Because of this variety and the long gap between infection and development of AD pathology, identifying associations between specific microbes and AD can be challenging. Moreover, individuals are exposed to many viruses throughout their lifetimes, but not all individuals develop AD; thus, determining why some exposures lead to AD is difficult. Epidemiological and registry-based studies are needed to help disentangle and evaluate

possible microbial causes of AD. Clinical trials that target patients who are HSV-positive and positive for other AD risk factors, including APOE, may also help to identify causal factors.

Session II: Herpes Viruses and AD—the Debate Continues

Session Chair: Maria Nagel, University of Colorado

A β and HSV1: a Model for Examining Infection and Innate Immunity in AD

William Eimer, Harvard University

The amyloid cascade hypothesis has long positioned amyloid accumulation as the cause of neurofibrillary tangles and neuroinflammation that lead to AD. However, the role of amyloid in AD etiology may be better articulated by considering amyloid as not merely a damaging byproduct of the brain, but as a part of the innate immune system that can serve an antimicrobial purpose as well as contribute to neurodegeneration. Such an approach to amyloid bridges investigation of its role in neuroinflammation with the pathogenic etiology of AD.

A β 42 has been observed to bind with surface glycoproteins in both bacterial and viral pathogens, preventing them from infecting host cells and agglutinating them into a seeding mechanism that results in amyloid plaques with pathogens trapped inside. Dr. Eimer and colleagues showed that overexpression of A β 42 slowed mortality of candida, salmonella, and HSV1 infections in several animal models, including cultured neurons, Drosophila, and 5XFAD and APP-KO mice, with agglutination progressing two to three times faster in the presence of herpes viruses compared to salmonella bacteria. While A β 42 can form plaques in the absence of pathogens, 3D cultured models of human neurons showed A β 42 was preferentially present on cells infected with HSV1, and rapidly formed far larger plaques in the presence of HSV1 cells than alone. The simultaneous presence of A β 42 and HSV1 in 3D cultures rapidly drove cytotoxicity to substantially higher levels than those caused by HSV1 infection alone, perhaps suggesting that A β 42 interaction with HSV1 slows pathogenesis by increasing mitophagy. These results suggest that A β 42 may act not only as an exterior antimicrobial peptide that agglutinates pathogens, but also as an integral modulator that alters the progression of immune response to pathogens.

Testing the Hypothesis on Viral Etiology of Late-Onset AD

Iliia Baskakov, University of Maryland

The Einstein Aging Study showed that cohorts born after 1929 have lower incidences of dementia at the same biological age compared to those born earlier, suggesting a potential causative role of infectious agents in AD that has been ameliorated by increased exposure to antimicrobial and antiviral agents. Dr. Baskakov and colleagues tested the hypothesis that infectious agents have a causative role in AD and explored whether APP or A β protect against viral infection of the CNS. They found that overexpression of APP in 5XFAD mice did not protect against HSV1 infection, and that HSV1 neither triggered A β plaques in young mice nor bound to existing plaques in old mice; however, brain regions in both 5XFAD mice and WT controls infected with HSV1 showed consistent presence of reactive microglia that engulf infected

neurons and HSV1 virus directly as well as upregulated phagocytic uptake. High microglia activity was primarily found in areas with a high density of amyloid plaques. Viral invasion was limited in these areas, but no evidence was found of virus entrapped by A β plaques. The team thus hypothesized that instead of pathogens directly triggering protective A β plaques, multiple exposures to pathogens across the lifetime may lead to chronic activation of microglia that contributes to plaque formation. Recent studies have suggested that microglia may build plaques through phagocytic uptake of A β peptide. To further explore whether infectious agents play a causative role in AD, Dr. Baskakov's team will examine the long-term consequences of viral infection and microglial activation in aged, humanized APP^{+/+} mice that do not spontaneously develop AD pathology.

Exposure to Infectious Agents and Cognitive Function: the Baltimore Epidemiologic Catchment Area Study Follow-Up

Adam Spira, Johns Hopkins University

Attempts to prevent AD through modifiable risk factors such as diabetes, depression, and physical inactivity neglect infections common in older adults that have been linked to cognitive impairment and that may be overlooked AD risk factors. Working with a sample of middle-aged and older adults followed since 1981 by the Baltimore Epidemiologic Catchment Area Study, Dr. Spira's team investigated how exposure to common infectious agents relates to cognitive performance and whether exposure to a greater number of infections is associated with lower cognitive performance. Participants' exposure to HSV1, CMV, Epstein-Barr virus (EBV), VZV, and toxoplasmosis was determined by immunoglobulin G antibody levels, and cognitive outcomes were assessed using a global cognitive function test and a delayed recall task. In a sample of 575 participants adjusted for age, sex, race, education, and APOe4 carrier status, results showed a statistically significant relation between higher numbers of positive antibody tests and lower global cognitive performance. Among individual infectious agents, only CMV exhibited a statistically significant association with lower cognitive performance. Further study of the relationship between cognitive performance and the accumulation and reactivation of multiple infectious agents should incorporate a broader range of infectious agents, including coronaviruses, and explore possible mechanisms (e.g., inflammation, epigenetic change) by which patterns of infection may lead to decline as well as how antimicrobial interventions and stress impact outcomes.

The Potential Role of Bacteroides in AD

Laura Cox, Harvard University

In addition to external viral and bacterial pathogens, AD may be caused by anaerobic infections, which typically arise when bacteria from the normal microbiota cause infection due to compromised host defenses. Changing gut microbiota-brain interactions can reduce immune function throughout aging, and immune dysfunction and physiological changes increase susceptibility to anaerobic infections. Dr. Cox and colleagues found that levels of *Bacteroides*, the most common gut bacteria, tripled in the gut microbiome of aging female AD mice and were correlated with A β 40 and A β 42 in the brain. Previous research in humans has shown that

Bacteroides levels increase with age and in AD patients. Dr. Cox's team administered *Bacteroides fragilis* (*B. fragilis*) to APP/PS1 mice at plaque onset and found that the bacteria contributed to A β plaque production, altered multiple genes that may be implicated in AD pathology, and decreased peripheral GM-CSF, a secreted factor that may activate the peripheral immune system to improve the ability of microglia to clear amyloid. Dr. Cox's team also found that *B. fragilis* modulated microglia genes involved in protein homeostasis and neuronal cell death and impaired the clearance of A β plaques by peripherally recruited macrophages, potentially contributing to AD. *Bacteroides* has diverse functions, many of which are beneficial, and it is likely only some strains contribute to AD. Further study of the molecular diversity of *Bacteroides* to identify those strains and explore their role in AD pathology could lead to molecular diagnostics for use in point-of-care settings. Such studies may also help develop targeted approaches to shaping the microbiome in order to eliminate pathological interactions that may contribute to AD not by inducing inflammation, but by altering amyloid processing and inhibiting central and peripheral immune responses to A β plaque.

Enterobacterial Infections as Drivers of Tauopathies

Irene Salinas, University of New Mexico

Shigella sonnei (*S. sonnei*) is an emerging species of enterobacteria that is highly prevalent in industrialized countries where lifespans are longer and AD incidence is high. *S. sonnei* primarily causes mild intestinal symptoms but can cause neurological symptoms, including rare cases of epilepsy, neurologic hospitalization, and encephalopathy. Previous studies have linked enterobacteria with AD and shown that early childhood infection with other *Shigella* strains that are less dominant in industrialized countries predicts cognitive delays in later childhood; however, little research has been done on the potential cognitive impacts of *S. sonnei*. Dr. Salinas and colleagues found that tauopathy patients have signatures of past *S. sonnei* infection at much higher levels than controls and hypothesized that either *S. sonnei* infection induces long-term neurodegeneration in a tau-dependent manner or neurons undergoing degeneration are more susceptible to *S. sonnei* invasion. They found that *S. sonnei* induced long-term enteric neuron loss in the colon in tau-producing mice, but not in tau-suppressed mice; caused pro-inflammatory cytokine signatures in the colon and cortex; and, in rare cases, directly invaded the cortex. *S. sonnei* infection was also strongly associated with long-term behavioral changes and tau pathology in male mice (likely due to a less robust antibody response than in females) and was shown to disrupt the CDK5 pathway in the cortex of tau-producing mice of both sexes. The cortex, lymph nodes, olfactory bulb, and olfactory epithelium of *Shigella*-infected tau-producing mice already undergoing neurodegeneration were almost exclusively dominated by *S. sonnei*, whereas the same tissues in tau-suppressed mice showed greater microbial diversity. Further study of the link between *S. sonnei* and tau pathology should explore effects in older mice, whether *S. sonnei* invades enteric or other neurons, and when individuals infected with *S. sonnei* start to develop markers of neurodegeneration.

Moderated Discussion of Session II

Moderator: Maria Nagel, University of Colorado

Challenges of Animal and Organoid Models

Modeling human disease in animals poses significant challenges, including the tension between using lower doses of infectious agents to simulate the conditions of human infection and using higher doses to simulate human pathology when studying agents that are optimized for human, not animal. Participants discussed the limitations of AD mouse models that were developed based on earlier assumptions about AD etiology and that are therefore not ideally suited for the study of immunomodulation. Organoid models offer a more manipulable environment to examine specific interactions, but because they do not represent a full immune system, global conclusions cannot necessarily be extrapolated from their use alone.

Clinical Trials and Defining Causative Agents

Some participants emphasized a need for more clinical trials to proceed in parallel with lab and epidemiology studies, noting that progress in lab research is unlikely to shift clinical approaches until it results in human interventions, such as vaccines, to protect against pathogens implicated in AD. However, the complexity of designing clinical trials that will yield actionable results is exacerbated by the interplay among pathogens, which makes defining causative agents difficult. Combining antivirals for synergistic effects and selecting subjects who are both APOE4 carriers and HSV1 seropositive may be a viable approach. Further study of bacterial and viral agents—including COVID-19—is needed, as are more inventive and longitudinal methods of capturing historical evidence of pathogens that cannot be traced postmortem.

Session III Part I: Pathogens and AD—Is there Evidence for Causation?

Session Chair: Adam Spira, Johns Hopkins University

The Brain Microbiome in Health and Disease

Richard Lathe, University of Edinburgh

Dr. Lathe and his colleagues seek to understand the impact of the brain microbiome on health and disease. They developed 64-mer probes using ribosomal RNA to detect microbial sequences in control and AD brain samples. No major differences were observed in abundances of bacteria, fungi, and chloroplastida, each of which were represented in all disease and control samples. Analyses of the 20 most abundant viral sequences detected few herpes viruses and showed that the most abundant reads were related to adenovirus type C. Overall, cellular microbes were found to be significantly more abundant than viruses; however, approximately one-third of individuals exhibited very low levels of microbes in the brain. Further analyses revealed that brain regions with high abundances of cellular microbes, viruses, and retroelements are strongly correlated. Using data from the Edinburgh Brain Bank, Dr. Lathe and colleagues found that the cingulate cortex appears to hold the most microbe-related reads, followed by the amygdala, hippocampus, and hypothalamus, but with relatively few microbes in the cortex; these findings are critical because many previous studies have focused solely on the cortex, likely missing important findings in other regions. Some participant samples exhibited microbes only in the cingulate cortex, whereas analyses of other samples indicated that microbes had spread from one brain region to another. Additional analyses will be performed to investigate brain regions related to cholinergic pathways. One challenge to these

types of studies is defining control and AD samples: some of the control subjects may eventually develop AD or may have been misdiagnosed. Further, some pathogens may be harmless and others pathogenic.

Single-Cell Dissection of Pathogen-Associated Changes in AD

Manolis Kellis, Massachusetts Institute of Technology

Using the ROSMAP cohort, Dr. Kellis and colleagues used single cell profiling of RNA and DNA regulatory regions to interrogate the relationship between HSV1- and CMV-positivity and AD. scRNASeq analyses revealed gene expression changes in cell types between AD and control brains and between male and female brains. Using transcriptomic profiles, Dr. Kellis and colleagues constructed a timeline of gene expression data to indicate when certain risk factors are upregulated and downregulated in relation to disease progression. They applied similar techniques to create spatiotemporal maps of AD progression based on changes in gene expression, neuronal and glial cell types, and pathology across specific brain regions. CMV-positive brains with AD exhibited higher abundances of oligodendrocytes and lower abundances of excitatory neurons than controls. HSV1-positive individuals displayed higher abundances of oligodendrocyte precursor cells (OPCs), than control subjects. CMV-positive subjects without AD displayed upregulated cellular stress responses in oligodendrocytes and microglial inflammatory responses, whereas non-AD subjects exhibited downregulation of synaptic transmission and cytokine-mediated signaling. AD subjects positive for both HSV1 and CMV exhibited upregulation of astrocytic responses to cytokines and microglial stress responses, whereas those who were only CMV+ (HSV1-negative and non-AD) displayed a downregulation of synaptic transmission, cellular assembly, and synaptic pruning pathways. Overall, CMV and HSV1 resulted in similar changes in the transcriptome of both AD and non-AD brains, indicating that these pathogens may contribute to the dysregulation of gene expression profiles through parallel pathways.

Periodontal Disease and Cognitive Aging in a Multiethnic Cohort: Findings from the Washington-Inwood Columbia Aging Project Ancillary Study of Oral Health

James Noble, Columbia University

Periodontal disease is a common gum infection that causes the gums to recede, reduces the underlying bone, and exposes the root of the tooth. Symptoms of periodontal disease include gum redness, bleeding during brushing or flossing, tooth loss, and recurrent infections. Many microbes have been implicated in causing periodontal disease in combination with environmental and genetic risk factors. This disease typically becomes more pronounced with age and has been associated with cognitive impairment and AD through cross-sectional studies. To further examine these associations, Dr. Noble and colleagues have worked with the Washington-Inwood Columbia Aging Project, which is a multi-ethnic longitudinal prospective study of cognitive aging in more than 6,000 older adults residing in northern Manhattan. The study incorporated an oral health study for 1,993 participants in 2013, which included full-mouth periodontal examinations and the collection of plaque and microbiota samples for further testing. Initial analyses indicated that approximately 75-80 percent of participants

exhibited moderate to severe periodontal disease and that multiple factors were associated with lower tooth count, including increasing age, lower educational attainment, tobacco use, and dementia. Analyses of the microbiome data identified microbes that are more associated with severe periodontal disease than mild disease, indicating that the microbiome changes as the disease progresses. MRI data analyses revealed that the number of teeth present is a protective factor against white matter hyperintensity, and AD MRI signatures and a review of the longitudinal data confirmed that number of teeth present is also protective against memory loss and complications in language and visuospatial processing. Next steps are to further investigate the mechanistic links between periodontal disease and AD, particularly because periodontal disease incidence is greatly influenced by health disparities. These investigations will include analysis of amyloid responses to chronic infections and inflammatory changes.

***Porphyromonas gingivalis* in AD Brains: Evidence for Disease Causation and Treatment with Small-Molecule Inhibitors**

Stephen Dominy, Cortexyme, Inc

Porphyromonas gingivalis (*P. gingivalis*) is associated with chronic periodontitis and has been detected in the brains of individuals with AD. Levels of gingipains, the cysteine protease virulence factors of *P. gingivalis*, correlate with AD diagnosis, as well as with tau pathology. Oral infection with *P. gingivalis* in wildtype mice results in the infection spreading to the brain, induction of A β 40 and A β 42, and eventual development of AD pathology, including brain inflammation, neurodegeneration, and A β aggregation. Studies by Dr. Dominy and colleagues showed that gingipain load in human brains correlate with AD diagnosis and that gingipains colocalize with tau tangles and intraneuronal A β aggregates. A follow-up study showed that *P. gingivalis* can infect neurons *in vitro* and persistently express active gingipains intraneuronally. Dr. Dominy and colleagues also found that gingipain inhibitors are able to block the induction of A β caused by *P. gingivalis* infections in a mouse model. These findings led Dr. Dominy and colleagues to develop COR388 (atuzaginstat), a potent, orally available, brain-penetrant gingipain inhibitor, which efficaciously and safely blocked gingipain-mediated A β induction in preclinical studies. A Phase I human trial in patients with mild to moderate AD indicated that a 28-day drug treatment paradigm resulted in decreased fragmentation of APOE in CSF. In 2019, the pivotal P2/3 GAIN Trial to evaluate atuzaginstat was launched and the trial results indicated that 100 percent of GAIN subjects (with mild to moderate cognitive impairments) exhibited systematic *P. gingivalis* exposure, more than 90 percent of subjects exhibited moderate to severe periodontal disease, and 84 percent of subjects exhibited typical AD CSF biomarker levels. Results from the GAIN trial also indicate evidence of CNS infections through measurements of anti-Pg immunoglobulin G levels. Topline data related to disease modifications in AD from the GAIN trial will be released during November 2021.

Clearance of A β in Toxoplasma-Infected Murine Models of AD

Melissa Lodoen, University of California, Irvine

Toxoplasma gondii (*T. gondii*) is an obligate intracellular eukaryotic parasite that affects approximately 30 percent of the global population and can infect the CNS. Infections are

typically asymptomatic, chronic, and result in dormant cysts within the brain; however, infections in immunocompromised individuals can be life-threatening. Studies using AD mouse models indicate that *T. gondii* infections reduce A β plaques, increase microglial activation and homeostatic proliferation, and improve cognition, indicating protection against AD phenotypes. To further explore the underlying mechanism of protection, Dr. Lodoen and colleagues developed a mouse model of *T. gondii* infection. By Day 15 post-infection, this model exhibits *T. gondii* cysts in the murine brain, inflammatory monocytes infiltration in the CNS, and microglial activation in regions infiltrated by monocytes. 5XFAD mouse models of chronic *T. gondii* infection also exhibit increased and sustained microglial activation, particularly surrounding A β plaques. In addition, upon *T. gondii* infection, A β plaques are significantly reduced and Iba1 expression (a marker of activated microglia) is increased in the hippocampus, cortex, and thalamus. CD68 is also increased within plaques of infected 5XFAD mice, indicating lysosomal and phagocytic microglial activity. Using longitudinal 2-photon imaging of amyloid plaque formation, Dr. Lodoen and colleagues found that A β plaque volume does not differ between control and AD mice at baseline and during acute infection, suggesting that A β plaque volume is not significantly reduced until chronic infection is achieved. These studies emphasize how the *T. gondii* model can reveal new insights into amyloid dynamics and interactions with microglia.

Microglia, Genetics, and Pathogens in AD

Elizabeth Bradshaw, Columbia University

Genetics studies have implicated microglia activity in late-onset AD (LOAD). Genetic variation can dampen the microglial activation response, leading to increased risk for AD. Dr. Bradshaw and colleagues sought to evaluate the interaction between microglial genetic variants, pathogens, and LOAD. LOAD-related genetic variation may modulate microglial responses to HSV-1; however, the mechanisms underlying this modulation are poorly understood. Dr. Bradshaw and colleagues developed an *in vitro* human microglia-like model derived from blood monocytes from hundreds of individual donors to explore genetic- and/or age-driven microglial dysfunction; this model involves incubating monocytes with multiple cytokines, including GM-CSF, IL-34, and NGF-beta, for 10-14 days in order to transform the cells into microglia-like cells. Microglial-specific genes are upregulated in these microglia-like cells, including HEXB, C1QA, GAS6, GPR34, PROS1, TGF-beta R1, BIN1, and CD39. To assess whether genetic variation leads to differential RNA and protein expression and altered disease risk, Dr. Bradshaw and colleagues performed eQTL analyses and identified that the CD33M isoform is upregulated in those with higher AD risk, whereas the CD33m isoform is downregulated in the microglia-like cell model. The microglia-like cells express pathogen recognition receptors (PRRs) and other signaling molecules. Dr. Bradshaw and colleagues treated these microglia-like cells, as well as monocyte-derived macrophages, with *P. gingivalis* lipopolysaccharides and found that the macrophages exhibited higher inductions of IL-1b and IL-6 than the microglia-like cells. Next steps are to evaluate the levels of induction for the monocyte-derived macrophages and the microglia-like cells across the context of genetic variation.

Cerebrospinal Fluid Immunity in AD

David Gate, Northwestern University

In addition to providing protection against brain trauma and injury, CSF provides immune protection to the brain. The predominant immune cells within CSF are CD4- and CD8-positive T cells and smaller populations of innate immune cells; overall, these cellular populations in the CSF are smaller than those within the blood. To study differences in immune responses in AD, Dr. Gate and colleagues analyzed peripheral blood mononuclear cells and CSF—collected from cognitively impaired and healthy control patients—using mass spectrometry (MS) methods. Analyses identified an upregulated CD8-positive T cell population and an upregulation in T cell receptor (TCR) signaling in the blood of AD subjects, compared to healthy controls. In a second cohort, Dr. Gate and colleagues found that these upregulated T cells are negatively associated with cognition in AD. Immunohistochemical analyses revealed that CD8-positive T cells localize near neuronal processes and A β plaques within the postmortem AD brain, enter the AD brain, and monitor the CSF. Dr. Gate and colleagues also found that many of the T cells in the CSF and hippocampus of AD patients express genes related to presenting antigens and eliminating pathogens. Network analysis of the specific TCR sequences across AD patients revealed that many AD patients share TCR sequences that are known to be responsive to EBV antigen EBNA3 and that the T cells that contain these sequences express factors associated with effector functions, including CCL5 and GZMK. Dr. Gate and colleagues have developed an in vitro T cell-neuron killing assay that uses (1) CD8-positive T cells that are known to respond to EBV that are incubated with granzyme B fluorescent substrate and (2) neurons incubated with EBV antigen and intracellular dye. These two cell types are then co-incubated, which reveals that the T cells begin killing the neuronal cells that express the EBV antigen.

Probable Involvement of Varicella Zoster Virus in AD via the Reactivation of Quiescent HSV1

Dana Cairns, Tufts University

Dr. Cairns and colleagues have identified that human induced neural stem cells (hiNSCs) can be infected by VZV and can also be coinfecting with HSV1. HSV1 alone or in combination with VZV can induce A β plaque-like formations in cultured cells. Independently, both HSV1 and VZV also induce gliosis and inflammation in cell culture. In order to identify whether VZV is able to reactivate quiescent HSV1 infections, Dr. Cairns and colleagues infected their cell culture model with HSV1, treated the cells with valacyclovir to stop the infection, and then treated with either VZV or mock infection. The VZV-infected cells exhibited a reactivation of HSV1 and expression of A β , whereas mock-infected cells did not.

Moderated Discussion of Session III Part I

Moderator: Adam Spira, Johns Hopkins University

Inflammatory Responses in AD

Participants discussed whether an interferon storm-like mechanism may be responsible for some of the expression profile changes associated with pathogen infection, noting that pro-

inflammatory signals in the periphery are known to be associated with periodontal disease; however, not all individuals with AD and periodontal disease exhibit this phenotype.

APOE Fragmentation and AD Pathology

Participants discussed how APOE fragmentation plays a role in AD pathology. Although the mechanism is poorly understood, participants suggested that A β proteins are bound to APOE fragments in the brain and thus when fragmentation is increased as a result of gingipain infection, the clearance of bound A β proteins is also increased.

Immune Responses to *T. gondii* in Mice and Humans

Associations between *T. gondii* and cognitive decline are significantly different in the human and mouse. Whereas many studies have indicated a negative relationship between *T. gondii* and human cognitive decline, infection in mouse models improves cognition. These differences may be caused by general differences in the human and mouse immune systems; in particular, the Toll-like receptors responsible for responding to *T. gondii* infection in mice are non-functional in humans.

Session III Part II: Pathogens in AD—Is there Evidence for Causation?

Session Chair: Avindra Nath, NINDS

HIV/AIDS, Aging, and AD

Eliezer Masliah, NIA

Neuropathogenesis of HIV involves the infiltration of macrophages through the blood-brain barrier and activation of microglia that release cytokines and chemokines, which in turn activate glial cells that, along with HIV-secreted proteins, trigger neurodegenerative processes. Even with antiretroviral treatment, latent HIV infections can lead to chronic inflammation. Both neuro-HIV and AD include proteinopathy phenotypes and studies have found that antiviral agents can disrupt microglial phagocytosis of A β aggregates.

HIV-associated neurocognitive disorder (HAND) is more severe in older adults, compared to younger adults, and is associated with accelerated aging processes. Evidence suggests that both HIV and AD lead to dysregulation of common pathways, including neuronal and synaptic function. HIV and aging also likely cause dysregulation in protein processing mechanisms (e.g., autophagy), leading to the accumulation and aggregation of proteins (including amyloid and tau) and eventual neurodegeneration. In addition, studies have found that HIV-1 Tat can increase A β levels through neprilysin-mediated lysosomal inhibition and interactions with A β precursor protein. Other studies have found that A β levels in patients with HAND are similar to those exhibited by AD patients, although middle-aged HIV patients with HAND do not exhibit increased A β levels. In addition to A β , tau deposition is also accelerated in older adults with HIV. Additional longitudinal studies with biomarkers will help to further explore the relationships and shared mechanisms underlying HIV and AD.

Virus and Olfactory System Interactions Accelerate AD Pathology

Maria Nagel, University of Colorado

VZV and HSV1 are human alpha-herpesviruses that typically become latent in older adults, but can be reactivated to cause neurological diseases. Both VZV and HSV1 recapitulate hallmark pathologies of AD, including dementia, cerebrovascular disease, neuroinflammation, A β and tau aggregation, and neuronal dysfunction, and antiviral use can reduce the risk of dementia after a HSV1 or VZV infection. VZV and HSV1 are unique among other pathogens because they can travel along neurites of ganglionic neurons to be directly deposited in the brain parenchyma and cerebral arteries. Dr. Nagel and colleagues found that VZV infections induce amylin expression and enrich pathways involved in AD pathologies; HSV1 infections do not induce amylin expression but do induce A β deposition. They also found that amylin-knockdown models led to a reduction of VZV transcripts; VSV-positive supernatant and plasma promoted A β aggregation; and VZV proteins caused protein misfolding in a dose-dependent manner and colocalized with A β proteins within cerebral vasculature. CSF collected from VZV patients exhibited elevated A β and amylin levels, indicating that VZV is amyloidogenic. Dr. Nagel and colleagues also evaluated simian varicella virus (SVV) infection in rhesus macaques, which causes an increase in serum amylin and A β . Infection of the pancreas by SVV leads to the presence of amyloid in the pancreas.

Brain areas involved in olfactory processing are also implicated in early AD neuropathology. Dr. Nagel and colleagues hypothesize that alpha-herpesvirus infections of the olfactory epithelium (OE) induce pathological processes in the olfactory system and hippocampus that are characteristic of early AD. To test this hypothesis, Dr. Nagel and colleagues will use sequencing and MS-based analyses to assess whether VZV infections of olfactory neurons *in vitro* and *in vivo* cause A β production, loss of smell, and olfactory dysfunction.

A Possible Role for Latent HHV-6A in AD

Chris Pröschel, University of Rochester

HHV-6 is uniquely able to integrate within chromosomes and thus can be inherited through the germline. It is associated with demyelinating diseases, such as multiple sclerosis and progressive multifocal leukoencephalopathy, but virions have not been identified. U94 is a protein that inhibits viral gene expression and replication to allow viruses like HHV-6A to remain in a latent state. Dr. Pröschel and colleagues hypothesize that latent HHV-6 impairs human OPC function, leading re-myelination activities to be insufficient to repair damage. During acute infection, OPCs undergo cell cycle arrest, resulting in syncytia formation, viral integration, expression of U94, and viral latency. Further analyses found that U94 causes attenuation of OPC migration and maturation, rendering OPCs unable to reach a lesion; similar attenuation of OPC functioning have been reported in AD studies. Other studies have found that alterations in OPC morphology and myelination activities precede clinical AD onset. Dr. Pröschel and colleagues identified that whereas U94 expression in immature neurons attenuates morphological maturation, expression in mature neurons attenuates synaptogenesis. To assess whether U94 impacts AD onset, they developed a novel transgenic

mouse model and found that mice expressing U94 and APP displayed significantly more A β plaque formation than mice overexpressing U94 or APP alone, suggesting that U94 enhances amyloidogenic APP processing. Together, these findings have led Dr. Pröschel and colleagues to hypothesize that latent HHV-6A is a disease-modifying factor that requires further study.

Effects of *Chlamydia pneumoniae* Infection on the Brain and Retina in AD

Timothy Crother and Maya Koronyo-Hamoui, Cedars-Sinai Medical Center

Chlamydia pneumoniae (*C. pneumoniae*) is a common obligatory respiratory pathogen that is associated with diseases involving chronic inflammation, including AD. *C. pneumoniae* RNA and DNA have been detected in human AD brains and anti-*C. pneumoniae* titers are increased in AD patients. *C. pneumoniae* infection complications are also associated with dementia prognosis. In mouse models, *C. pneumoniae* infection leads to an increase in A β plaques in wildtype mice. Drs. Crother and Koronyo-Hamoui have evaluated chronic and acute *C. pneumoniae* infections through bioinformatic and omics approaches using transgenic mouse models and found that infection can induce inflammasome activity and aberrant T-cell responses; investigators will further explore these findings through a treatment study against inflammasomal activity and mouse models of antibody depletion. Additional analyses showed that *C. pneumoniae*-infected mice exhibit a robust A β aggregation response and anti-chlamydia antigens in the brain soon after infection and a reduction in soluble A β during acute infection, which is likely caused by macrophage activity and IL-12. Drs. Crother and Koronyo-Hamoui also observed chlamydial antigens and increased A β plaques in retinal samples from AD patients, compared to controls.

Polymicrobial “Lichenoid” Cerebritis in AD and Preclinical Models

David Corry, Baylor College of Medicine

Studies using immunofluorescence methods have detected fungi within the brain of AD patients. To further investigate the impact of these fungi on AD, Dr. Corry and colleagues developed an acute infection mouse model, in which mice are intravenously injected with 25,000 colony forming units of *Candida albicans* (*C. albicans*), which is a sublethal dose. These mice display A β peptide accumulations on fungi and fungal-induced glial granulomas. Based on these and other findings, Dr. Corry and colleagues hypothesize that *C. albicans* infections of the brain initiate through gut colonization, metastasis, and cerebral mycosis through blood-brain barrier infiltration; upon brain entry, the fungi induce A β 's antimicrobial functions, microglial recruitment, and eventual dementia. To assess other fungi-like pathogens and AD, Dr. Corry and colleagues identified a novel polymicrobial lichenoid through mouse brain-derived cell cultures. Investigators confirmed that this lichenoid species is a combination of *C. albicans*-produced cellulose matrices and staphylococcus. The species was also detected in AD and control human brain cultures. These findings indicate that *C. albicans* is a ubiquitous, highly adapted human fungal parasite that can avoid sterilizing immunity, leading to long-term infection of the brain, and suggests the hypothesis that fungal-directed polymicrobial lichenoid infections are causal of AD.

Repurposing of Existing Vaccines for Alzheimer's Prevention

Svetlana Ukraintseva, Duke University

Many pathogens have been linked to AD and related traits, suggesting that no one pathogen alone causes AD and that a variety of pathogens in combination with compromised immunity may be the true cause. Dr. Ukraintseva and colleagues hypothesized that some existing vaccines may have a protective effect against AD through beneficial off-target effects. To test this hypothesis, they used longitudinal data from the Health and Retirement Study (HRS), as well as Medicare administrative records, to evaluate whether adult vaccinations against shingles and/or pneumonia can reduce the risk of AD. Shingles, HSV, pneumonia, and recurrent mycoses were each associated with increased AD risk, whereas vaccination against shingles and pneumonia was associated with decreased AD risk and mortality. These findings support the hypothesis that compromised immunity may contribute to AD risk, and that vaccines with beneficial off-target effects may aid in prevention of AD. Live-attenuated shingles and pneumonia vaccines are promising candidates to repurpose for AD prevention.

Moderated Discussion of Session III Part II

Moderator: Avindra Nath, NINDS

Heterogeneity in Pathogens and AD

The causes of AD may be as heterogeneous as the disease itself. Studies have emphasized that many pathogens are associated with AD; although until recently many researchers asserted that the brain existed in a sterile, pristine environment, it now is known to have a robust immune system that encounters pathogens regularly. Participants thus discussed the need to integrate research focusing on individual pathogens into a combined framework that explains AD pathogenesis and provides a comprehensive basis for future therapeutic development.

Pathogens in the Periphery

Participants identified two hypotheses that were highlighted during the workshop: (1) pathogens in the periphery can infect the brain during aging to trigger AD pathology and (2) pathogens that enter the periphery and prime the immune system, but do not enter the brain, can prime the brain's immune system to the pathogen and induce protective functions.

Sample Collection and Contamination

Participants stressed the importance of obtaining high-quality brain tissues and samples with optimized collection and processing procedures to ensure that pathogens identified in those samples are not caused by contamination.

Final Discussion

Moderator: Avindra Nath, NINDS

Shared Mechanisms

Participants agreed that sufficient evidence was presented during this workshop to support the hypothesis that pathogens can cause AD, noting that next steps in these studies must be to identify shared mechanisms leveraged by different pathogens to assess how many pathogens

can cause AD. Some possible shared mechanisms may be transmission to the brain across nerves from areas of high microbe abundance, such as the gut or mouth, or factors that lead to transneuronal degeneration and death as well as breakdown of the blood-brain barrier.

Nasal Route of Entry

One shared factor in pathogen-initiated AD may be route of entry into the human body. Studies have found that nasal entry can cause latent HSV1 infections in the hypothalamic nuclei (which are associated with AD-related sleep disorders). In addition, the olfactory bulb is located adjacent to the entorhinal cortex, where AD tau pathology begins developing, and which has terminal connections with the hippocampus' dentate gyrus, where amyloidosis typically begins.

Gaps and Challenges

One major challenge to current AD research is contradictory evidence. Participants postulated that contradictory findings may be caused by molecular and cellular interactions within specific populations, including individuals with different genetic backgrounds or environmental risk factors. A major data gap is the lack of microbe-relevant phenotypes captured in longitudinal datasets; electronic health records are optimized for acute care and typically do not include sufficient information on infections that could help generate new hypotheses. Another challenge is the length of time required to develop a mouse model of infection and AD; one possible solution to this challenge is to create mouse models of multiple infections (e.g., HIV and HHV) to accelerate the neurodegenerative process.

Appendix 1: Agenda

Day 1: October 5, 2021

- 10:00 am** **Welcoming Remarks**
Eliezer Masliah, NIA
- 10:10 am** **Workshop Objectives**
NIA DN Staff
- 10:20 am** **Keynote Presentation: AD Pathology—An Orchestrated Innate Immune Response of the Brain?**
Rudolph Tanzi, Harvard University
- 10:55 am** **Break**
- 11:00 am** **Session I: Herpes Simplex and AD—The Epidemiological Perspective**
Session Chair: Steven Jacobson, NINDS
- Speakers:**
- Hugo Lövheim, Umeå University
 - Christian Schnier, University of Edinburgh
 - Ben Readhead, Arizona State University
 - Steven Jacobson, NINDS
- Moderated Discussion**
- 12:30 pm** **Break**
- 1:00 pm** **Session II: Herpes Viruses and AD—The Debate Continues**
Session Chair: Maria Nagel, University of Colorado
- Speakers:**
- William Eimer, Harvard University
 - Ilia Baskakov, University of Maryland
 - Adam Spira, Johns Hopkins Bloomberg School of Public Health
 - Laura Cox, Harvard University
 - Irene Salinas, University of New Mexico
- Moderated Discussion**
- 2:30 pm** **Adjourn Day 1**

Day 2: October 6, 2021

- 9:55 am** **Welcome to Day 2**
NIA DN Staff
- 10:00 am** **Session III Part I: Pathogens and AD—Is there Evidence for Causation?**
Session Chair: Steven Jacobson, NINDS
- Speakers:**
- Richard Lathe, University of Edinburgh
 - Manolis Kellis, Massachusetts Institute of Technology
 - James Noble, Columbia University
 - Steven Dominy, Cortexyme
 - Melissa Lodoen, University of California, Irvine
 - Elizabeth Bradshaw, Columbia University
 - David Gate, Northwestern University
 - Dana Cairns, Tufts University
- Moderated Discussion**
- 12:15 pm** **Break**
- 12:45 pm** **Session III Part I: Pathogens and AD—Is there Evidence for Causation?**
Session Chair: Avindra Nath, NINDS
- Speakers:**
- Eliezer Masliah, NIA
 - Maria Nagel, University of Colorado
 - Chris Proschel, University of Rochester
 - David Corry, Baylor College of Medicine
 - Timothy Crother, Cedars-Sinai Medical Center
 - Maya Koronyo-Hamaoui, Cedars-Sinai Medical Center
 - Svetlana Ukraintseva, Duke University
- Moderated Discussion**
- 3:00 pm** **Break**
- 3:10 pm** **Final Discussion**
Moderator: Mack Mackiewicz, NIA
- 4:10 pm** **Closing Remarks**
Eliezer Masliah, NIA
- 4:15 pm** **Adjourn Day 2**

Appendix 2: Speakers and Moderators

Invited Speakers and Moderators

Ilia Baskakov, University of Maryland
Elizabeth Bradshaw, Columbia University
Dana Cairns, Tufts University
David Corry, Baylor College of Medicine
Laura Cox, Harvard University
Timothy Crother, Cedars-Sinai Medical Center
Stephen Dominy, Cortexyme, Inc.
William Eimer, Harvard University
David Gate, Northwestern University
Steven Jacobson, NINDS
Manolis Kellis, Massachusetts Institute of Technology
Maya Koronyo-Hamaoui, Cedars-Sinai Medical Center
Richard Lathe, University of Edinburgh
Melissa Lodoen, University of California, Irvine
Hugo Lövhelm, Umeå University
Mack Mackiewicz, NIA
Eliezer Masliah, NIA
Maria Nagel, University of Colorado
Avindra Nath, NINDS
James Noble, Columbia University
Chris Proschel, University of Rochester
Benjamin Readhead, Arizona State University
Irene Salinas, University of New Mexico
Christian Schnier, University of Edinburgh
Adam Spira, Johns Hopkins University
Rudolph Tanzi, Harvard University
Svetlana Ukraintseva, Duke University