

**National Institute on Aging
Division of Neuroscience**

Can Autism Spectrum Disorders Tell Us Anything About Alzheimer's Disease?

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Acronym Definitions

AD	Alzheimer's disease
ADNP	activity-dependent neuroprotective protein
ADRD	Alzheimer's disease and related dementias
APP	amyloid precursor protein
ASD	autism spectrum disorders
A β	amyloid beta
DN	Division of Neuroscience
E/I	excitation-inhibition
FMRP	Fragile X mental retardation protein
FTD	frontotemporal dementia
FXS	Fragile X syndrome
GARS	Gilliam Autism Rating Scale
HAT	histone acetyltransferase
HDAC	histone deacetylase
ID	intellectual disability
IPSC	induced pluripotent stem cell
LTD	long-term depression
LTP	long-term potentiation
MCI	mild cognitive impairment
mGluR	metabotropic glutamate receptor
mTOR	mammalian target of rapamycin
NIA	National Institute on Aging
NO	nitric oxide
NT	Neurotypical
SNO	S-nitrosylated
TAND	TSC-associated neuropsychiatric disorder
TSC	tuberous sclerosis complex

Executive Summary

Neurodevelopment and neurodegenerative disease both have heterogeneous symptom presentation, occur at a specific stage of life, and currently have no disease-modifying treatments. In addition, both disease classes affect cognition and behavior, sometimes in strikingly similar ways, and share certain molecular characteristics. To survey gaps and opportunities in understanding the connection between Alzheimer's disease (AD) and autism spectrum disorders (ASD), the National Institute on Aging (NIA) Division of Neuroscience (DN) held a virtual workshop March 8-9, 2022. Researchers convened to discuss how AD and ASD studies can inform each other. The workshop was organized into four sessions, each focusing on a different aspect of the connection between AD and ASD.

The first session discussed clinical and epidemiological approaches to examining the connection between AD and ASD. Participants presented data that strongly suggest that individuals with ASD have an increased risk of developing dementia as they age, and that this connection may be driven by elevated genetic risk factors. In addition, the severity of ASD-like behaviors in older adults can predict both cognitive decline and postmortem neuropathology. Medicaid and Medicare datasets may be useful in exploring this connection further. Longitudinal studies of aging with ASD will be essential to identify vulnerability and protective factors and to explore what aspects of ASD-like behaviors are most associated with dementia risk. The field would benefit greatly from the development of diagnostic tools to identify ASD in older adults and dementia in older adults with ASD.

The second session focused on connections between ASD and AD-related proteins amyloid precursor protein (APP) and tau. In genetic forms of ASD, hyperactive activity-elicited APP production may drive aberrant synapse formation during development. In addition, Fragile X mental retardation protein (FMRP), a protein associated with ASD, may facilitate A β -driven stress responses, causing network and synaptic deficits. Further, mutations in *TSC1*, which are implicated in a genetic form of ASD, may also cause neurodegeneration, driven by the hyperacetylation of tau. Wild-type tau may also enable certain genetic and environmental triggers to elicit excitation-inhibition imbalance and corresponding deficits in ASD and AD. A thorough examination of APP metabolites and processing secretases, FMRP, and all splice isoforms of tau, in disease and over the lifespan, will be critical to understanding this relationship.

The third session examined signaling pathways dysregulated in both AD and ASD. The speakers presented data highlighting the importance of aberrantly high mTOR activity in both disorders. In models of both AD and ASD, the accumulation of nitric oxide drives S-nitrosylation of mTOR proteins, causing molecular and behavioral deficits that can be rescued via mTOR or NO inhibition. In addition, high mTOR activity in both AD and ASD models causes high levels of translational regulator DJ-1, disrupting the expression of calcium channels. Fly and mouse models of Fragile X syndrome and familial AD have similar molecular and behavioral deficits that can be rescued via treatment with cAMP inhibitors, suggesting a possible therapeutic avenue. Future research should focus on the development of reliable biomarkers for elevated brain activity of mTOR and cAMP, which could be used in longitudinal studies to examine these signaling pathways in aging with and without dementia.

Building on these rich findings, the fourth session explored the relationship between genes and environment in AD and ASD. In both AD and ASD models, modulating epigenetics via histone acetylation can suppress deleterious phenotypes. In addition, inflammation plays a prominent role in both AD and ASD. In these disorders, DNA damage may elicit aberrant immune activation that causes uncontrolled

cell death. Epidemiologically, pollutant exposure links ASD and AD. Pollutant exposure is associated with ASD risk and can even cause AD-like neuropathology. To connect these ideas, future studies should explore the idea of microglial priming, in which early exposure to a pollutant may prime microglia for toxic reactivity to a second exposure later in life, causing disease.

Meeting Summary

Welcome Remarks, Day 1

Eliezer Masliah, M.D. – Director, National Institute on Aging Division of Neuroscience

NIA has been interested in how genetics, environmental factors, and homeostatic mechanisms interact with proteinopathies to cause synaptic damage. To gain insights in this area, aging and dementia researchers have started to draw some of the biology from other fields, such as cancer biology, immunology, and neuropsychiatric disorders.

To advance our understanding of neurodegenerative disorders even further, the field now turns to neurodevelopmental disorders, including ASD. This workshop begins to explore insights in this area that can be used to move the field forward.

Workshop Objectives

Lisa Opanashuk, Ph.D. – Program Director, National Institute on Aging Division of Neuroscience

Dr. Lisa Opanashuk shared the objectives for this workshop, which were as follows:

1. Consider whether ASD can tell us anything about Alzheimer's disease and related dementias (ADRD).
2. Discuss epidemiological approaches to look at the potential association of ASD with ADRD.
3. Highlight basic research on the intersection of cell biological mechanisms contributing to ASD and ADRD.
4. Identify research opportunities to further understand ADRD based on the knowledge of ASD.

The workshop was split into four sessions. Questions were taken in moderated discussions at the end of each session, rather than after each speaker.

Session 1: Clinical/Epidemiology

Aging With Autism Spectrum Disorder: Longitudinal and Cross-Sectional Approaches to Cognitive, Brain, and Genomic Data

B. Blair Braden, Ph.D. – Arizona State University

Dr. B. Blair Braden presented results from a longitudinal cognitive and brain aging study on a cohort of middle-aged and older adults with ASD. Because the first children who were described as autistic are just now becoming elderly adults, very little is known about aging with ASD. The world population is

aging, and estimates suggest that in 2050 there will be over 1 million elderly adults with ASD.¹ However, older people remain underrepresented in ASD literature.² In addition, there is mounting evidence that individuals with ASD have an increased risk of early-onset dementias.³ There is a pressing need for research about aging with ASD to adequately meet the unique needs of this community.

To address this gap in knowledge, Dr. Braden's group started their own cognitive and brain aging study in ASD. In their first cohort – men ages 40-70 with ASD but no intellectual disability (ID) plus age-matched neurotypical (NT) adults – they measured cognition, brain structures, brain activity, and connectivity, as well as ASD-related behaviors. In a cross-sectional comparison of baseline differences between the ASD and NT groups, the ASD group had significantly worse executive function, reduced fractional anisotropy in bilateral regions around fimbria/fornix (suggesting demyelination), and smaller hippocampi.⁴ These results suggest individuals with ASD may have a weakened hippocampal system at middle age. Future research will characterize the trajectory of this region across the lifespan.

Previous cross-sectional data had conflicting conclusions about whether ASD increased vulnerability for or protected against cognitive aging. Concerned about possible cohort effects in cross-sectional data, Dr. Braden's group collected a comprehensive longitudinal dataset to address these discrepancies. In this dataset, participants were seen every two years for cognitive and neuroimaging assessments. Cross-sectional analysis of the data showed that ASD was protective against age-related decline in short-term memory. However, a longitudinal analysis of the same data shows that middle-aged adults with ASD were more likely to show clinically meaningful declines in short-term memory compared to the NT group. In a mixed cross-sectional and longitudinal analysis, the ASD group showed steeper declines in long-term memory and hippocampal shrinkage compared to the NT group. These novel longitudinal findings establish short-term verbal memory, long-term visual memory, and hippocampal volume as vulnerable to aging in ASD and suggest that cohort effects likely drive discrepant literature results.

Dr. Braden and her colleagues next sought to determine whether any brain structure measurements could be used as biomarkers to predict which adults with ASD were most vulnerable to accelerated cognitive decline and potential neurodegeneration. They found that extracellular free water measurements of most regions in the hippocampus and fornix at baseline could predict declines in long-term visual memory over time. Combining several measures in a principal component analysis (PCA), the research team produced a PCA component with strong predictive power for future long-term memory changes.

Finally, Dr. Braden discussed genetic contributions to accelerated aging in ASD. In their dataset, Dr. Braden's group found that individuals with ASD are more likely to carry the *APOE4* allele than controls and that the allele has a stronger effect on hippocampal volume over time in ASD compared to NT individuals. These data suggest that AD genetic risk may be elevated in ASD and may be an important factor in moderating cognitive aging outcomes.

Prevalence and Pathologic Underpinnings of Autism-Like Behaviors in a Community-Based Dementia Research Cohort

Elizabeth Rhodus, Ph.D., OTR/L – University of Kentucky

As an occupational therapist, Dr. Elizabeth Rhodus noticed similar behaviors in individuals with neurodevelopmental and neurodegenerative disorders. Although the literature describes some

anatomical and behavioral overlap between these groups, the possibility of late-life emergence of ASD has been poorly explored. Dr. Rhodus sought to 1) determine if ASD-like symptoms were quantifiably present in older adults with ADRD, 2) identify the relationship between ASD-like behaviors and psychiatric symptoms in dementia, and 3) examine the neuropathology and neuroanatomy of people with ADRD and ASD-like behaviors.

To test whether ASD-like behaviors are present in ADRD, Dr. Rhodus and colleagues surveyed participants of people with mild cognitive impairment (MCI) or ADRD enrolled in the University of Kentucky ADRC. ASD-like symptoms were measured with the Gilliam Autism Rating Scale, Second Edition (GARS-2), which has been validated for ages 3–22. Dr. Rhodus noted that the GARS-2 was used because there is currently no validated observation-based tool to assess ASD-like behaviors in an older population. ASD-like behaviors were present in a variety of dementia types including AD, vascular dementia, Lewy body dementia, frontotemporal dementia (FTD), and MCI. In addition, ASD-like behaviors in dementia corresponded with a different clinical trajectory. Compared to adults with dementia but no ASD-like behaviors, those with ASD-like behaviors were significantly younger at the age of cognitive impairment onset and had more advanced cognitive impairment, as measured by the CDR Sum of Boxes score.⁵

To determine the association between ASD-like symptoms and the classical behavioral and psychological symptoms in dementia, the participants also completed the neuropsychiatric inventory (NPI) survey. Analyses of the responses found that ASD-like behaviors and dementia-related neuropsychiatric behaviors significantly overlap. In a hierarchical linear regression, the NPI scores predicted 10% of the variance in cognitive impairment. Incorporating the GARS-2 scores (ASD-like behaviors) explained 6% additional variance in dementia severity.⁶ These data suggest that ASD-like behaviors mediate differences in cognitive impairment levels independent of neuropsychiatric symptoms.

Individuals with ADRD and ASD-like symptoms had more severe dementia-related pathology in a postmortem analysis. Those with ASD-like behaviors and ADRD had increased neurofibrillary tangle density and tau burden in the frontal and temporal cortices and increased amyloid burden in the occipital and parietal cortices compared to individuals with ADRD and no ASD-like behaviors. These findings suggest that antemortem ASD-like behaviors are associated with postmortem neuropathology.

National Data Approaches to Examining the Connection Between Alzheimer's Disease and Autism Spectrum Disorder

Lindsay Shea, M.S., DrPH., and Giacomo Vivanti, Ph.D. – Drexel University

Dr. Lindsay Shea and Dr. Giacomo Vivanti described their research, which uses population-level data exploring the relationship between ASD and dementia. Their research explores the prevalence and co-occurrence between ASD and dementia as well as implications for the service system, such as expenditures. In a study examining Medicaid beneficiaries (30-64 years of age), they found that the incidence of early-onset dementia in those who had either ASD only, or ASD with ID, was double, compared to the general population⁷. This model adjusted for age, psychiatric disorders, cardiovascular risk factors, and other possible confounding factors.

Next, Dr. Shea and her research group used a larger dataset that included both Medicaid and Medicare systems. Medicaid is a joint federal-state funded insurance option that is available across the lifespan and delivers a wide array of services. Medicare is federally funded by the Centers for Medicare and Medicaid Services. Most adults in the United States enroll in Medicare when they become eligible at age 65; however, individuals who meet specific criteria can be eligible early. Combined, these services generate data from over 100 million Americans. Linking data across these two systems allows researchers to observe many people over time, providing a valuable opportunity to understand the experiences and needs of different populations to inform clinical practice and program planning.

In younger populations, claims data has a high positive predictive value for ASD diagnosis by community clinicians. The field could benefit tremendously from a similar effort to compare claims data and community-based diagnoses in older adults. Medicaid confers the additional benefit of covering an exceptionally diverse group of people. The data from this system could be used to help the fieldwork toward addressing health inequities. This is especially relevant to ASD research, which has struggled to engage diverse groups.

Dr. Shea and Dr. Vivanti presented data describing service usage from 2014 to 2016, examining individuals with ASD, ID, or both who were 40 and older at the end of 2016. For the Medicaid system, the cohort included 800,606 individuals and for the Medicare system, it included 412,223 individuals. Their analysis found that a higher proportion of people with ASD live in urban areas, which has distinct implications for access to health care. A majority of individuals in all three groups were dual-eligible for Medicaid and Medicare, suggesting that resource allocation and processes for enrollment across these two systems are critical. These results indicate a need for both Medicaid and Medicare to ensure that there are accessible tools for dementia detection, services, and support in these communities.

An analysis of age differences across all three diagnosis groups over time revealed that in the Medicaid system, most individuals present with dementia diagnosis before age 65, when they could also be eligible for Medicare. In addition, the group of individuals with ASD and ID largely enrolls in Medicare before age 65, likely to gain access to additional services and clinical care needs for these new diagnoses. These data indicate that these services may be needed younger for individuals with ASD, ID, or both. Future analyses will stratify these age groups by an array of characteristics to understand whether there may be opportunities for age-specific detection strategies and the need for services packages across age groups.

Individuals enrolled in Medicaid with ADRD had a significantly higher risk of mortality if they had ASD, ID, or both. These findings point toward the need to understand mortality rates and causes of death to inform opportunities for clinical care later in life as well as research to identify protective factors. The data from Medicare indicate lower rates of mortality in individuals with ADRD, ASD, and ID compared to those with ADRD and ID or ADRD and ASD. These findings suggest there may be clinical or service access differences in need of further examination. A series of logistic regression analyses on all individuals in the dataset with ASD found that increased age, co-occurring ID, female sex, and urbanicity significantly increased the likelihood of a dementia diagnosis.

These datasets can also be used to compare expenditures across diagnostic groups. Analyses to this end found that the highest mean costs are for individuals with ASD, ID, and dementia. In addition, inpatient costs drive spending across all groups, which may suggest that community-based care methods could both reduce costs and improve quality of life. Studying effective care strategies will be essential to understanding these cost differences and to thinking about how federal or state resources may be allocated to support care for these groups.

Overall, these data suggest that there is a higher prevalence of dementia in individuals with ASD. The risk factors associated with increased dementia prevalence in the general population are similarly associated with increased dementia risk in ASD. Increased dementia in ASD may result from genetic differences or lifestyle-related or service need factors, including barriers to accessing intellectual, educational, and social opportunities. Although the mechanism of this relationship still needs to be addressed, these findings emphasize the importance of developing guidelines and tools for the diagnosis of dementia in ASD and addressing the service needs in the ASD population with dementia.

Moderated Discussion

Susan Bookheimer, Ph.D. – University of California, Los Angeles

Q: Dr. Susan Bookheimer asked Dr. Braden to begin by talking about ascertainment bias related to older individuals who may have ASD but were never diagnosed as such.

A: Dr. Braden noted that it is a challenge to identify ASD in middle-aged and older adults. Although their approach of ensuring that those diagnosed with ASD meet certain criteria on the Autism Diagnostic Observation Schedule score seems rigorous, that test was not designed for older adults. Current younger adults have had a lot of services, while older adults have not. Older individuals with ASD have gained compensatory skills that may mask their symptoms of both ASD and cognitive decline. As such, the field needs improved diagnostic tools to identify ASD in older adults and to identify dementia in older adults with ASD.

Q: Dr. Bookheimer asked the speakers to address the skill by which geriatricians can diagnose ASD.

A: Dr. Braden noted that adding a self-report of ASD symptoms to cognitive aging studies may be a strong first step to engage geriatricians.

A: Dr. Vivanti added that to diagnose dementia in someone who has ASD, it is critical to have multiple sources of clinical information. For example, it is essential to have an idea of the independent skills the individual had before the possibility of dementia-related decline. Clinicians should involve family members to report any changes in functioning and conduct assessments that take into account the familiarity of the setting.

Q: Dr. Bookheimer mentioned that there are several large-scale efforts to collect longitudinal data on cognitive aging, such as the Alzheimer's Disease Neuroimaging Initiative (ADNI) and the Human Connectome Project in Aging (HCP-A), and that an ASD self-report question would be a simple addition to these studies. Dr. Bookheimer asked Dr. Shea whether such additions could be made in Medicare and Medicaid systems data collection.

A: Dr. Shea reiterated that child-based data to validate claims in ASD have yielded high predictive value. This should be repeated in older adults once the field establishes how to diagnose ASD in this population.

Q: A workshop attendee asked about social vulnerability. It is well-established that for NT older adults, maintaining good social contacts protects against AD. Given the known social deficits in ASD, the attendee wondered whether lack of social contact in people with ASD may be putting them at additional risk of dementia.

A: Dr. Vivanti responded that there is a growing body of literature on early interventions for ASD (< 5 years) and that most of these focus on social engagement and social learning. However, there are no interventions for older individuals with ASD.

A: Dr. Rhodus agreed that it is interesting to consider a lifespan approach. In addition to designing interventions for older adults with ASD, researchers should consider the group of older adults who have ASD but were never diagnosed, who may need additional support.

Q: Dr. Bookheimer asked the speakers whether it is possible that ASD symptoms could be revealed by the onset of dementia and to speak to the idea of biological vulnerability.

A: Dr. Braden described work from a doctoral student in her lab who designed a way to get around sex differences in camouflaging behavior. They have some evidence to suggest that the ability to camouflage behavior was decreased in the older cohort and as a function of longitudinal time.

Q: Workshop attendees asked whether ADNI or HCP-A collect data on ASD features.

A: Dr. Bookheimer responded that although they do not currently collect this data, they should begin doing so.

Q: Dr. Bookheimer asked the speakers how to distinguish ID from dementia in older adults with ASD.

A: Dr. Rhodus responded by emphasizing the need to incorporate historical behaviors into diagnostics to decide whether to pursue dementia biomarkers and other tests.

A: Dr. Vivanti noted that it is important for clinicians to be aware that a person with ASD facing age-related changes in behavior might be experiencing a cognitive decline – clinicians cannot always assume cognitive or behavioral symptoms are a function of the ASD.

Q: A workshop attendee asked whether there are any peripheral diseases that increase the risk of dementia in people with ASD.

A: Dr. Shea responded that this information can be discerned in Medicaid and Medicare data. Her team is working out how to rigorously perform these analyses.

Q: A workshop attendee asked whether there has been any stratification of ASD with epilepsy and the likelihood of developing dementia.

A: Dr. Vivanti responded that, in their data, those with ASD plus seizures were at even higher risk of dementia and that epilepsy alone also increased the risk of dementia.

A: Dr. Bookheimer added that there is a lot of epilepsy in AD that often goes undiagnosed.

Q: Dr. Bookheimer asked all of the speakers from Session 1 whether the severity of ASD symptoms correlates with the risk of developing dementia, as well as how the field can better understand which aspects of the autism spectrum are most associated with AD. She believes that such studies may help us better understand the pathological underpinnings of AD, as ASD pathology is quite diverse.

A: Dr. Braden responded that they have not examined symptom severity as a moderator of cognitive decline. However, they do not observe an effect of symptom severity on intervention response in several of their studies.

A: Dr. Vivanti responded that insurance claims data do not contain information on the severity of ASD symptoms. However, researchers can discern the intersection of ASD with comorbidities, which is important because most existing data does not consider individuals with ASD and other diagnoses as well. As these individuals are at even greater risk, research for appropriate care is warranted.

Q: A workshop attendee asked whether anyone is collecting genetic data.

A: Dr. Shea responded that there are some diagnosis codes and procedure codes that are indicative of the presence of certain genetic conditions or procedures to detect them. They can also determine who received genetic testing or genetic counseling, although they cannot observe genes directly.

Q: A workshop attendee asked Dr. Braden whether other groups have similar data showing greater incidence of the *APOE4* allele in the ASD population and whether there are other kinds of biomarkers of AD pathology, such as serum biomarkers in the ASD population.

A: Dr. Braden commented that in addition to their sample, there is an older study (10-15 years old) that showed the same results. There is a paper that shows methylation differences of the *APOE4* gene associated with ASD and some literature evidence that serum levels of the *APOE4* protein are different.

Q: Dr. Bookheimer asked how clinicians can confidently diagnose dementia type in individuals with ASD.

A: Dr. Braden responded that there are several papers describing elevated Parkinsonian pathology in individuals with ASD and dementia. In addition, the ASD brain phenocopies the FTD brain in many ways, but there are no published findings on how this similarity plays out in terms of FTD incidence in the ASD population.

A: Dr. Rhodus added that sensory-based behavioral interventions from ASD adolescents could be applied to older adults with FTD.

Key Opportunities

- Design longitudinal studies of aging with ASD that measure cognition, behavior, and AD biomarkers (imaging, CSF, blood) across the lifespan, in addition to AD-related genetic and environmental risk factors.
 - Identify vulnerability and protective factors from these research cohorts.

- Explore what aspects of ASD-like behavior are most associated with dementia risk.
- Develop methods to increase retainment of individuals with ASD in research as they age.
- Determine whether medical claims data has high positive predictive value for community-based ASD diagnoses in older adults.
- Develop improved diagnostic tools to identify ASD behaviors in older adults (with and without dementia) as well as to identify dementia in older adults with ASD.

Session 2: Amyloid/Tau

Fragile X and APP: A Decade in Review, a Vision for the Future

Cara Westmark, Ph.D. – University of Wisconsin

Dr. Cara Westmark presented background information and key findings on the relationship between Fragile X mental retardation protein (FMRP) and amyloid precursor protein (APP). FMRP is an RNA-binding protein involved in protein synthesis, excitatory-inhibitory balance, and synapse development. The absence of FMRP causes Fragile X syndrome (FXS), the leading known genetic cause of autism⁸. APP is an integral membrane protein concentrated at synapses that functions as a cell surface receptor and can give rise to pathogenic A β . Dr. Westmark also introduced the metabotropic glutamate receptor 5 (mGluR5), as a key mediator of the interaction between APP and FMRP. mGluR5 is an excitatory G protein-coupled receptor expressed on post-synaptic membranes. mGluR5 signals upstream of FMRP and has been the main therapeutic target for FXS for almost two decades. These proteins form a signaling nexus that is dysregulated in both FXS and AD.

In 2007, Dr. Westmark and her colleagues discovered that the APP mRNA is regulated by mGluR5 and FMRP. FMRP binds a regulatory element in the coding region of APP mRNA and inhibits translation when bound. The translation of the APP mRNA is regulated by additional factors as well, including hnRNP C, nucleolin, YB1, microRNAs, and cellular structures such as P-bodies. How the regulatory elements and RNA binding proteins interact to modulate APP mRNA localization and protein synthesis remains to be determined. FMRP binds APP mRNA and inhibits its translation under basal conditions, but when mGluR5 signaling is activated this binding is lost, leading to APP synthesis.

After APP is positioned across the cell membrane, it is processed by secretases through one of two pathways. In amyloidogenic processing, β -secretase cleaves extracellularly to generate the N-terminus of A β , and γ -secretase cleaves intracellularly to form the C-terminus. In non-amyloidogenic processing, α -secretase cleaves extracellularly instead of β -secretase. Because α -secretase cleaves within the A β sequence, the formation of the amyloidogenic peptide is prevented. These pathways generate numerous protein metabolites of interest. In amyloidogenic processing, A β and soluble APP β are released into the extracellular space. In non-amyloidogenic processing, soluble APP α is released into the extracellular space. In both pathways, C-terminal fragments generated by γ -secretase cleavage are released into the cytoplasm. While A β is the best studied of the APP metabolites, studying the other fragments could provide critical insights into disease pathology.

Dysregulated APP metabolite levels are present in both FXS and AD. Dr. Westmark's laboratory observed that the *Fmr1* knockout mouse, the most widely used model of FXS, has increased levels of APP,

supporting the current model of FMRP inhibiting APP translation. Genetically restoring APP to wild-type levels in the *Fmr1* knockout mouse rescues ASD behaviors, the ratio of mature to immature dendritic spines, mGluR long-term depression (LTD), neocortical hyperexcitability, epileptiform discharges, and seizures. In addition, the overexpression of APP in the *Fmr1* knockout mouse increases seizures. These data suggest that APP directly contributes to FXS pathology.

Fmr1 knockout mice also have increased levels of soluble APP α starting three weeks after birth – a critical period for synapse stabilization. In addition, they found upregulation of the α secretase ADAM10. Modulating ADAM10 can restore translational control, synaptic morphology, and behavior in the *Fmr1* knockout mice. These data suggest a model in which the *Fmr1* knockout mouse has increased non-amyloidogenic processing of APP, leading to changes in synapse formation in development and corresponding changes in behavior. Work quantitating APP metabolites in ASD patient plasma has found increased soluble APP α , decreased soluble APP β , and decreased A β . Studies of FXS have found elevated soluble APP, as is present in ASD. However, there is also increased soluble APP β and A β , suggesting some differences between ASD and FXS. Future studies should examine how APP metabolite and secretase levels in these conditions change over the lifespan.

mGluR signaling sits upstream of FMRP and APP and triggers APP production when active. The mGluR theory posits that in FXS, lack of FMRP causes exaggerated responses to mGluR activation, which directly cause aberrant synapse formation during development and corresponding symptoms. mGluR antagonists have been the focus of drug repurposing efforts for FXS. Although there is strong evidence of the efficacy of mGluR5 antagonists for FXS in multiple preclinical models, results from clinical trials have been disappointing. The reasons for this lack of translatability should be thoroughly explored. Dr. Westmark noted that diet can significantly impact seizure frequency, suggesting that this and other exposome factors may impact FXS and AD outcomes. There is also evidence that mGluR5 inhibition can rescue disease phenotypes, such as seizures, cognitive deficits, and hyperexcitability, in AD mouse models.

In both AD and FXS models, the over- or underexpression of APP increases seizure propensity, suggesting that APP may function as a rheostat to regulate synaptic balance in the brain. Dr. Westmark proposed a model in which, under wild-type conditions, FMRP binding is regulated by phosphorylation, and APP acts as a variable resistor to maintain an appropriate excitation-inhibition (E/I) balance. In the context of the deficient FMRP and excess APP, the circuit is constitutively on, overloading the system and causing hyperexcitability. Dr. Westmark concluded by emphasizing that ASD and AD are both associated with aberrant APP processing. Addressing gaps in knowledge regarding APP biology will inform both disorders. APP metabolites and secretases, as well as FMRP and related signaling proteins, should be investigated as potential biomarkers and therapeutic targets for both FXS and AD.

The Function of Fragile X Protein FMRP in A β Pathology

Nien-Pei Tsai, Ph.D. – University of Illinois, Urbana-Champaign

Dr. Nien-Pei Tsai discussed a mechanism in which A β triggers translation suppression through a pathway involving FMRP. In AD, A β causes cellular stress that leads to a translational repression response.⁹ Because FMRP is a well-known translational repressor,¹⁰ Dr. Tsai sought to determine whether it is involved in A β -driven translational repression. Treating primary neuronal cultures with A β peptide

increased FMRP protein expression compared to treatment with a scrambled control. In addition, the APP/PS1 AD mouse model has elevated FMRP levels, although Dr. Tsai noted that this result has varied between labs.

In a puromycin assay to visualize newly synthesized proteins, A β treatment elicited global translational repression. This repression was lost in the *Fmr1* knockout mouse, suggesting that FMRP is required for A β -induced translational suppression. Next, Dr. Tsai and colleagues sought to identify the translational factors through which FMRP represses translation. A β treatment increased phosphorylation of eIF2 α and eEF2, two factors that inhibit translation. This effect was lost in *Fmr1* knockout mice, suggesting that elevated FMRP caused by A β treatment is contributing to the phosphorylation of these factors.

Because FMRP represses translation, Dr. Tsai's group tested the hypothesis that FMRP inhibits the translation of phosphatases, causing increased phosphorylation of eIF2 α and eEF2. In support of this model, they found that A β treatment downregulates PP1 and PP2A phosphatase expression and this effect is lost in *Fmr1* knockout neurons. Next, they used specific inhibitors to determine which phosphatase acts on each translational factor. Inhibition of PP2A activity caused increased phosphorylation of eEF2, and this increase was dependent on FMRP. Unfortunately, the PP1 inhibitor had off-target effects and could not be used in further studies.

The remaining experiments focused on discerning how the A β -FMRP-PP2A-eEF2 pathway contributes to neurodegeneration. A β treatment of wild-type neuronal cultures caused reduced spike frequency and synchronicity, established features of neurodegeneration. In *Fmr1* knockout cultures, A β triggers the reduction of spike rate but does not decrease synchronicity. This suggests that A β -induced hypo-synchrony is dependent on FMRP. Inhibiting PP2A rescues the A β -induced decrease in synchrony. Overall, these data support a model in which A β -induced reduction of spike rate occurs independently of FMRP, but A β -induced reduction in synchronicity is dependent on FMRP and PP2A. In addition, these data suggest that FMRP may play a detrimental role in AD, bolstering A β -driven desynchronization.

As another measure of neurodegeneration, Dr. Tsai and colleagues counted synapses in cultured neurons labeled with pre- and postsynaptic markers. In this assay, synapse number is measured by counting puncta with colocalization of these markers. In wild-type neurons, A β -treatment reduced synapse number. However, this effect was lost in *Fmr1* knockout neurons. Treatment with PP2A inhibitor failed to rescue A β -induced decrease in synapse number. These data suggest that A β -induced reduction in synapse number is dependent on FMRP but independent of PP2A.

The data presented by Dr. Tsai suggest a model in which A β induces FMRP-dependent translational suppression via PP2A and eEF2, which reduces neural network synchronicity. Future studies will examine how A β promotes FMRP expression and will establish the relevance of this pathway in vivo.

The Molecular Link Between Tuberous Sclerosis Complex, Alzheimer's Disease, and Other Tauopathies

Aimee Kao, M.D., Ph.D. – University of California, San Francisco

Dr. Aimee Kao presented her work connecting *Tsc1*, a gene mutated in genetic forms of ASD, with the accumulation of tau, a protein associated with neurodegeneration. Dr. Kao's group became interested in this connection when a 55-year-old man came into their clinic with symptoms of behavioral variant FTD,

including emotional flattening, social withdrawal, loss of empathy, confusion, and forgetfulness.¹¹ The patient had family members with cognitive and seizure disorders, suggesting a possible genetic cause. Whole exome sequencing of the patient revealed a novel, loss-of-function variant of the *Tsc1* gene.

Mutations in *TSC1* or *TSC2* are associated with tuberous sclerosis complex (TSC), a genetic disorder that causes numerous benign tumors, epilepsy, ID, autism, and other neuropsychiatric features. TSC1 acts in a complex with TSC2 to mediate mammalian target of rapamycin (mTOR) activity. The mTOR pathway regulates cell metabolism by promoting growth and inhibiting autophagy. TSC1/TSC2 inhibits mTOR activity and loss-of-function mutations in *Tsc1* cause increased mTOR activity. Before Dr. Kao's patient, mutations in *TSC1* had not been previously associated with neurodegenerative disease.

A review of the literature on TSC found that the disorder is categorized as an infantile tauopathy, one of several neurodevelopmental disorders with p-tau accumulation. In addition, recent literature has described a syndrome associated with TSC called TSC-associated neuropsychiatric disorder (TAND). Many of the symptoms of TAND overlap with symptoms of behavioral variant FTD. These findings emphasized that *TSC1* may be a gene that links neurodevelopmental and neurodegenerative disorders.

Dr. Kao's group gathered a clinical cohort of adults with mild TSC and compared them to adults with FTD and healthy controls. The TSC and FTD groups had similar scores of TAND severity, executive function, and language. Interestingly, the TSC group exhibited elevated cerebrospinal fluid p-tau levels and positive tau PET scans.¹² The Kao group also found that mutations in TSC1 are overrepresented in tauopathies. In one tauopathy, progressive supranuclear palsy, they found mutations in the PEST domain of TSC1, which is known to control protein degradation. Interestingly, these variants were also found in individuals with early-onset AD. Cell lines expressing these variants of TSC1 revealed that they have shortened half-lives compared to wild-type TSC1.¹³ Overall, these data suggested that lower levels of TSC1 may predispose to tauopathy. Indeed, Dr. Kao found that *Tsc1* loss-of-function mutations cause tau accumulation in cell lines, induced pluripotent stem cell (iPSC)-derived neurons, and mice.

To begin to uncover the mechanism connecting low TSC1 to tau accumulation, Dr. Kao's group surveyed the molecular characteristics of the tau that accumulated in their models. They found increased acetylated tau (Ac-tau) in *Tsc1* heterozygous iPSC-derived neurons as well as brain tissue from a cohort of adults with *TSC1* heterozygous mutations. In addition, *Tsc1* heterozygous cells had increased p300 acetyltransferase activity and decreased SIRT1 deacetylase activity. Pharmacological inhibition of acetyltransferase activity or enhancement of deacetylase activity rescues tau levels in *Tsc1* heterozygous cells.¹³ These data suggest a model in which low levels of TSC1 cause increased acetylation and decreased deacetylation, causing accumulation of Ac-tau.

To discern why increased Ac-tau leads to tau accumulation, Dr. Kao's group performed assays measuring the presence of Ac-tau in lysosomes and chaperone-mediated autophagy. Data reveal that Ac-tau is excluded from chaperone-mediated autophagy and lysosomes.¹³ Overall, these data suggest a model in which low levels of TSC1 cause hyperactive mTOR, which leads to increased p300 acetyltransferase activity and decreased SIRT1 deacetylase activity. These enzymatic changes cause tau to become hyperacetylated, excluding it from autophagy mechanisms, leading to accumulation and pathology. *Tsc1* loss-of-function mutations constitute a wild-type tauopathy, as the tau that accumulates is normal,

linking ASD and neurodegeneration. The mechanism of tau accumulation in this condition provides novel potential biomarkers and therapeutic targets.

Using Tau Reduction to Counteract Excitation/Inhibition Imbalance – Potential Benefits in Alzheimer's Disease and Autism Spectrum Disorders

Lennart Mucke, M.D. – Gladstone Institutes and University of California, San Francisco

Dr. Lennart Mucke discussed how, in both neurodevelopmental and neurodegenerative diseases, tau appears to enable E/I imbalance, making it an attractive biomarker and therapeutic target. Tau is a complex protein, as it undergoes alternative splicing that produces six isoforms and undergoes extensive post-translation modifications. Many uncertainties remain regarding tau function, including its physiological roles, the purpose of its post-translational modifications, cell-specific effects, its most pathogenic forms, and the impact of its spread. In neurodevelopmental diseases, a genetic trigger can cause network dysfunction, which acts via the PI3K pathway and mTOR to cause behavioral and cognitive deficits. Tau inhibits PTEN, the phosphatase that counteracts PI3K activity, enabling further mTOR activity and corresponding pathologies. In AD, tau is present as neurofibrillary tangles in neurons and in soluble forms both inside and outside neurons.

Most tau research discusses a pathway in which pathogenic triggers cause tau to gain a function (aggregation, spread, etc.) that leads to neuronal dysfunction and degeneration. Contrastingly, Dr. Mucke and colleagues investigated the possibility that normal tau could enable pathogenic signaling pathways to contribute to disease. The addition of a human APP transgene causes epileptiform activity in mice. However, knocking out one or both copies of wild-type tau eliminates this effect, suggesting that tau is required for APP-triggered epileptiform activity.¹⁴ Interestingly, subclinical epileptic activity occurs in > 40% of patients with sporadic AD and those with epileptic activity experience faster cognitive decline over time.¹⁵ These data suggest that tau may enable E/I imbalance and network dysfunction in AD and that normal network function may be restored via tau reduction.

Tau may also play an enabling role in ASD. Dravet syndrome is a severe form of epilepsy caused by mutations in the gene encoding sodium channel NaV1.1 and associated with ASD symptoms. A mouse model of Dravet syndrome has increased sudden unexpected death, stereotypic movements, and social deficits. In this mouse model, genetically reducing tau rescues these deficits. Tau reduction could also prevent excessive stereotypic movements in a mouse model of focal cortical dysplasia, another ASD.¹⁶ Dr. Mucke emphasized that tau could be a common aspect or mechanism of ASD, AD, and related disorders, making it an attractive therapeutic target. His lab tested the therapeutic potential of an antisense oligonucleotide that targets tau mRNA. Treatment of Dravet syndrome model mice with this antisense oligonucleotide at postnatal day 10 could restore premature mortality, epileptiform activity, and stereotypic behaviors to wild-type levels. Physiology data suggests that tau reduction prevents hypersynchrony that results from overexcitation. In addition, cell-specific knockouts of tau in excitatory or inhibitory neurons revealed that the beneficial effects of tau ablation take place in excitatory neurons.

Tau may also mediate inflammation-related changes in gene expression. Adding a human APP transgene causes epileptiform activity and increased inflammatory gene expression in old (> 26 month) mice. Genetic ablation of tau prevents both effects. Interestingly, the gene expression changes prevented by tau ablation and those prevented by antiepileptic drugs significantly overlap and include a set of complement proteins.¹⁷ These data suggest that network dysfunction and immune dysfunction engage in a vicious cycle that causes synaptic dysfunction and loss, and that the cycle may be broken with drugs that prevent seizure or reduce tau.

Tauopathy in the Young Autistic Brain: Novel Biomarker and Therapeutic Target: The Case of the ADNP Syndrome and NAP (Davunetide)

Illana Gozes, Ph.D. – Tel Aviv University

Dr. Illana Gozes described the function of activity-dependent neuroprotective protein (ADNP) and its potential as a therapeutic target. ADNP interacts with the SWI/SNF chromatin remodeling complex and acts as a transcription factor in the nucleus, while also binding microtubules in the cytoplasm. Loss-of-function mutations in ADNP are associated with a form of ASD called ADNP syndrome, characterized by delayed development, motor dysfunction, ID, speech delays, sleep problems, and ASD-like behaviors.¹⁸ In addition to ADNP syndrome, the *ADNP* gene is also mutated in certain regions of the postmortem AD brain. The accumulation of mutations in each region is associated with increased tauopathy. RNA-seq analyses suggest somatic mutations that accumulate in AD are enriched for genes associated with ASD and especially long genes.¹⁹

Dr. Gozes and her colleagues found that a small peptide within ADNP, called NAP, was neuroprotective, and tested whether NAP (also called davunetide) could be used therapeutically. Data show that NAP can effectively treat deficiencies in the *Adnp* heterozygous mouse model of ASD, including disrupted dendritic spine formation, reduced glutamatergic transmission in the hippocampus, developmental delays, speech delays, ASD-like behaviors, and cognitive dysfunction.²⁰ Another mouse model, harboring the most common mutation found in ADNP syndrome, has disrupted visual-evoked potentials; altered gene expression; and caused defective syntax, developmental delays, gait deficits, disrupted microbiome, and Alzheimer's-like tauopathy. All these deficiencies can be rescued with NAP treatment.²¹

Evidence suggests that NAP may exert its therapeutic effects by stabilizing microtubule dynamics. The microtubule end-binding motif of ADNP is present in the NAP peptide. Several assays show that NAP enhances microtubule dynamics and subsequent spine formation in cells with either wild-type or mutated ADNP. In addition to ADNP, microtubule end-binding protein also interacts with tau, enhancing its binding to the microtubule shaft. Data support a model in which NAP enhances the interaction between tau and the microtubule shaft, preventing hyperphosphorylation and tauopathy.²² Immunohistochemistry on postmortem brain tissue from children with ADNP syndrome shows tauopathy in many brain regions.²³ NAP treatment reduces tauopathy in cell cultures with mutated *Adnp*.

Preclinical research and clinical trials support the use of ADNP/NAP as a biomarker and as therapeutic. ADNP levels are significantly reduced in serum from individuals with AD, and concentration of ADNP

correlates with performance on IQ tests. In a Phase 2 clinical trial for amnesic MCI, NAP treatment significantly improved performance of a short-term memory task.²⁴

Moderated Discussion

Lennart Mucke, M.D. – Gladstone Institutes and University of California, San Francisco

Q: A workshop attendee asked Dr. Westmark if her laboratory has explored the relationship between APPs/A β and premutations in *FMR1*, which appear to be more variably associated with differences in FMRP relative to full mutations, but predictive of severe neurodegeneration.

A: Dr. Westmark responded that her lab has not examined premutation models yet, but it is a topic they would like to pursue.

Q: Dr. Kao was asked whether there is a relationship between *TSC2* mutations and tauopathies, as is seen with *TSC1*.

A: Dr. Kao replied that in their cell models, TSC1 co-immunoprecipitates with tau while TSC2 does not. Similarly, tau does not accumulate but does have increased phosphorylation in a *Tsc2* knockdown cell model. In postmortem neuropathology, *TSC2* mutation carriers had increased acetylated tau and phosphorylated tau. Dr. Kao hypothesizes that TSC1 and TSC2 can interact in different combinations with each other, tau, and chaperone proteins, which complicates results interpretation.

Q: A workshop attendee asked Dr. Mucke to what extent tau was reduced with antisense oligonucleotide treatment in the Dravet syndrome model.

A: Dr. Mucke responded that they observed a 70-80% reduction. His group is particularly interested in finding when the treatment might be most effective. Treatment at postnatal day 20 decreases mortality but does not confer the same behavioral benefits, and, as such, may be too late. Treatments may need to occur before the brain is aberrantly wired in development.

Q: Dr. Gozes asked Dr. Mucke whether he believes that all tau mutations cause the gain of a toxic function.

A: Dr. Mucke replied that he does consider all detrimental tau mutations to be the gain of function. Most findings showing detrimental effects of tau loss-of-function mutations have been refuted. Namely, genetic knockout of tau does not lead to destabilization of microtubules or significant other effects in the brain. He views tau-driven destabilization of microtubules as a gain-of-function effect.

A: Dr. Gozes added that similar reasoning may hold with APP. Haploinsufficiency of APP is dangerous, but so is the gain of function. She suggested that because tau has many alternative splice isoforms, the concepts of gain and loss of function may be more nuanced. She stressed that that gain or loss of function of tau may elicit different effects in different developmental periods.

A: Dr. Mucke responded that his group has knocked down tau at many different stages of development and still observe no detrimental effects. He noted that removal of APP also has

minimal effects. This suggests that both tau and APP may have mechanisms to compensate for their loss.

A: Dr. Gozes noted that both APP and tau levels increase with trauma, but it is unknown whether this is a pathological or a protective mechanism. Dr. Mucke stressed that the physiological roles of tau need to be investigated more thoroughly.

Q: Dr. Thomas Jongens asked Dr. Kao whether older TSC patients still have elevated mTOR activity.

A: Dr. Kao responded that they have not yet examined this. Her group believes that some effects of aging are mediated through the lysosome, which becomes less efficient and struggles to maintain an acidic pH with age, predisposing proteins like tau to accumulate. In their neuropathology study, tau accumulation only occurred in the older individuals, not those in their 20s and 30s, suggesting a strong effect of aging. Adding to the previous discussion of whether detrimental tau mutations are all gains of function, Dr. Kao noted that many point mutations of tau impair its lysosomal proteolysis, causing it to have a longer half-life, which would be considered a gain of function.

Q: A workshop attendee asked Dr. Kao whether she has examined cell cycle reactivation, a hallmark of AD, in conditions or models with decreased TSC1 expression.

A: Dr. Kao responded that they have not explored this, as they are only just starting to examine effects of *Tsc1* deficiency on neurodegenerative processes.

Q: A workshop attendee asked where high-functioning, cognitively gifted children with ASD fit into the relationship between ASD and AD.

A: Dr. Braden responded that even children with ASD and high IQs may have a difficult time with other aspects of cognition, such as executive function. It may be an oversimplification to say that there is a cohort of children with ASD who perform above average on all aspects of cognitive function.

A: Dr. Mucke added that in some neurodegenerative diseases, remarkable new talents can emerge and that this may connect with the idea of savantism in ASD.

Q: Dr. Jongens asked Dr. Gozes whether she has examined the impact of NAP treatment on vesicle or mitochondrial transport in the ADNP model.

A: Dr. Gozes responded that they have only examined axonal transport and that it is inhibited in heterozygous ADNP mice and can be rescued by NAP treatment.

Dr. Mucke summarized that this session identified signaling and neurotransmission pathways involving tau or A β that are impacted by ASD and AD. In addition, the session explored possible therapeutic entry points that might be targeted to advantage in both conditions.

Key Opportunities

- Investigate the following as potential biomarkers and therapeutic targets in neurodevelopmental and neurodegenerative disease:

- APP metabolites and APP-processing secretases
- Acetylated tau
- FMRP and related signaling proteins
- Wild-type tau
- Explore physiological and pathological functions of each splice isoform of wild-type tau at different stages of development and across the lifespan.
- Rigorously explore the impact of exposome elements, like diet, on common outcome measures for AD and ASD mouse models such as seizure frequency and performance on memory tests.

Welcome Remarks, Day 2

National Institute on Aging Division of Neuroscience Staff

Dr. Opanashuk reiterated the main goal of the workshop, along with the four major objectives. She encouraged workshop attendees to use the Q&A feature to ask questions and invited all the workshop panelists to participate in the moderated discussions.

Session 3: Signaling Pathways

Nitric Oxide and mTOR Signaling: Shared Pathological Mechanisms for Autism and Alzheimer's Disease

Haitham Amal, Ph.D. – Hebrew University

Dr. Haitham Amal discussed a shared mechanism between ASD and AD that involves aberrant activation of mTORC signaling triggered by nitric oxide (NO). In recent decades, NO has been associated with neurodegenerative disease and found to drive pathological changes in the brain. In one established pathway, NO causes the S-nitrosylation of proteins, causing signaling alterations that may drive disease.²⁵

The Amal lab has established that there are increases in NO signaling in the *Shank3* (*InsG3680*) ASD mouse model and the *Tau*(*P301S*) AD mouse model. Because AD and ASD have shared clinical manifestations, they hypothesized that NO signaling may contribute to a common mechanism. Dr. Amal and colleagues used SNOTRAP mass spectrometry proteomics to identify all S-nitrosylated (SNO) proteins in cortical brain tissue from the *Shank3* and *Tau* mutant mice. SNO proteins common to both mouse models were enriched for proteins in the mTORC1 signaling pathway. In the *Shank3* mutant mouse, AGTR1, the insulin receptor; p38 MAPK; and TSC2 were S-nitrosylated. In the *Tau* mutant mouse, WNT11, FZD10, and RAC1 were S-nitrosylated. These data support a model in which NO causes the S-nitrosylation of mTORC1 pathway proteins and corresponding signaling changes in both disorders.²⁶

One of the SNO proteins in the *Shank3* mutant model was TSC2, a protein that dimerizes with TSC1 to inhibit mTORC1 signaling. S-nitrosylation of TSC2 triggers ubiquitination and subsequent degradation and also inhibits the dimerization TSC1 and TSC2. As such, Dr. Amal's group hypothesized that mTORC1 is hyperactive in the *Shank3* mutant model. They found that a downstream target of mTORC1, RPS6, was

significantly more phosphorylated in both the *Shank3* and the *Tau* mutant models, suggesting overactive mTORC1 in both. Pharmacological inhibition of mTORC1 or neuronal NO synthase could rescue spine density, sociability, and anxiety deficits in the *Shank3* mutant model of ASD. Future studies will examine the ability of these inhibitors to rescue deficits in the AD mouse model.

Synaptic Dysfunction: Intersection of Autism and Dementia

Kimberly Raab-Graham, Ph.D. – Wake Forest University

Dr. Kimberly Raab-Graham discussed a mechanism shared by AD and ASD in which high mTORC1 signaling causes increased expression of an RNA-binding protein, ultimately leading to decreased calcium channel expression and dysregulated calcium dynamics. Early presentations have asked whether the emergence of ASD-like behaviors in cognitive impairment are caused by undetected ASD in the older population. Dr. Raab-Graham presented data that suggests an alternative model, in which the overactive mTOR signaling characteristic of neurodegenerative diseases triggers a molecular switch leading to ASD-like behaviors.

In tuberous sclerosis, *Tsc1* mutations cause overactive mTOR signaling. High mTOR activity is also present in AD and other neurodegenerative diseases. mTOR signaling in neurons is essential for memory formation, driving changes in protein synthesis that change synaptic strength. Most studies discuss proteins that have mTOR activity-driven increases in expression. However, Dr. Raab-Graham discovered that mTOR activity also represses the translation of a set of mRNAs. Synaptic proteins were isolated from mice treated with vehicle or mTOR inhibitor rapamycin. Proteomics analyses identified proteins with mTOR-driven expression changes. Dr. Raab-Graham found that the expression of an almost equal number of proteins increased with mTOR inactivity as it did with mTOR activity.²⁷

Dr. Raab-Graham and colleagues constructed a protein-protein interaction network using their data of mTOR-sensitive proteins and databases of proteins associated with AD, ASD, and epilepsy. They used the network to identify proteins in their dataset likely to be dysregulated in all three disorders. One such protein, DJ-1, is a known mTOR-sensitive translational regulator.²⁷ Computational analysis revealed that several calcium channel subunits contain DJ-1 binding motifs. Dr. Raab-Graham and colleagues conducted RNA immunoprecipitation using DJ-1 antibodies and found that DJ-1 strongly binds *Cacna1c* and *Cacna2d2* mRNA in the *Tsc1* and APP/PS1 mouse models of disease, but not in wild-type. Further analyses focused on *Cacna1c*, which encodes CaV1.2 – a subunit of the L-type voltage-dependent calcium channel.

The AAV-driven overexpression of DJ-1 in mouse hippocampus caused a reduction in CaV1.2 expression and blocked activation of dendritic calcium signaling. These data suggest a disease model in which mTOR activity increases DJ1 expression, which inhibits CaV1.2 translation, causing disrupted calcium signaling. Indeed, hyperactive mTOR signaling, high DJ-1 expression, and low CaV1.2 expression occur in human AD, mouse models of AD, and mouse models of ASD. Mouse models of both AD and ASD had reduced calcium signals in dendrites. Using computational methods,²⁸ Dr. Raab-Graham's lab identified BayK as a drug that could be repurposed to reduce DJ-1 levels and restore the expression of DJ-1-regulated proteins. In the *Tsc1* mouse model, a single BayK injection rescued DJ-1 levels and calcium channel activity.

Brain Insulin Signaling Alterations in Models of Fragile X and Alzheimer's Disease and Their Link to Cognitive Deficits

Thomas Jongens, Ph.D. – University of Pennsylvania

Dr. Thomas Jongens described his work in drosophila models examining signaling pathways common to FXS and AD. Drosophila has a well conserved homolog of FMR1, *Dfmr1*. Like the mouse and human proteins, *Dfmr1* is an RNA-binding protein involved in positive and negative translational regulation. The drosophila *dfmr1* null model of FXS has phenotypes in common with the mouse *Fmr1* knockout model, including seizures, cognitive and social deficits, circadian deficits, and neuroanatomical defects.

Dr. Jongens and colleagues found enhanced mGluR signaling in *dfmr1* null flies. mGluR antagonists restored short-term memory in this model, as assessed by courtship assays.²⁹ Contrastingly, mGluR antagonists could not rescue circadian rhythm behavior in *dfmr1* null flies. To determine the circuit disrupted in the *dfmr1* null flies causing this defect, the Dr. Jongens group restored FMR1 protein in subsets of neurons in the brain. They found that restoring FMR1 protein in a set of insulin-producing neurons in *dfmr1* null flies fully restored both circadian behavior and short-term memory.

Next, Dr. Jongens and colleagues assessed the activity of insulin signaling in *dfmr1* null flies, using several markers. They found that insulin signaling was elevated in these brains and that reducing insulin signaling back to wild-type levels could rescue circadian behavior and short-term memory.³⁰ Dr. Jongens identified a genetic interaction between this pathway and the gene encoding presenilin in a model of familial AD. Presenilin is the catalytic subunit of γ -secretase, which cleaves APP to produce the A β peptide. Familial AD is caused by mutations in a single allele of Presenilin 1, Presenilin 2, or APP.

Fly models of familial AD with missense mutations in the gene encoding presenilin, *psn*, cause disrupted learning at memory, as measured with a courtship assay, at 30 days of age. Flies heterozygous for both *dfmr1* and *psn* had significantly worse short-term memory than either single mutant, suggesting they act via a common signaling pathway. Because mGluR antagonist treatment was effective in the *dfmr1* null flies, the Jongens group tested this in the *psn* flies. They found that mGluR antagonist treatment prevents the defects in learning and memory that occur at 30 days of age in these flies. Interestingly, treatment given at 30 days of age, after memory loss onset, can reverse these deficits as well.³¹

Psn heterozygous flies had phenotypes that hinted at dysregulated insulin signaling, such as increased body weight and increased glycogen stores compared to wild type. When Dr. Jongens and colleagues examined insulin signaling in the brain, they found a marked increase in PI3K activity. They found that treatment of control flies with a γ -secretase inhibitor could phenocopy the increased PI3K activity, suggesting that defects in γ -secretase activity in the *psn* flies was driving this phenotype. Treatment of *psn* heterozygous flies with PI3K inhibitors rescues memory deficits, linking increased insulin signaling to memory loss in this model. These data suggest that in drosophila models of FXS and AD, FMR1 and PSN act in a common pathway to mediate memory deficits.

Fragile X Syndrome and Alzheimer's Disease: Points of Intersection

Sean McBride, M.D., Ph.D. – Choi McBride Medical Group

ASD etiology is extremely complex, resulting from a combination of several hundred predisposing genetic mutations and environmental factors. FXS is an informative disorder to study, as it is monogenic, but recapitulates features of ASD. In addition, FXS has established outcome measures and strong biomarker candidates to use in trials.

Fly models of both FXS and familial AD have age-dependent impairment of learning and memory, suggesting that the investigation of the pathways at play in both models may be similar. Several groups of researchers have shown that FMRP promotes cAMP signaling. PSN, which is mutated in familial AD, regulates IP3 and GSK3b activity, which are both present in the cAMP signaling pathway. cAMP levels are deficient in FXS models and patients, suggesting that restoring this critical second messenger may be beneficial. Dr. McBride presented data demonstrating that cAMP upregulation via lithium or PDE-4 inhibitors can ameliorate disease phenotypes in fly and mouse models of FXS and AD.

In the *dfmr1* null fly, lithium treatment rescued immediate recall memory, short-term memory, and long-term memory no matter when administered, as well as brain morphology if given during development.³¹ In the FXS mouse model, lithium treatment reduced aberrantly high mGluR LTD, total and phosphorylated tau, dendritic spine morphology, social preference, and other behavioral phenotypes.³² Although one open label trial for lithium has shown that treatment improves RBANS list learning, the results have not gotten a lot of traction, possibly due to misconceptions about lithium.

In both the Psn and Tau overexpression fly models of AD, lithium treatment can rescue memory deficits, even after the onset of impairment.³³ In a 3xTg AD mouse model, lithium treatment re-establishes late-phase, long-term potentiation (LTP) and improves memory in the Morris water maze. Biochemically, lithium treatment increased GSK3 phosphorylation at an inhibitory site and increased CREB phosphorylation at an activating site in these mice. There have been a handful of small clinical trials for lithium use in AD, all showing no or little effect. However, the University of Pittsburgh is currently recruiting for a randomized control trial to examine lithium use in AD at a larger scale.

Inhibition of phosphodiesterase PDE4 can also elevate cAMP levels, presenting another therapeutic target. Treating FXS fly models with either of two different PDE4 could rescue long- and short-term memory, even when treatment started in adulthood. When given during development, treatment also rescued normal brain morphology.³⁴ In the mouse model of FXS, PDE4 inhibition rescued overactive mGluR LTD and normalized tau phosphorylation. Behaviorally, PDE4 inhibitors normalized hyperarousal, social interaction, nesting behavior, and dendritic spine morphology in these mice. A Phase 1 trial for a PDE4 inhibitor in FXS demonstrated that the drug was safe and well-tolerated. A Phase 2 trial revealed that the drug improved cognition in a battery of assessments, paving the way for a Phase 3 trial.

In a fly AD model that overexpresses wild-type tau, PDE-4 inhibition could also rescue short- and long-term memory deficits. In a 3xTg mouse model of AD, a chronic two-month treatment with PDE-4 inhibitor rescued late-phase LTP deficits and memory as measured by the Morris water maze. As in the mouse model of FXS, PDE-4 inhibition increased GSK3 phosphorylation at an inhibitory site and

increased phosphorylation of CREB at an activating site.³⁵ A Phase 2 clinical trial examining a PDE-4 inhibitor in AD found no effect; however, the investigators think that data from the larger dosage arm of the trial may trend toward effect, leading them to pursue this idea further.

Moderated Discussion

Sean McBride, M.D., Ph.D. – Choi McBride Medical Group, Rowan University

Q: Dr. Abel asked how other functions downstream of mTOR, such as cytoskeleton remodeling, are impacted by *Tsc1* mutation.

A: Dr. Raab-Graham commented that it is well established that mTOR is involved in the cytoskeletal changes that drive synapse formation.

A: Dr. Amal added that increased mTOR activity reduces spine density. Conversely, when mTOR is inhibited, there is an increase in spine density.

A: Dr. Abel noted that they are measuring protein synthesis during sleep deprivation, both globally and with immunoprecipitation for Raptor and Rictor as a way to answer this question. Interestingly, they observe selective effects of sleep loss on 4EBP rather than on S6K. They are able to rescue the effects of sleep deprivation by manipulating 4EBP phosphorylation. He also noted that one of the difficulties of using spine number as a metric is that spine-counting methods do not always give consistent results. Additionally, in the hippocampus, spines near the soma and those in the CA1 region are substantially different. He suggests that in addition to considering different spine morphologies, investigators should examine trends in spine changes along the dendritic tree, as this could be a source of discrepant results.

A: Dr. Jongens added that current studies examine CLIP (RNA crosslinking and immunoprecipitation assay) targets in the soma versus the dendrites. These studies have found that targets are generally translationally inhibited in the absence of FMRP in the soma and proximal dendrites, while translation is promoted in more distal regions. The impact of cellular location is a topic the whole field should consider, as it will improve the current understanding of how proteins are regulated with respect to activity and synaptic function.

A: Dr. Raab-Graham added that spine density metrics do not necessarily reflect the presence of functional synapses. To get at this, investigators should examine pre- and postsynaptic engagement. Spine number should not be considered a metric of synaptic communication.

A: Dr. Abel commented that one technique to visualize synaptic engagement is STED with three or four colors labeling pre- and postsynaptic receptors; however, this technique is only pragmatic for in vitro models.

A: Dr. Graham added that her lab has a video in preparation to be published in the Journal of Visualized Experiments (JoVE) as well as a publication in Molecular Psychiatry describing a proximity ligation assay for synaptic engagement.

Q: A workshop attendee asked the speakers whether they have assayed mTOR activity in ASD models other than *Tsc1* or *Tsc2*.

A: Dr. McBride responded that Dr. Zukin has shown high mTOR activity in FXS.

A: Dr. Amal added that in the *Shank3* and *Casper2* mouse models of ASD, there is both increased mTOR activity as well as increases in NO and S-nitrosylated proteins. Future studies will examine mTOR activity in the *Btbr* mouse model of idiopathic ASD.

Q: A workshop attendee also asked Dr. Amal and Dr. Raab-Graham whether they observed any sex differences in their assays.

A: Dr. Amal responded that there were no differences between males and females in the *Shank3* or *Casper2* mouse models in the numerous biochemical and behavioral assays they performed.

A: Dr. Gozes noted that her group has found sex differences in axonal transport and autophagy in ASD models. She also noted that to find sex differences, investigators may have to consider differences in brain regions as well as differences in genetic background.

A: Dr. McBride added that sex differences should be more thoroughly investigated in clinical trials for ASD.

Q: Dr. Mucke noted that his research group generally regards PTEN phosphatase activity as beneficial, in that it signals upstream of mTOR and can prevent its aberrant overactivation of mTOR in the ASD model. He was surprised to find a report where synaptic deficits in the APP/PS1 model were suppressed by a very strong PTEN inhibitor. Dr. Mucke asked his fellow speakers how this finding might fit into what had been discussed.

A: Dr. McBride suggested that these unexpected findings could be explained by differences in mTOR dysfunction with age. He suggested that it is possible the mice for which PTEN inhibition was beneficial may have decreased mTORC activity, such that PTEN inhibition may lead to increased mTOR activity and rescue of deficits.

A: Dr. Raab-Graham added that cycles of mTOR signaling in ADRD and related models may complicate the system.

A: Dr. McBride agreed that hyper- and hypoactive levels of mTOR may both be contributing to pathologies in AD and ASD. He emphasized the need for more longitudinal studies in AD as well as in animal models of AD.

Q: Dr. John Lukens asked the group to discuss the roles of non-neuronal cells in AD and ASD. Given that many therapeutic treatments presented in this workshop globally activate or deplete pathways, these treatments may affect cell populations outside the brain, such as the immune system. He suggested that strategies to target neurons in more specific ways may lead to better outcomes.

A: Dr. Raab-Graham added that rapamycin studies exemplify Dr. Lukens' point well. Rapamycin treatment is effective in patients with TSC but there are a lot of side effects. Researchers are currently designing a version of rapamycin that is selectively brain permeable. She suggested that targeting proteins that are expressed in the brain but not the periphery may also be a useful strategy.

A: Dr. Mucke added that a benefit to the tau reduction technique is that it does not seem to change baseline mTOR activity. He suggested that strategies that modulate target pathways indirectly could be an effective approach as well.

Q: An attendee asked Dr. Abel whether cAMP may be involved in the delayed GABAergic transition in FXS.

A: Dr. Abel responded that although he was unsure, there have been some studies examining Baclofen, an activator of the GABA_B receptor, in FXS.

Dr. McBride commented that the FDA may have different approval routes depending on the age of the treatment population and the duration of treatment. Considering these routes may be beneficial to designing translational research studies, Dr. McBride added.

Key Opportunities

- More thoroughly examine sex differences in AD and ASD mouse models and clinical trials.
- Develop a reliable biomarker of brain mTOR activity and use in longitudinal studies to examine mTOR signaling in aging, in individuals with AD, ASD, neither, or both.
- Consider the targeting of non-neuronal proteins and pathways in AD and ASD.
- Examine impact of increased nitric oxide in models of neurodegenerative and neurodevelopmental disease.

Session 4: Other

Epigenetic Regulation of Activity-Dependent Gene Expression: Links between Neurodevelopment and Neurodegenerative Disorders

Ted Abel, Ph.D. – University of Iowa

Dr. Ted Abel highlighted the epigenetic regulation of activity-dependent gene expression as a connection between neurodevelopmental and neurodegenerative disorders. Neuronal activity, seizure, and experience can induce changes in gene expression. Mechanisms of activity-dependent gene expression have been studied by numerous groups. Initially, these studies focused on CREB, a transcription factor that, when phosphorylated, binds elements in DNA and drives expression. Phosphorylation of CREB is driven by a variety of kinases that are activated in response to neuronal activity or experience.

Several studies identified signals that caused CREB phosphorylation but did not activate gene expression, leading to the discovery of CREB co-activators, such as CBP/p300. Co-activators integrate multiple signals via post-translational modifications and activate changes in expression through enzymatic activity. CBP is an acetyltransferase that acts on histones and other factors. Acetylated histones are associated with active transcription, as they open nucleosomes and create binding sites for bromodomain-containing chromatin readers that activate transcription. Histone acetylation is just one element within the field of epigenetics – the idea of long-lasting cellular memory that can be maintained

in the absence of changes in DNA sequence. Epigenetic mechanisms include histone modifications, DNA methylation, RNA-mediated gene silencing, and protein conformational changes.

The Abel lab has focused on the role of histone acetylation in memory. Histone acetyltransferases (HATs) add acetyl groups to histones while histone deacetylases (HDACs) remove the acetyl group. Mutating HATs blocks activation of gene expression, resulting in deficits in contextual memory. Mutating HDACs causes increased acetylated histones and enhances memory. The Abel group and others have studied an HDAC called SIN3A, which interacts with REST and several other proteins to create a complex that represses gene expression. Knocking out *Sin3a* causes enhancements in memory and plasticity that occur via mGluR5 signaling and scaffolding proteins such as HOMER1. Because reducing SIN3A activity enhances memory, it can be thought of as a memory suppressor gene, as mentioned by Dr. Raab-Graham.³⁶

Several studies have examined the role of HDACs in neurodegeneration. HDAC inhibitors can arrest polyG-driven neurodegeneration in flies. Additionally, histone acetylation decreases in age and in mouse models of ADRD. In these models, HDAC inhibition can rescue cognitive deficits. Dr. Abel and his lab have examined the efficacy of CBP activators in a tau model of neurodegenerative disease. CBP activators can rescue spatial, long-term memory deficits in this mouse model. Subsequent studies have found that CBP activation drives the expression of the transcription factor NR4A1 via acetylation. NR4A1 then activates the expression of chaperone genes that function in endoplasmic reticulum to coordinate the presentation post-synaptic receptors. This pathway is defective in tau mutant neurons and can be rescued by modifying NR4A1 activity.³⁷

Extensive genetic studies of neurodevelopmental disorders have found that mutations in genes encoding epigenetic regulators are enriched, including p300, CBP, and SIN3A. Mechanisms involving these proteins in AD may be relevant to ASD. Mutations in CBP cause different syndromes with ASD-like behaviors according to which domain of the 300 kD protein is mutated.³⁸ This suggests that mutations in different domains of proteins involved in epigenetic regulation may give rise to different clinical presentation. Overall, data discussed reveal the critical relevance of overlapping epigenetic mechanisms in AD and ASD and suggest that manipulating histone acetylation may be a useful therapeutic avenue.

Role of DNA Damage Surveillance by the Innate Immune System in Neurodevelopment

John Lukens, Ph.D. – University of Virginia

During development, the innate immune responses trigger cell death, or dieback, to support the formation of a healthy brain. The under- or overactivation of immune signaling during development causes pathology. Underactivation can cause defects in developmentally programmed cell death and synaptic pruning. Similarly, inducing inflammation during development can also cause issues. Many of the same immune pathways that are involved in neurodevelopment can drive neuroinflammation and neurodegeneration later in life.³⁹

In the context of AD, microglia are essential to protect neurons from neurotoxic material, such as protein aggregates. In addition, microglia can partition off neurons from these neurotoxic materials. However, overactivation of these pathways is associated with inflammation, cell death, and synapse

loss. Immune signaling in the brain uses many of the same pathways in early and late life, such as TREM2 signaling, the complement system, and inflammasomes.

Inflammasomes are multiprotein complexes that regulate proinflammatory cytokines and cause cell death. Studies suggest that inflammasome activation is a critical player in AD, inducing neuroinflammation and spreading A β oligomeric seeds. DNA damage can trigger inflammasome activation via immune molecules known as DNA damage sensors. Dr. John Lukens' lab sought to determine whether DNA damage sensors play a role in neurodevelopment and behavior.

A large body of research shows that maintenance of genomic integrity is required for CNS health. Individuals with mutations in DNA damage response genes not only get cancer later in life, but also develop early onset neurological disorders and, in some cases, early onset neurodegenerative diseases. Early in development, replicative and metabolic stress can cause extensive DNA damage. The Lukens lab studied the role of DNA damage sensor AIM2 in the maintenance of genomic integrity during development. AIM2 senses double stranded DNA (dsDNA) in the cytoplasm of the cell, which indicates DNA damage, and triggers inflammasome activation, cytokine production, and cell death.

Early in development, *Aim2* homozygous knockout mice have high levels of dsDNA damage accumulation that colocalizes with markers of active inflammasomes. Behaviorally, these mice had increased anxiety in the elevated plus maze and the open field test, and deficient motor coordination on the Rotarod test. In addition, the mice had vocalization and socialization issues, phenotypes that may be relevant to ASD symptoms.

Next, the Lukens lab sought to discern whether phenotypes in the *Aim2* knockout mice were driven by their role in cytokine release or membrane pore formation. They found that AIM2 regulation of cytokines IL-1 and IL-18 were not driving the phenotype. Rather, they found that deletion of Gasdermin-D, a molecule involved in pore formation and cell death, phenocopies the *Aim2* knockout mice. Further, DNA damage promotes inflammasome activation and cell death in wild-type, but not *Aim2* knockout cells, suggesting that AIM2 is required for these processes. In addition, mice lacking AIM2 or Gasdermin-D had significantly greater numbers of neurons in certain brain regions, particularly in the cerebellum.⁴⁰

These data suggest that during neurodevelopment, DNA damage triggers AIM2 activation, which causes inflammasome formation, driving Gasdermin-D-mediated cell death. Further, this occurs as a mechanism by which the brain purges itself of cells with irreparable DNA damage. When this pathway is deficient, there is impaired removal of these cells, causing behavioral abnormalities and altered brain maturation. Recent evidence suggests that inflammasome activation occurs in the AD brain and may be a critical driver of pathologies.

Air Pollution and Autism: Connections With Alzheimer's Disease?

Marc Weisskopf, Ph.D., Sc.D. – Harvard University

Dr. Marc Weisskopf discussed his research examining the epidemiology of air pollution and ASD and potential connections with AD. Air pollution is often measured by examining levels of particulate matter (PM) in the air. Most research today focuses on PM_{2.5} particles, which are less than 2.5 microns in diameter. Even in locations without visible air pollution, particulate matter can have biological consequences, such as inflammation, that can drive disease in the lungs, heart, and even brain.

EPA models of air pollutants in different counties show that higher levels of pollutants in the perinatal location of the mother tracked with higher rates of ASD in children. Previous case control studies found that children with ASD had higher levels of certain pollutants in their blood and urine. However, because ASD changes how individuals interact with the environment, these findings were not surprising or informative. EPA models of pollution levels are based on monitors throughout the country, as well as knowledge of sources of pollution such as traffic, industry, and wildfire levels. As such, investigators can assign exposures to cases and controls independent of disease status. Dr. Weisskopf suggests that this model could be applied to other types of national exposure data, not just air pollutants. In this large dataset, Dr. Weisskopf and colleagues found that postnatal exposure to traffic pollutants is associated with an increased risk of ASD, even after correcting for several possible confounding factors.⁴¹

As was previously described in this session, microglia play an essential role in brain development. In addition, the aberrant activation of microglia has detrimental effects on normal development. As the resident immune cells of the brain, microglia are the first responders to external insults such as air pollutants. ASD brains often show enrichment of genetic variants of synaptic and neuronal genes, but not microglial genes. However, there is evidence of altered expression of microglial genes in ASD brains, indicative of a potential role for environmental factors.

Respiratory intake of pollutants increases blood cytokine levels, which can damage the blood-brain barrier and lead to altered neurotransmission, neuroinflammation, oxidative stress, and other pathologies in the brain. These brain pathologies related to air pollutant exposures are also evident in neurodegenerative diseases.⁴² Neuropathology on highly exposed dogs shares many characteristics of AD pathologies, including degenerating neurons, plaques and tangles, tau-positive neurons, activated microglia, and vascular abnormalities.⁴³ Meta-analyses of publications examining air pollution exposure and dementia largely suggest a small but significant association.⁴⁴ Literature suggests that the toxic activation of microglia may be playing a role in aberrant synapse elimination in dementia. Future studies in this area should explore critical exposure windows for both dementia and neuro-developmental disorders. In addition, investigators should explore the idea of microglial priming, in which early exposure to a pollutant may prime microglia for toxic reactivity to a second exposure later in life, causing disease.

Moderated Discussion

Ted Abel, Ph.D. – University of Iowa

Q: Dr. Gozes asked Dr. Lukens whether he saw an accumulation of DNA mutations in addition to DNA damage in his *Aim2* model.

A: Dr. Lukens responded that he hopes to learn more about how neurons and glial cells regulate their genomic material. He noted that neurons have more endogenous retroviral genes and increased transposition compared to other cell types, warranting further investigation.

Q: Dr. Abel asked Dr. Lukens about the possibility of other, non-microglial immune mechanisms, such as circulating interleukins coming from T-cells, playing a role in neurodevelopmental disease and dementia.

A: Dr. Lukens noted that there is certainly evidence for non-microglial mechanisms, although what specific immune cell they come from is still unknown.

A: Dr. Abel added that although most ASD studies focus on the brain, individuals with ASD have many nonbehavioral medical issues that affect other organs, which could be mediated by immune dysfunction.

A: Dr. Lukens noted that the gut and microbiome certainly communicate with the brain, and that this connection is worth exploring further in ASD. Interestingly, symptoms sometimes wane when children with ASD have a fever. Although this phenomenon is not well understood, it is likely to have an immune-mediated mechanism.

A: Dr. Jongens noted that many genetic causes of autism present as syndromes; however, researchers rarely investigate outside of the brain. He suggests the field could benefit from examining the underlying mechanisms of gastrointestinal problems, heart problems, and other nonbrain features in ASDs. Dr. Abel added that targeting these other medical issues could improve quality of life for people with ASDs, while brain mechanisms are still being explored. Dr. Gozes added that mutations causing syndromic autism often occur in genes encoding epigenetic regulators that function in many organ systems.

Q: Dr. Gozes asked Dr. Weisskopf whether the COVID-19 pandemic could be used to study the potential protective effects of avoiding pollutant exposure.

A: Dr. Weisskopf responded that although the pandemic did cause a decrease in overall pollution, there are better-controlled examples of this.

Q: An attendee asked Dr. Weisskopf whether there may be a way to treat microglial-mediated damage in ASD and AD.

A: Dr. Weisskopf responded that mitigating exposures to air pollutants may be a more efficient approach than repairing the effects of the damage. He stressed the importance of studies identifying vulnerability and resilience factors in pollutant-mediated deficits.

Q: Dr. Abel asked Dr. Weisskopf whether studies of maternal smoking exposure and DNA methylation could be used as a model for creating an epigenetic biomarker for air pollutant exposure.

A: Dr. Weisskopf responded that creating an epigenetic fingerprint of air pollutant exposure would be extremely valuable to the field.

A: Dr. Gozes added that in addition to epigenetics, the accumulation of particular somatic mutations could be used as a marker for exposure.

A: Dr. Weisskopf agreed that this may be a useful metric and added that they are exploring the idea that paternal exposure could be equally important as maternal exposure in determining ASD risk. In addition, somatic mutations in sperm are understudied and may help explain the contribution of paternal age to ASD risk.

Dr. McBride commented that both basic scientists and clinicians should avoid treating ASDs as a homogenous group, and that parsing out the differences between ASDs is essential to the field. Treating ASDs as distinct disorders will also help researchers discern possible connections with AD.

Q: Dr. Abel asked Dr. Weisskopf about how health disparities connect to differences in exposure. Particularly, Dr. Abel was interested in whether differences in diagnostic rates could change how epidemiologists perceive or measure the impact of environmental exposures.

A: Dr. Weisskopf replied that issues relating to who has access to care and who gets diagnosed are extremely problematic. He noted that air pollution is quite different in rural versus city environments, not only in the amount of exposure but in the components as well.

Key Opportunities

- Develop an epigenetic fingerprint for each type of environmental exposure that may contribute to AD and ASD risk.
- Examine the role of mobile genetic elements in driving mutational burden in AD.
- Investigate DNA-damage-driven inflammation as a potential biomarker and therapeutic target in AD and ASD.
- Use national exposure datasets to draw connections between environmental exposures and AD/ASD risk, looking specifically for critical exposure windows that drive risk.

Final Discussion

At the start of the final discussion, the moderators of the Day 1 sessions reviewed their key findings.

Dr. Bookheimer first reviewed major points from Session 1. The first session focused on working with individuals with AD and ASD and described a variety of different methods to measure shared incidence. A considerable amount of evidence suggests that ASD, not ID or other features of neurodevelopment disorders, has a specific relationship with AD. Studies examining the relationship between ASD and AD often have serious cohort effects, driven by difference in when individuals were diagnosed. The availability of large datasets such as Medicare and Medicaid data can help researchers examine the comorbidity of AD and ASD, as well as related symptoms. Many other large datasets, such as ADNI and the human connectome study in aging, lack measures of ASD-like symptoms, which may be helpful to add. Several large studies do have blood from individuals with AD and ASD, providing an opportunity to explore some of the genetic risks that have been discussed, including the increased incidence of *ApoE4* in the ASD population. Similarly, blood AD datasets could be used to examine genetic risk factors related to ASD. With coordination for AD and ASD researchers with large datasets, the field could address specific genetic and mechanistic questions raised in this workshop. In addition, recent advances in blood AD biomarkers enables researchers to perform cost-effective, minimally invasive, widescale assessments of individuals across the lifespan.

Dr. Mucke summarized key findings from Session 2. The amyloid and tau session highlighted that studying ASD and AD together can provide fascinating insights into both fields. AD-related proteins, tau and APP, can be implicated in ASD pathogenesis. Similarly, signaling abnormalities in ASD play critical roles in AD pathways. Network function and E/I ratios are dysregulated in both conditions and their inhibition may have therapeutic benefit. APP, its metabolites, and tau protein can all be implicated in ASD and AD in various ways, especially within the mTOR and glutamate signaling pathways. In both aggregated and nonaggregated forms, tau can contribute to ASD-related deficits. This is likely also true

for APP in the context of FXS. Exploring methods to modulate levels of these proteins could be beneficial to both AD and ASD. All the talks presented strong entry points for further exploration and therapeutic intervention.

Q: Dr. Gozes noted that targeting amyloid or tau may never be an effective therapeutic strategy, considering the presence of DNA damage. She asked whether decreasing environmental exposures such as air pollution may be a more effective intervention that could prevent the accumulation of DNA damage.

A: Dr. Mucke added that people with early stages of AD have two times the dsDNA damage in neurons and astrocytes. He noted that GWAS studies of AD suggest inflammation-related processes play a huge role.

A: Dr. Gozes added that de novo mutated genes in ASD are not necessarily related to inflammation but may influence the expression of inflammation-related genes.

A: Dr. Bookheimer noted that inflammation-related genes are implicated in polygenic, syndromic ASDs.

A: Dr. Jongens added that mitochondrial genes are also linked to ASD and PD.

Q: An attendee asked, given that there is a substantial subgroup of people with ASD, AD, and epilepsy, are differences in this population compared to the ASD-only population.

A: Dr. Mucke replied that individuals with certain forms of epilepsy and AD decline more rapidly, and this may also be true for people with ASD. It remains unknown, however, whether epilepsy contributes to severity of disease or whether it occurs in individuals with more severe disease. This distinction would inform whether the suppression of epileptiform activity could be used as a therapeutic avenue.

A: Dr. Jongens added that certain monogenetic forms of ASD lead to a higher incidence of seizures during childhood but disappear at adolescence. He wondered whether seizures reappear in these individuals as they progress toward AD.

A: Dr. McBride replied that although seizures classically improve in children with FXS, there are other ASD-related single gene disorders where the seizures never improve.

Dr. McBride added that many individuals with AD and ASD see community doctors, never go to academic centers, and never undergo exome sequencing. In addition, the percentage of people with ASD who remain in longitudinal studies as they get older is low. He wondered how academic centers can better retain these individuals.

Q: Dr. Mucke commented that another important issue from Session 2 to consider is the idea of a therapeutic window of opportunity and asked speakers to comment on this topic. For both AD and ASD, studies could be designed to determine the time period in which treatment can modify the disease, not just treat symptoms. He said that he suspects that, for AD, treatments may have to be initiated a decade or more before the disease becomes clinically detectable. The idea that pathogenic processes in ASD and AD could be reversible with epigenetic or other manipulations is an interesting possibility.

A: Dr. McBride noted that in fly and mouse models, cognitive impairment can be reversed, although he was uncertain whether that finding will translate to humans. He noted that at least for AD, current treatments are not disease modifying, suggesting interventions must occur years, perhaps decades, prior to symptom onset. However, with the right drugs, it may be possible to have cognitive gains after the onset of impairment.

A: Dr. Gozes added that in ASD, although in utero treatments are unlikely, early behavioral interventions seem to provide more benefits.

A: Dr. Jongens added that mTOR antagonist treatment during development can rescue age-related learning deficits in FXS flies. He suggested this is an example of preempting neurodegeneration before the onset of the phenotype.

Q: A workshop attendee asked how chronic treatment with mTORC inhibitors may affect individuals as they age.

A: Dr. Abel noted that this will be interesting to investigate in certain syndromic forms of neurodevelopmental disorders.

Dr. Abel noted that although the workshop covered many different models of ASD, it is still a challenge to determine what behavioral readouts in these animals are relevant to human symptoms. In the context of ADRD, assays to model cognitive deficits are more well established. For animal experiments to be effective, researchers need to know when the relevant phenotypes occur and then perform a time course with the therapeutic intervention to discover when it may be most effective.

Q: A workshop attendee asked whether reserve and resilience, an active area of research in AD, is also being explored in ASD.

A: Dr. Abel commented that sex differences may be relevant to that question, considering the resistance of females to genetic alternations in neurodevelopmental disorders.

A: Dr. Weisskopf added that they are exploring vulnerability and resilience to the effects of the same environmental exposure.

A: Dr. Mucke added that even the same mutation can manifest very different forms of ASD, suggesting that there is significant modulation by environmental factors.

Key Opportunities

- Add measures of ASD symptoms to established longitudinal studies of aging, such as ADNI and HCP-A.
- Use available samples from large studies of individuals with ASD to look for alterations in AD risk-related genes and protein biomarkers. Similarly, use large AD datasets to look for alterations in genes related to ASD risk.
- Investigate the contribution of epilepsy to cognitive/behavioral symptom severity in AD and ASD.
- Establish behavioral assays in mice and flies that are relevant to human ASD symptoms.

Closing Remarks

Lisa Opanashuk, Ph.D. – Program Director, National Institute on Aging Division of Neuroscience

Dr. Opanashuk thanked the speakers, especially for their lively discussion of gaps and opportunities in the field. She noted that future workshops on this topic are planned and will involve other institutes, including the National Institute of Neurological Disorders and Stroke and the Eunice Kennedy Shriver National Institute of Child Health and Human Development. Dr. Opanashuk acknowledged Dr. Rachel Sare for coming up with the focus of this workshop, as well as recruiting speakers for each area of interest. Lastly, Dr. Opanashuk thanked Dr. Eliezer Masliah, the director of the Division of Neuroscience at NIA, and adjourned the workshop.

Appendix A: Agenda

Day 1: March 8, 2022

1:00 p.m.	Welcome Remarks Eliezer Masliah, M.D. – Director, NIA DN
1:10 p.m.	Workshop Objectives Lisa Opanashuk, Ph.D. – Program Director, NIA DN

SESSION 1: CLINICAL/EPIDEMIOLOGY	
1:20 p.m.	Aging With Autism Spectrum Disorder: Longitudinal and Cross-Sectional Approaches to Cognitive, Brain, and Genomic Data B. Blair Braden, Ph.D. – Arizona State University
1:40 p.m.	Prevalence and Pathologic Underpinnings of Autism-Like Behaviors in a Community-Based Dementia Research Cohort Elizabeth Rhodus, Ph.D. – University of Kentucky
2:00 p.m.	National Data Approaches to Examining the Connection Between Alzheimer's Disease and Autism Spectrum Disorder Lindsay Shea, Dr.PH. – Drexel University
2:20–2:50 p.m.	Moderated Discussion Moderator: Susan Bookheimer, Ph.D. – University of California, Los Angeles

BREAK – 10 min

SESSION 2: AMYLOID/TAU	
3:00 p.m.	Fragile X and APP: A Decade in Review, a Vision for the Future Cara Westmark, Ph.D. – University of Wisconsin
3:20 p.m.	The Function of Fragile X Protein FMRP in Aβ Pathology Nien Pei-Tsai, Ph.D. – University of Illinois, Urbana-Champaign

3:40 p.m.	The Molecular Link Between Tuberous Sclerosis Complex, Alzheimer's Disease, and Other Tauopathies Aimee Kao, M.D., Ph.D. – University of California, San Francisco
4:00 p.m.	Using Tau Reduction to Counteract Excitation/Inhibition Imbalance – Potential Benefits in Alzheimer's Disease and Autism Spectrum Disorders Lennart Mucke, M.D. – Gladstone Institutes and University of California, San Francisco
4:20 p.m.	Tauopathy in the Young Autistic Brain: Novel Biomarker and Therapeutic Target: The Case of the ADNP Syndrome and NAP (Davunetide) Illana Gozes, Ph.D. – Tel Aviv University
4:40–5:00 p.m.	Moderated Discussion Moderator: Lennart Mucke, M.D. – Gladstone Institutes and University of California, San Francisco

Day 2: March 9, 2022

1:00 p.m.	Welcome Remarks NIA DN Staff
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SESSION 3: SIGNALING PATHWAYS	
1:10 p.m.	Nitric Oxide and mTOR Signaling: Shared Pathological Mechanisms for Autism and Alzheimer's Disease Haitham Amal, Ph.D. – Hebrew University
1:30 p.m.	Synaptic Dysfunction: Intersection of Autism and Dementia Kimberly Raab-Graham, Ph.D. – Wake Forest University
1:50 p.m.	Brain Insulin Signaling Alterations in Models of Fragile X and Alzheimer's Disease and Their Link to Cognitive Deficits Thomas Jongens, Ph.D. – University of Pennsylvania
2:10 p.m.	Fragile X Syndrome and Alzheimer's Disease: Points of Intersection Sean McBride, M.D., Ph.D. – Choi McBride Medical Group
2:30–2:50 p.m.	Moderated Discussion Moderator: Sean McBride, M.D., Ph.D. – Choi McBride Medical Group

BREAK – 10 min**SESSION 4: OTHER**

3:00 p.m.	Epigenetic Regulation of Activity-Dependent Gene Expression: Links Between Neurodevelopment and Neurodegenerative Disorders Ted Abel, Ph.D. – University of Iowa
3:20 p.m.	Role of DNA Damage Surveillance by the Innate Immune System in Neurodevelopment John Lukens, Ph.D. – University of Virginia
3:40 p.m.	Air Pollution and Autism: Connections With Alzheimer's Disease? Marc Weisskopf, Ph.D., Sc.D. – Harvard University
4:00 p.m.	Moderated Discussion Moderator: Ted Abel, Ph.D. – University of Iowa
4:20 p.m.	Final Discussion
5:00 p.m.	Closing Remarks Lisa Opanashuk, Ph.D. – Program Director, NIA Division of Neuroscience

Appendix B: Participant List

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