Understanding mechanisms and seeking cures for Alzheimer's disease:
Why we must be “extraordinarily diverse”

Madhav Thambisetty, MD, PhD

Clinical and Translational Neuroscience Unit,
Laboratory of Behavioral Neuroscience,
National Institute on Aging (NIA),
National Institutes of Health (NIH),
Baltimore, MD, USA

E-mail: thambisettym@mail.nih.gov
Tel: 410-558-8572
Fax: 410-558-8674
After more than a century since Dr. Alois Alzheimer first described the pathological hallmarks accompanying the defining clinical features of the disease, we have yet to deliver any meaningful disease-modifying treatments to our patients. In this article, we present a rationale for the need to be “extraordinarily diverse” in seeking effective ways to treat or prevent this devastating disease. Our approach is based on applying a systems-biology perspective at the population level, using a diverse array of ‘OMICS’ methodologies to identify molecular mechanisms associated with well-established AD risk factors including systemic inflammation, obesity and insulin resistance. We believe that applying this strategy to understand longitudinal changes in human physiology during aging is of paramount importance in identifying meaningful opportunities to intervene effectively in AD.
Alzheimer’s disease (AD) is one of the major global public health challenges of our time. In this article, we present a rationale for the need to be “extraordinarily diverse” in seeking effective ways to treat or prevent this devastating disease. The phrase “extraordinarily diverse” was recently used by a senior colleague in the intramural program of the National Institute on Aging (NIA) when reviewing our research progress. Although in the overall context of the positive review we received, we have little doubt that this phrase was complimentary of our broad portfolio of studies, it leaves just enough unsaid to merit an unequivocal justification of our approach. Here, we argue the importance of “extraordinary diversity” in the way we study AD with a view to developing effective interventions against it.

The most compelling argument for a comprehensive overhaul of the way we study AD is the sobering reality of our inability to treat it. Thus, after more than a century since Dr. Alois Alzheimer first described the pathological hallmarks accompanying the defining clinical features of the disease (14), we have yet to deliver any meaningful disease-modifying treatments to our patients. Currently, the only medications routinely used in treating AD patients are barely symptomatic or palliative interventions that do not alter the relentlessly progressive trajectory of the disease. The last of these drugs to be approved by the FDA was Memantine, an NMDA antagonist in 2003.

Despite significant advances in our knowledge of risk factors for AD and the molecular pathways underlying AD pathology, these advances have not yet translated into tangible benefits for patients.

While the reasons for the repeated failures of experimental AD treatments are many, the unifying theme that runs through the grim statistics behind failed AD therapeutics is a striking “lack of diversity.” We use this phrase to describe two specific phenomena:

i. The almost single-minded focus of drug development on the two primary pathological features of AD i.e. the amyloid plaque and neurofibrillary tangle through experimental treatments targeting the Aβ peptide or tau protein (25) (29). Thus, most current disease-modifying trials in AD rely upon Aβ as the pharmacological target, seeking to enhance its clearance from the brain, inhibit its accumulation or block its aggregation. Between 2002 through 2012, 145 of 221 disease-modifying trials (>65%) were targeted at Aβ (16). This is despite the fact that Aβ as a disease target is unvalidated, and no class of agents has yet shown efficacy for this target in human clinical trials. Numerous clinical trials of anti-Aβ treatments in AD, testing a variety of distinct approaches, ranging from both active and passive immunotherapy, inhibition of Aβ-aggregation, and modulation/inhibition of γ-secretase and β-secretase have uniformly failed to
show any clinical benefits (40). Similarly, although numbering fewer than anti-
Aβ treatments, clinical trials of interventions targeting tau have also failed (41).

The overwhelming reliance on targeting the two key pathological hallmarks of
AD in human clinical trials has been fueled in large part, by animal models that
faithfully replicate these pathologies and “respond” to experimental treatments
that clear them or prevent their development (7, 47). Preclinical AD research
using transgenic mouse models has thus generated hundreds of publications,
many of them in high-impact, high-profile journals wherein a candidate
Alzheimer’s drug has dramatically cleared the brain of AD pathology. The
observation that many animal models of amyloidosis show biological and
behavioral benefits from anti-Aβ agents, has created a yawning “translational
gap” between human and animal studies (32) (15), wherein the stark reality is
that these same experimental treatments have proven to be resounding failures
in human clinical trials.

ii. A “lack of diversity” similarly afflicts human clinical research, albeit in another
way. If the translational gap between the promise of preclinical AD research and
the grim reality of failed human clinical trials is to be bridged, one solution is to
build experimental AD therapeutic discovery programs from rigorous and high
quality human studies that use a diverse array of methods. This goal brings with
it a distinct set of challenges. Investigators working at the human end of the
translational research spectrum have all too often defined their careers and their
science through the individual methods they use, rather than the problems they
seek to solve. Thus, epidemiologists ‘observe,’ imagers ‘image,’ biomarker
scientists ‘assay,’ geneticists do ‘GWAS’, clinicians ‘treat’ and neuropsychologists
endlessly debate which test of executive function is most sensitive to cognitive
impairment. A concerted effort to comprehensively study mechanisms
underlying AD pathogenesis and risk in human subjects, with the ultimate goal
of testing whether such mechanisms may be valid treatment targets, requires a
seamless joining of forces across disciplines and between scientists who have
traditionally preferred to remain within comfort zones defined by the respective
tools of their trades. It was precisely this coming together of a diverse array of
expertise and methods that Norman Geschwind referred to when he spoke of
“the need for the marshalling of a full array of disciplines for the understanding of
behavior” (24)

In our research, we have embraced the twin challenges of adopting diversity in
terms of both studying potentially modifiable disease mechanisms as well as in
the array of tools used to identify such mechanisms in AD. We believe that
applying this strategy to understand longitudinal changes in human physiology during aging is of paramount importance in identifying meaningful opportunities to intervene effectively in AD. Recognition that the preclinical prodrome of AD may begin several years, if not decades before the onset of cognitive impairment, and the identification of distinct components of an “Alzheimer’s pathophysiological signature” during presymptomatic stages of the disease, has allowed us to imagine for the first time in a generation, the possibility of preventing AD or targeting potential treatments in asymptomatic at-risk individuals during the earliest stages of disease progression (54) (55). However, in order to fully exploit this invaluable window of therapeutic opportunity, we believe that a concerted attempt must also be made to discover the causal biological drivers of disease progression during the earliest stages of AD. The underlying assumption, arguably a reasonable one, in our own efforts to uncover such mechanisms underlying AD pathogenesis in presymptomatic individuals, is that the evolution of AD neuropathology and the eventual expression of symptoms represent the culmination of sustained perturbations in human physiology over several years. These studies therefore test whether we can reliably measure such abnormalities in human physiology and unambiguously relate them to both severity of AD pathology in the brain and risk of AD progression.

Our effort to identify disease mechanisms that may present plausible opportunities for intervention in AD therefore utilizes longitudinal clinical data from well-characterized cohorts of older individuals who are cognitively normal at baseline and are followed over several years during which some develop incident AD or mild cognitive impairment (MCI) due to AD. In ‘top-down’ studies, we begin by testing associations of established AD risk factors in these cohorts with distinct components of the AD pathophysiological signature and then proceed to identify the molecular bases of such associations using a variety of ‘omics’ methods in brain and blood tissue samples. Conversely, in ‘bottom-up’ studies, we first seek to identify specific metabolite or proteomic correlates of AD pathology in the brain and then ask whether systemic alterations in these proteins or metabolites are also associated with distinct endophenotypes of AD and risk of disease progression in presymptomatic individuals.

In the following sections, we specify the essential elements integral to the design of such studies citing specific examples from our recent work.

1. *Beginning at the beginning: what is normal aging?*
In 1958, William W. Peter, a retired U.S. Public Health Service officer and missionary doctor, met with Nathan Shock, Chief of the Gerontology Branch at the National Institutes of Health (NIH), to ask whether he could make a contribution to science by donating his body for research after his death. Dr. Shock proposed what was at the time, a radical alternative-participation in a research study that sought to understand normal aging by repeatedly evaluating the same people over time over several years. The Baltimore Longitudinal Study of Aging (BLSA), which is now among the longest running studies of normal aging in North America, arose from this conversation and was initiated by Dr. Shock to “observe and document the physical, mental, and emotional effects of the aging process in healthy, active people (22) (51).” Dr. Peter went on to become the first of more than three thousand participants that have since been studied in the BLSA with the current enrollment being more than 1300 individuals. BLSA participants ranging in age from their 20s to 90s are studied every two years with detailed assessments of virtually every aspect of human physiology. They undergo a complete physical exam, tests of mobility, body composition, muscle strength, bone density and geometry, cardio-respiratory function, nervous system anatomy and function, glucose metabolism, inflammation, hormonal status, and more. Core laboratory evaluations include oral glucose tolerance tests, complete blood counts, and comprehensive metabolic profiles. Standardized tests to assess cognitive performance began in 1960 and adjudicated diagnoses of AD/MCI by consensus case conferences using standardized criteria started in 1990. In 1994 Dr. Susan Resnick established the BLSA-neuroimaging substudy (BLSA-NI), prioritizing BLSA participants for admission based on health considerations and the amount of previous cognitive data available for each individual (43, 44). At enrollment, participants were free of central nervous system disease (e.g., epilepsy, stroke, bipolar illness, dementia), severe cardiac disease (e.g., myocardial infarction, coronary artery disease requiring angioplasty or coronary artery bypass surgery), pulmonary disease, or metastatic cancer. Multi-modal neuroimaging data in BLSA-NI include structural magnetic resonance imaging (MRI) including diffusion tensor imaging (DTI) with quantification of white matter lesion volumes, $^{15}$O-water positron emission tomography (PET), and since 2005, in vivo amyloid imaging by $^{11}$C-Pittsburgh Compound-B (PiB) PET. Since 2015, participants have also been enrolled into a tau PET imaging protocol with $^{18}$F-AV-1451. Archived blood plasma, serum and urine samples collected at each visit from every BLSA participant are stored for future analyses in a core biorepository. Genome-wide genotyping data is available in a majority of participants. Approximately half of the BLSA
participants also enroll in an autopsy program and postmortem examination of the brain is performed by an expert team of neuropathologists led by Dr. Juan Troncoso at Johns Hopkins University. Routine studies of post-mortem brain tissue include assessment of AD pathology and quantification of plaque and tangle pathology by CERAD and Braak criteria respectively (39).

2. Tracking preclinical Alzheimer's disease in cognitively normal at-risk individuals

Just as the concept of studying normal aging through serial assessments of healthy individuals was ahead of its time in 1958, the intramural program of the National Institute of Mental Health (NIMH) set out to study the progression of preclinical AD by longitudinal evaluations of cognitively normal at-risk individuals in 1995. The Biomarkers of Cognitive Decline Among Normal Individuals (BIOCARD) study was initiated by Dr. Trey Sunderland in the Geriatric Psychiatry Branch at NIMH and enrolled 349 cognitively normal individuals, a majority of whom had a first degree relative with dementia (26). These participants consented to undergo annual clinical and cognitive evaluations and bi-annual brain MRI and lumbar puncture for cerebrospinal fluid (CSF) analyses. The overarching goal of the BIOCARD study was to identify predictors of progression from normal cognitive status to mild cognitive impairment and/or AD. Considering that the earliest formal recommendation to incorporate imaging and CSF biomarker data in the diagnosis of AD was made by Dubois and colleagues in 2007 (20), and consensus criteria for preclinical AD were first proposed in 2011 (54), the BIOCARD study was a pioneer in its goal of charting this unmapped territory more than a decade earlier. BIOCARD was terminated in 2005 for administrative reasons, and in 2009, the NIA funded Dr. Marilyn Albert at Johns Hopkins University to re-enroll the cohort and continue the study. The entire cohort has since been re-established and initial analyses of CSF, cognitive and MRI data completed (37, 38, 53). Archived blood and CSF samples from BIOCARD participants have been curated and stored in a biomarker core laboratory. Of the 349 participants who entered the original study, 307 provided CSF at baseline, with 199 individuals providing serial CSF samples. Using immunoassay methods (xMAP-based AlzBio3; Innogenetics, Ghent, Belgium) standardized with the Alzheimer's Disease Neuroimaging Initiative (ADNI) biomarker core, assays of t-tau, p-tau and AB42 have been performed on CSF samples in BIOCARD and confirm evolution of the CSF pathophysiological signature prior to the diagnosis of incident MCI (38, 53).
3. **Understanding risk in AD: the advent of biological epidemiology**

The most well established risk factors for AD to emerge from observational studies include obesity, hypercholesterolemia, diabetes, low levels of education, depression, and traumatic brain injury (67) (2, 8, 36, 64) (35). In addition to these environmental/lifestyle risk factors, large-scale genome-wide association studies (GWAS) have identified dozens of common genetic variants associated with risk of AD, besides APOE (17). While these studies are not particularly useful in assessing an individual’s lifetime risk of AD (11, 30), and in the case of non-genetic factors, may provide a basis for recommending lifestyle modifications to preserve brain health, rigorous analyses of the precise molecular mechanisms mediating their associations with AD pathogenesis are lacking. This is best illustrated in the case of environmental/lifestyle risk factors where, a practicing clinician often relies upon an especially thin evidence base to make recommendations about exercise and diet when counseling patients about ways in which they might reduce their risk or slow the progression of AD. It is thus common to hear doctors in memory clinics use phrases such as “what is good for the heart is good for the brain” in response to patients anxious to hear about how they can act to preserve cognitive health. Such nebulous statements aptly reflect the reluctance of researchers to systematically study the biological basis of factors underlying risk of and resilience to AD that have been repeatedly identified by decades of epidemiological research. We are thus incapable of advising our patients on how much, how often, or what type of exercise is most beneficial against AD or what it is about the Mediterranean diet that might be neuroprotective (56). Although one could argue that a “healthy diet” or “regular exercise” are safe and inexpensive interventions that do not require further rigorous studies on their mechanisms of action, this indifference has resulted in a lamentable state of missed opportunity where we have failed to extend traditional epidemiology beyond mere observation and towards precise, actionable intelligence for our patients. Although the reasons for this neglect are numerous and beyond the scope of this article, it is worth introspecting whether our research priorities and the remit of funding agencies supporting them are overly influenced by the single-minded focus of the pharmaceutical industry to invent the one magic formulation that will cure AD.

This is not to deny that there are also other tangible challenges that make such mechanistic studies difficult to undertake in humans. Scientists studying molecular mechanisms of disease rely upon simple biological systems where single gene(s) or protein(s) in a transgenic animal or cell culture model can
be efficiently manipulated within a biological pathway of interest and the
effects of such alterations read out precisely and consistently. However,
these reductionist approaches may be particularly unsuitable in the context
of understanding the biological basis of risk and resilience in AD, a disease of
the aging brain that is likely to represent the culmination of decades of
complex gene-environment interactions.

In our own studies, we have taken on this challenge by combining the deep
phenotyping of human physiology in well characterized cohorts such as the
BLSA/BIOCARD with high-throughput tools of modern biology that can be
applied to understand the molecular mechanisms underlying transitions
from normal aging to cognitive impairment and AD. In these studies, our
emphasis is to be varied in framing our questions and agnostic in our search
for answers, relying upon combining insights from multiple methods and
allowing the data to direct a deeper understanding of specific environmental
or genetic risk factors that drive the evolution of AD in humans at the
population level. We have applied this strategy to study the associations of
novel genetic risk variants such as CLU, CR1 and PICALM with specific AD
endophenotypes including resting state cerebral blood flow (rCBF), brain
amyloid deposition and the age-at-onset (AAO) (59, 60, 62). In the case of the
CLU gene, these studies have aligned especially well with our previous
discovery using mass spectrometry-based proteomic analyses of plasma in
combination with neuroimaging (structural MRI and 11C-PiB PET), that
concentration of clusterin (also called apolipoprotein-J/apol) is related to
severity of cognitive impairment in AD and reflects core features of AD
neuropathology (58). In the BLSA, we have leveraged the rich longitudinal
MRI data in the neuroimaging substudy to show that plasma clusterin
concentrations are also related to longitudinal rates of brain atrophy in
cognitively normal individuals converting to MCI (58). These findings have
been widely replicated and have also been extended by other groups to
demonstrate an interaction between CSF concentrations of clusterin and Aβ42
that influence longitudinal rates of atrophy within the entorhinal cortex in
AD (18).

Are there therapeutic implications of these findings on clusterin, a multi-
functional extracellular chaperone protein and complement modulator that
may be relevant for patients with AD? Looking further afield at advances in
experimental therapeutics in oncology, the development of custirsen, an
antisense oligonucleotide to clusterin has been a promising advance for
patients with castration-resistant prostate cancer (CRPC) (9, 10, 34). The
observation that the secreted isoform of clusterin, sCLU exerts anti-apoptotic activity in cancer cells led to the development of anti-sCLU based oligonucleotide therapy as an experimental approach in prostate cancer (31). Whether similar experimental manipulations altering the ratio of the nuclear:secreted clusterin (n:CLU:sCLU) may be a relevant therapeutic strategy in AD is worthy of testing in experimental models and a rational extension of human studies such as ours, as well as previous observations in animal models, implicating this multifunctional protein in AD pathogenesis.

Within the realm of non-genetic risk factors for AD, we have used a similar strategy in the BLSA, leveraging multiple methodological approaches to study relationships between insulin resistance (IR) and midlife adiposity with AD pathology. While IR and obesity are traditionally grouped under the common umbrella of “vascular” risk factors, our studies indicate that they each impact AD risk through distinct mechanisms. In our analyses, we used longitudinal oral glucose tolerance data in combination with neuropathological assessments and in vivo amyloid imaging to show that IR does not appear to impact the severity of either amyloid or neurofibrillary pathology in AD, suggesting that its role may be downstream of both Aβ deposition and tau phosphorylation (61, 63). In contrast to IR, midlife adiposity is associated with greater severity of neurofibrillary pathology and lowers the age-at-onset of AD, with each increment in BMI at 50 years of age being associated with a lowering of the AAO by almost 7 months (12). Our results provide strong evidence for long-lasting effects of midlife obesity on accelerating the course of AD and severity of associated neuropathology. We believe that these findings are of considerable public health importance as they reveal a long window of opportunity when lifestyle interventions against obesity may be initiated to slow the trajectory of AD progression.

While these studies are important in delineating distinct routes to AD associated with specific risk factors, we are also interested in understanding the neurobiological basis of behaviors related to such “lifestyle” risks. This is important to develop meaningful and effective behavioral modifications against lifestyle-related risk factors such as obesity. However, the biological bases of obesity-related behaviors are poorly understood. Popular culture and the media perpetuate a ‘headless, hungry and unhealthy’ stereotype of the overweight individual as weak-willed, susceptible to the temptation of high calorie foods and prone to ill health (42). However, it is unclear whether a common biological mechanism underlies both a predisposition to obesity as well as impulsive behavior and a preference for calorie-dense foods.
have studied the common fat-mass and obesity-associated (FTO) gene and its relationships with trajectories of adiposity and several other intermediate phenotypes including brain function (through $^{15}$O-water PET), impulsivity (through analyses of longitudinal personality measures from the NEO Personality Inventory; NEO-PI-R) and macronutrient intake (from longitudinal dietary records) during aging (13) (figure-1). We first confirmed previous studies by showing that risk allele carriers of FTO show higher trajectories of BMI relative to non-carriers. Then, using $^{15}$O-water PET in the BLSA neuroimaging substudy, we showed that FTO risk allele carriers show longitudinal decreases in brain function within the ventro-medial prefrontal cortex (vmPFC) in regions known to be important for impulse control (21, 66) and taste responsiveness (45, 46). Complementing the neuroimaging results, we found that risk allele carriers also show greater impulsivity during aging as well as a preference for high calorie foods. Taken together, our results suggest that a common neural mechanism may underlie obesity-associated impulsivity and increased consumption of high-calorie foods during aging.

The examples of the studies above illustrate the utility of combining epidemiological analyses of observational data with deep phenotyping of longitudinal changes in human physiology to understand how risk for AD impacts its progression during the earliest stages of disease. In the following sections, we outline the design of ongoing studies where we also combine high-dimensional data from multiple “omics” methods (transcriptomics, proteomics and metabolomics) to further enhance these studies in the pursuit of a complete understanding of mechanisms underlying early stages of AD progression.

4. “OMICS” to the fore: when biology meets epidemiology

We have initiated a series of proteomics and metabolomics studies on archived frozen brain tissue material from the autopsy sample of the BLSA to identify the molecular correlates of AD pathology, map them to known signaling pathways and establish their roles in the progression of AD. These analyses are also aimed at better understanding the molecular mechanisms underlying cognitive resilience in the presence of AD pathology. Although the presence of amyloid plaques and neurofibrillary tangles in the brain are the defining hallmarks of AD and provide confirmatory evidence at death to support a clinical diagnosis of AD, several studies have shown that these pathological features may be present in upto 50% of older individuals who did not show any signs of cognitive impairment during life (3). In the BLSA,
we have previously characterized these individuals as 'asymptomatic AD' (ASYMAD) to denote the presence of significant AD pathology in the brain without accompanying clinical features of AD during life (28). While the majority of mechanistic studies in AD have been directed towards understanding the molecular basis of AD risk, surprisingly few have attempted to characterize the biological basis of resilience to AD pathology.

We have acquired proteomic and metabolomic data in brain tissue samples from three groups of BLSA participants- 'controls' i.e. cognitively normal during life with no evidence of AD pathology at autopsy, ‘Alzheimer’s disease’ i.e. with a clinical diagnosis of probable/possible AD during life with autopsy confirmation and 'ASYMAD' i.e. without cognitive impairment during life but with significant AD pathology at autopsy. We have sampled three brain regions (middle frontal gyrus; MFG, inferior temporal gyrus; ITG and the cerebellum). Our rationale was to study brain regions both vulnerable to distinct core pathological features of AD (i.e. MFG; amyloid accumulation and ITG; tau deposition) as well as resistant to classical AD pathology (i.e. cerebellum) (33). In these samples, we have acquired metabolite and protein data using the following methods:

i) Quantitative and targeted metabolomics: BIOCRATES P180 Absolute IDQ™ platform assaying 180 metabolites in various classes including glycerophospholipids, sphingolipids, acylcarnitines, biogenic amines and hexoses.

ii) Global, untargeted metabolomic profiling: Ultra-performance liquid chromatography coupled with mass spectrometry (UPLC-MS) for lipid species, hydrophilic interaction-chromatography (HILIC-MS) for polar metabolites and Gas chromatography-mass spectrometry (GC-MS) for compounds such as prostaglandins, bile acids, amino acids and small peptides.

iii) Global, untargeted proteomic profiling by LC-MS/MS and label free quantification: These data were acquired in the MFG and precuneus regions. (data acquired through collaboration with Nicholas Seyfried and Allan Levey, Emory University and available at https://www.synapse.org/#!Synapse:syn3606086).

In parallel analyses, we have also acquired targeted and global metabolomics data using the same methods as above in serum samples from nearly 200 BLSA participants. While all these participants were cognitively normal at baseline, approximately half converted to MCI/AD at follow-up. By assessing serum metabolite levels before and after conversion in the "converters" in comparison to "non-converters," we are able to identify distinct peripheral metabolite signatures of AD in both the preclinical and symptomatic stages of disease progression (4). Equally importantly, by integrating analyses of large scale 'omics' datasets in brain and blood, we are able to derive unique insights into central-peripheral
metabolite fluxes that may be key drivers of AD pathogenesis. In primary analyses performed in brain samples, we thus aim to identify brain-metabolite signatures of AD pathology, and ask if serum levels of the same metabolites in a separate group of individuals, signal disease progression. Conversely, we can derive systemic/peripheral signatures in the serum associated with *a priori* risk factors of interest such as insulin resistance or systemic inflammation and ask whether these metabolites are also associated with regionally specific measures of AD pathology.

These studies have yielded novel findings including the association of brain fatty acid concentrations to severity of AD pathology and symptom onset (52) and the discovery of alpha2 macroglobulin, an acute phase protein as a sex-specific marker of neuronal injury associated with tau phosphorylation states in preclinical AD (65) (23) (figure-2). In ongoing studies, we are examining the role of brain glucose dysregulation and its relationship to longitudinal trajectories of serum glucose concentrations decades prior to the onset of AD symptoms. In these analyses, we combine brain tissue quantification of glucose levels by targeted metabolomics and proteomic assays of the neuronal (GLUT3) and astrocytic (GLUT1) glucose transporters with longitudinal serial oral glucose tolerance test (OGTT) measures available in the BLSA.

*Strength in diversity: the future of AD therapeutics*

The broad consensus emerging from repeated failures of AD clinical trials is that these interventions are being tested too late in the disease process and that targeting them in high-risk individuals during the preclinical stages of the disease is likely to be more successful (57). A similarly valid concern in anti-Aβ treatment trials is that their therapeutic efficacy may have been masked due to inclusion of patients who did not show evidence of amyloid accumulation in the brain (49). While these are among several improvements in the design of future clinical trials that must be implemented to ensure that we maximize the reliability and interpretability of results, there appears to be a worrying lack of acknowledgment within the AD research community that the original premise of anti-amyloid treatments, i.e. that Aβ is a causal driver of AD may in itself be wrong (27, 50) (1). Even as we await read-outs from pivotal clinical trials like the ‘Anti-Amyloid Treatment in Asymptomatic Alzheimer’s’ (A4) study which will test the efficacy of Aβ immunization using solanezumab, a monoclonal antibody to Aβ as a disease-modifying treatment in preclinical AD (55), the recent failure of solanezumab in the EXPEDITION-3 trial may be an ominous portent for the future of the amyloid cascade hypothesis as the principal driver of AD pathogenesis in sporadic, late-onset AD (48). EXPEDITION-3 was designed after a pooled subgroup analysis of two previous failures of solanezumab, EXPEDITION-1 and EXPEDITION-2 (19) showed that AD patients with “mild” symptoms may have shown some slowing of cognitive decline on the drug compared to those receiving placebo. EXPEDITION-3 therefore enrolled 2,000 mild-moderate AD patients with
imaging-confirmed evidence of brain amyloid plaques and randomized to placebo or
monthly injections of 400 mg solanezumab for 80 weeks. The global study, conducted in 11
countries and 210 study sites failed to show any significant separation between
solanezumab and placebo on the ADAS-Cog14, a combined assessment of cognition and
function that was the study's primary endpoint. These results further highlight a key
unresolved and deceptively simple question at the very heart of our quest for effective AD
treatments (5, 6):

Does AD cause plaques and tangles in the brain or do these lesions cause the disease?

We propose that the most effective strategy that can address this question and move us
closer to disease-modifying treatments is one that employs a systems level approach in
human subjects without \textit{a priori} assumptions about the nature or identity of molecular
mechanisms leading to the evolution of AD symptoms and neuropathology. This calls for a
combining of forces across disciplines, a rejection of dogma, openness to pose a broad
range of questions, the willingness to use a diverse array of tools and to go fearlessly where
the data leads us. Our success in implementing such an integrated and \textit{extraordinarily}
diverse framework for translational research in AD will eventually determine whether or
not we will overcome one of the defining global public health challenges of our times.

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Legends for figures

Figure-1
A2M is a sex-specific marker of neuronal injury in preclinical AD (A) Using the BIOCARD and ADNI studies, we explored associations between A2M and CSF measures of preclinical AD pathophysiology and risk of MCI/AD. (B) Using publicly available gene expression data acquired from the GTEx project (https://www.gtexportal.org/home/), we tested the association between blood and brain A2M gene expression. (C) Using publicly available gene expression data acquired from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) we explored global gene networks driving A2M gene expression (C.I.) and brain specific gene expression correlations (C.II.). Protein expression data from autopsy samples in the BLSA was used to validate gene expression findings (C.III.).

Figure reproduced from Varma VR et al. Alpha-2 macroglobulin in Alzheimer’s disease: a marker of neuronal injury through the RCAN1 pathway. MOLECULAR PSYCHIATRY 22(1):13-23 (2017)

Figure-2
The Fat mass- and obesity-associated gene, FTO is associated with (A) adiposity, longitudinal changes in brain function (B), impulsivity and dietary patterns (C) during aging.

A) We first tested whether FTO genotype (rs1421085 single nucleotide polymorphism; obesity-risk allele-C) influenced trajectories of adiposity during aging in the BLSA B) We then examined the association between FTO genotype and longitudinal changes in brain function, measured by serial resting state cerebral blood flow (rCBF) through $^{15}$O-water PET imaging in the neuroimaging substudy of the BLSA (BLSA-NI) C) Finally, based on our longitudinal rCBF results implicating brain regions involved in impulse control and taste responsiveness to food, we tested whether FTO genotype influenced longitudinal changes in impulsivity and macronutrient intake patterns during aging.

Figure reproduced from Chuang Y-F et al. FTO genotype and aging: pleiotropic longitudinal effects on adiposity, brain function, impulsivity and diet. MOLECULAR PSYCHIATRY 20(1):133-139 (2015)


a. Is serum/plasma A2M concentration associated with preclinical AD?

Data: Primary: BIOCARD (n=303) Replication: ADNI (n=566)

- CSF AD biomarkers
- Risk of MCI/AD
- Cognitive performance

- A2M
- Neuronal injury (t-tau & p-tau)

b. Does serum/plasma A2M concentration reflect brain A2M concentration?

Data: GTEx (n = 921)

mRNA

Hippocampus

Serum A2M

Brain A2M

c. What are the molecular pathways underlying association between A2M and neuronal injury?

I. Data: GEO (n = 4755)

mRNA

ABCAB8

SLIT2

MYH11

SPARCL1

APOC5

C2

A2M

II. Data: GEO (n = 281)

Entorhinal cortex

Hippocampus

RCAN1

Calcineurin

Brain A2M

III. Data: BLSA (n = 47)

Middle frontal gyrus
a. *FTO* and longitudinal changes in BMI during aging

- BLSA (N=697)

1958, 1960, **every 2 years**... 2012

Baseline

b. *FTO* and longitudinal changes in brain function

- BLSA (N=697) → BLSA-NI
  - N=69; 560 scans; follow-up interval 8.1 years.

- 

- 

- [80] water PET scans

1958, **1994**, Baseline

c. *FTO* and longitudinal changes in impulsivity and dietary patterns during aging

- Impulse control regions

- Secondary taste area

- BLSA (N=692)

1958, 1981, **every 2 years**... 1993

- NEO-PI-R

- 7-day dietary record
A. Is serum/plasma A2M concentration associated with preclinical AD?

Data: Primary: BIOCARD (n=303)
Replication: ADNI (n=566)

B. Does serum/plasma A2M concentration reflect brain A2M concentration?

Data: GTEx (n = 921)

C. What are the molecular pathways underlying association between A2M and neuronal injury?

I. Data: GEO (n = 4755)
II. Data: GEO (n = 281)
III. Data: BLSA (n = 47)
a  
*FTO and longitudinal changes in BMI during aging*

BLSA (N=697)  
1958 1960 *** every 2 years *** 2012  
Baseline

b  
*FTO and longitudinal changes in brain function*

BLSA  
BLSA-NI  
N=69; 560 scans; follow-up interval 8.1 years.

[c]  
*FTO and longitudinal changes in impulsivity and dietary patterns during aging*

BLSA (N=692)  
1958 1989 *** every 2 years *** 1994  
Baseline  
NEO-PI-R

Secondary  
Impulse control  
taste area

7-day dietary record

Excitement seeking