UNIVERSITY OF CALIFORNIA,
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Metabolic Systems Dyshomeostases Characterize Alzheimer's Disease:
Diverse Plasma Metabolomic Evidence from
LOAD, DS-AD, and ADAD

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Biomedical Sciences

by

Thomas Jeffrey Gross

Dissertation Committee:
Dr. Mark Mapstone, Co-Chair
Dr. Christine Gall, Co-Chair
Dr. Elizabeth Head
Dr. Brian Cummings
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DEDICATION

To the kid who so often looked to this moment
With trepidation, but then resolve
For so many long years

These pages are for you
And your Kind

And all those who suffer and have suffered
neurodegenerative diseases of abnormal aging

To my sister Sarah,
I've looked up to you so much
In these past few years

In jungles of poisonous plants strut the peacocks,
Though medicine gardens of beauty lie near.
The masses of peacocks do not find gardens pleasant,
But thrive on the essence of poisonous plants.

In similar fashion the brave Bodhisattvas
Remain in the jungle of worldly concern.
No matter how joyful this world's pleasure gardens,
These Brave Ones are never attracted to pleasures,
But thrive in the jungle of suffering and pain.
-*The Wheel of Sharp Weapons,* Verses 1-2

WHEN ONE CHOOSES TO WALK THE WAY OF THE MANDALORE,
YOU ARE BOTH HUNTER AND PREY.
HOW CAN ONE BE A COWARD IF ONE CHOOSES THIS WAY OF LIFE?
—The Armorer, *The Mandalorian (2019)*

Get beyond love and grief:
Exist for the good of Man
-Miyamoto Musashi*, The Book of Five Rings (1643)*

GATE, GATE
PARAGATE, PARASAMGATE
BODHI SOHA!
-Prajnaparamita Mantra

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VITA

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ABSTRACT OF THE DISSERTATION

Metabolic Systems Dyshomeostases Characterize Alzheimer's Disease:
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Thomas Jeffrey Gross
Doctor of Philosophy in Biomedical Sciences
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Professor Mark Mapstone, Co-Chair
Professor Christine Gall, Co-Chair

Alzheimer's disease (AD) has proven remarkably refractory to proposed and approved therapies, none of which has strongly demonstrated the capability to halt, sustainedly decelerate, or reverse cognitive decline in emerging disease. Although much translational research in AD has targeted amyloid plaque and tau proteopathies, burgeoning metabolomics technologies in the past decade have enabled the large-scale survey of the peripheral plasma metabolome in these vulnerably aging individuals. This is advantageous because substantial evidence exists that AD can be described as a complex biological system of peripherally evident, metabolic dyshomeostases in the process of abnormal cognitive aging. It is substantially less clear, however, how personalized-medicine-relevant individual differences in AD etiology and cognitive staging map (as jeopardized CNS-peripheral axes) onto this diversity of interconnected and embedded metabolic networks.

To explore this question, sporadic late-onset AD (LOAD) participants at the preclinical stage of disease were profiled using genome-scale metabolic network modeling over features of the plasma metabolome altered relative to controls. This revealed a

dysmetabolic signature (including lipids) which significantly overlapped with that of an independent cohort of preclinical LOAD participants. Further experiments in Down syndrome AD (DS-AD) suggested a similar alteration of lipids in manifest disease, but also central carbon metabolites vital to cellular bioenergetic homeostasis. To more closely examine this peripheral dysmetabolic heterogeneity in more comparable cognitive terms, Preclinical LOAD and preclinical familial, autosomal dominant AD (ADAD) plasma were compared and found to demonstrate modest, significant overlap. To assess the specificity of this finding, preclinical plasma was also compared to that of those with objective cognitive deficits across both LOAD and ADAD. This again demonstrated significant, modest pathway overlap, and similar metabolic pathways emerged from correlational analyses between metabolomic features and estimated mutation carrier years until diagnosis.

Because of this highly complex degree of residually non-shared, semantically dense information in the plasma metabolome across individual, clinical differences in AD, these biochemicals were mapped to inferred metabolic topics from *de novo* metabolic network modeling using natural language processing (NLP) approaches. Through these same topics, pairwise AD phenotypic comparisons were thus proportionally associated with clusters of biochemicals and enzymes. The fitted, metabolic Topic 4 intriguingly implicated hexosamine/aminoglycan metabolism, which was particularly pronounced in comparisons involving "supernormal," older adults in the highest percentiles of resilient cognitive aging. In continuing to explore these clinical phenotypic- peripheral metabolic mappings in the peripheral metabolome, these efforts will afford increasingly precise, semantic level insights into the biochemical diversity of AD pathobiology. In addition to informing further,

targeted mechanistic research, this will also translationally nominate contextually rich, empirically ascertained biomarker and therapeutic target candidates.

INTRODUCTION

Alzheimer's disease (AD) remains a chronic, progressive, age-associated neurodegenerative illness poorly controlled or remediated by currently FDA-authorized pharmaceuticals [1, 2]. This includes recently approved amyloid-attenuating antibody therapies which will require further clinical investigation in coming years. Unless successfully abated by effective therapeutics nominated by the field, AD threatens to affect 13.8 million Americans alone by 2050 at an estimated cost of 1.1 trillion dollars [3]. Predominantly gene-centric, proteopathic models of AD have proven limited in their capacity to address this unmet need through novel avenues of translational research. It remains unclear, however, if normalizing the disordered metabolism of these protein aggregates can bring about the reliable attenuation of emerging cognitive deficits in AD [4]. Key amongst these organizing frameworks has been the amyloid cascade hypothesis of AD originally proposed by Hardy, Selkoe, Higgins, and colleagues [5-8].

Engineered antibody therapeutics informed by this model and directed against beta-amyloid can attenuate its cortical deposition over trajectories of abnormal cognitive aging. Rigorous, randomized controlled clinical trials have also, however, demonstrated the limited efficacy of these compounds to halt, reverse, or stabilize cognitive decline in emerging and manifest AD [1, 4, 9]. The field continues to await further, imminent data clarifying the efficacy of these therapies within the protracted, early preclinical phase of AD progression [4]. These findings accompany increasing and historical arguments within the field, however, that AD represents a systemically pervasive, complexly heterogeneous pathophysiological process in abnormal cognitive aging substantially involving dysmetabolism [10]. In this capacity, data-intensive, high-throughput "-omics" methodologies (e.g., transcriptomics,

proteomics, metabolomics) broadly surveying peripheral biofluids may prove disproportionately useful to inform and direct an actionable translational systems biology of AD [11-14]. Critically, such aims may soon prove vital to the successful identification and biological contextualization of effective AD biomarkers and therapeutic targets [15-17].

Moreover, the distribution of these targets across vulnerably aging physiology may involve metabolism at multiple, hierarchical biological scales of organization [18-23]. The growing need to better translationally profile this complexity aligns substantially with recent proposals for new, agile mechanisms for accelerated translational biomedical research leveraging these "-omics" technologies to further personalized/precision healthcare [24]. It is thus not surprising that dynamic and sensitive changes to peripheral blood plasma biochemistry associated with AD (and appreciable through metabolomics) suggest promising, minimally invasive biomarker candidates informing upon emerging dementia [11-13, 25-32]. In parallel, metabolically targeted therapeutics (including inexpensive and minimally risk-prone lifestyle and dietary interventions) may provide novel avenues to support cognitively healthy aging [33, 34].

Cortical amyloid and tau proteopathies historically better studied in AD, however, remain definitional of the disease at autopsy and increasingly inform upon its antemortem monitoring (e.g., through CSF/ peripheral blood proteomics, neuroimaging) [35-45]. These dissociable patterns of neuropathology in relation to systemic dysmetabolism and attributes of the AD cognitive/ clinical phenotype, however, remain unclear and understudied to date [46]. Concretely, the stratification of peripheral blood plasma metabolomic composition according to AD clinical phenotype (in terms of its progressive development, discrete predisposing etiologies) may suggest novel biomolecular inferences regarding the disease in

explicitly systems biological terms of translational value. If these differing metabolomic profiles can be linked to dissociable clinical phenotypic attributes of AD, these mappings can then be contextualized and translationally targeted as discrete elements of known biological networks. In doing so, this strategy will rationally and semantically advance candidate AD biomarkers and therapeutic/interventional targets. In all cases, such findings would result from the antemortem characterization of emerging AD in vulnerably aging adults themselves and exist posed in relation to corresponding, dissociable attributes of the clinical disease phenotype (e.g., progressing cognitive status, differing predisposing etiologies).

1.1 The Amyloid Cascade Hypothesis: *A Historically Dominant Translational Model*

Central to the initial descriptions of the disease by Alois Alzheimer himself, neuritic, dense-core beta amyloid plaques and neurofibrillary tangles (NFTs) within the cortex, by definition, characterize AD in addition to its antemortem trajectory of cognitive decline [47, 48]. In the second half of the 20th century, cortical plaque proteopathies in AD were identified as amyloids through ultrastructural microscopy [49]. This broader class of pathologically disordered protein aggregates also associate with (if not drive) disease across diverse human tissues and organ systems (many of them intensively metabolic) [50-63]. It was not until the findings of Glenner, Wong, and colleagues in the mid 1980s that the composition of these plaques was identified molecularly as beta amyloid [64-66]. Parallel efforts in diverse clinical populations at substantially, genetically elevated risk of primary cortical amyloidosis (autosomal dominant Mendelian AD, **ADAD**; Down syndrome AD, **DS-AD**) soon thereafter implicated several genes possessing amyloid processing functions in AD. Productive genetic linkage mapping experiments in the late 1980s and early 1990s associated ADAD with highly penetrant Mendelian coding mutations of the amyloid precursor protein (*APP*) and

presenilin 1-2 (*PSEN1-2*) genes [67-73]. These amyloid-specific perspectives gained support from independent observations that those with Down syndrome (most often caused by trisomy of chromosome 21) experience AD earlier and disproportionately more compared to euploid, same-aged peers [46, 64, 74-78]. Because the human APP gene localizes to chromosome 21 and many of these genes are expressed at an approximately 3:2 trisomic dosage in Down syndrome (DS), genetically amyloid-centric hypotheses of AD proposed a compelling rationale for the shared burden of early-onset cortical amyloidosis and progressive cognitive deficits experienced by both familial (ADAD) and DS (DS-AD) aging cohorts [46].

Hardy, Higgins, Selkoe, and colleagues thus advanced a distinctly molecular and gene-centric model of AD proposing that aging-associated pathological alterations of amyloid metabolism causatively precipitate subsequent neurofibrillary tauopathy, with these proteopathies further resulting in neurodegeneration [5-8, 79]. From a systems biological perspective, the amyloid cascade hypothesis represents a feedforward pathobiological sequence which progresses linearly in both its mechanistic and temporal sequence. Dementia critically represents the terminal outcome of this proposed, neuropathologically cascading process. Although highly biologically finite and parsimonious, the amyloid cascade hypothesis has proven limited in its ability to nominate effective AD therapeutic targets promoting distinct cognitive benefits even when amyloidosis is successfully attenuated [1, 4, 59, 61, 80-87]. This suggests substantial opportunities for AD translational systems biology to more broadly ask novel questions regarding pathological aging in AD as ascertained from cognitively vulnerable, aging populations themselves and quantified through emerging, "-omics"-scale experiments. In multiple respects, translational systems

biology is uniquely equipped to leverage the clinical diversity of AD much as some have recently called for the future of "-omics"-driven AD research to be "extraordinarily diverse" in its own multidisciplinary bases, biological approaches, and research methods [88].

The biological relationship of amyloid cascade attributes to antemortem cognitive functioning remains an active area of research [38-40, 89]; however, its conceptualization strictly in terms of the amyloid cascade presents at least two challenges. First and because the amyloid cascade contextualizes dementia as the terminal outcome of AD proteopathies, this perspective belies the disease's extended biological and phenotypic dynamism as an evolving pathological process during the antemortem period of emerging illness [6, 35, 37, 90, 91]. Second, although the amyloid cascade draws support from multiple, distinct clinical populations at elevated risk of early-onset disease (ADAD, DS-AD), these populations nonetheless constitute limitedly representative, highly penetrant genetic etiologies predisposing relatively few aging adults to elevated dementia risk. In this capacity, the amyloid cascade hypothesis does not directly account for the greater than 95% of individuals who will develop AD due to no other singular risk factor than advanced age itself (i.e., sporadic, late-onset AD; **LOAD**) [46, 92].

Despite the ultimate ubiquity of amyloid and tau proteopathies in AD, a marked diversity of causatively upstream etiologic burden (i.e., LOAD, DS-AD, ADAD) confers jointly elevated risk of AD-associated pathological hallmarks and cognitive decline in abnormal aging [46]. This suggests that multiple systems pathobiological processes implicated in abnormal cognitive decline (which might be enumerable and experimentally dissociable) somehow converge upon cortical beta amyloidosis and neurofibrillary tauopathy in the ultimate course of AD [38-40, 89]. The systems biologically organizing principle (or

principles) accounting for these observations currently remain unclear to the detriment of translational biomarker and therapeutic discovery efforts within the field. If this pattern of ultimately convergent etiopathological burden cannot be taken for granted in the progressive sequence of AD cognitive decline, the amyloid cascade model alone provides limited insight into the basis for its observation across a range of diverse, at-risk clinical populations aging vulnerably.

AD might instead be described as a complex, hierarchically distributed disease process of unclear biological scope and extent in abnormal aging. If so, then such biological change systemically anticipating frank cognitive decline may be quantitatively and semantically interpretable in terms of antemortem clinical phenotype within aging adults themselves, where "-omics" methods make possible and enrich these efforts [93-95]. Such dissociations in terms of discrete, predisposing etiologies (LOAD, DS-AD, ADAD) and cognitive status will ideally inform upon why (in biochemical and molecular terms) AD represents a process of systems-biologically-collapsing diversity through progressing illness despite the initial diversity of its drivers. Critically and distinct from the amyloid cascade hypothesis, the contemporary National Institute on Aging- Alzheimer's Association (NIA-AA) model proposes an alternative framework which explicitly frames AD as an antemortem, dynamic systems biological process in abnormal aging [35, 37, 39, 40, 91, 96, 97]. As empirically pursued in this dissertation, the NIA-AA model of advancing disease considers the progressive cognitive change in AD as a systems biological phenomenon to be understood in terms of discrete, dementia-associated biological processes. This will be enabled, if not made possible because of, emerging translational systems biological approaches and methods including "-omics" methods including metabolomics.

1.2 The NIA-AA Model: *AD as an Antemortem, Dynamical Systems Biological Process*

In contrast to the amyloid cascade hypothesis, the NIA-AA model describes the ultimate occurrence of frank dementia in AD as the outcome of substantially extended "metastable" biological change antemortem [98, 99]. Specifically, the dynamics of these emerging deficits are characterized by clinical phenotypic resilience to cognitive decline despite accompanying systems biological instability in emerging AD. In the progression of abnormal age-associated cognitive impairment, this may suggest a disease process organized around incomplete and ultimately abortive compensations for emerging failure. These patterns of systems biological compromise may, in turn, contribute to pathological, feedforward failures of homeostatic and functional compensation in a compoundingly antagonistic, increasingly futile cycle characterizing the frank cognitive deterioration of manifest AD [15, 100-104]. Dissociable from the large-effect-size genetic factors (i.e., trisomy 21, autosomal dominant protein-coding mutations) which inform the amyloid cascade hypothesis (through DS-AD and ADAD respectively), these pathologically constrained trajectories of attempted compensation suggested by the NIA-AA model do not represent processes categorically distinct from aging itself. As initially described by Sperling and colleagues, the NIA-AA model instead understands AD as a continuous and pathological trajectory departing from healthy cognitive aging to an accelerating degree with progressing decline [90, 91]. That the NIA-AA model specifically underscores protracted biological change substantially anticipating frank cognitive change in disease progression reiterates the significance of this antemortem period to AD, particularly for the development of early biomarkers and effective therapies.

The fact that AD constitutes a substantially dynamical and complex antemortem systems biological process thus represents an important, unique emphasis of the NIA-AA model strongly supported by more than a decade of research in living human participants employing CSF proteomics, diverse neuroimaging measures, and peripheral blood biomarkers. Phenotypically stratified according to detailed neuropsychological measures ascertained from these same individuals, translational AD research informed by NIA-AA aims to understand the disease in a top-down capacity (e.g., from attributes of clinical phenotype to dissociably implicated pathobiology) within vulnerably aging adults themselves. This confers a unique advantage compared to efforts primarily informed by the amyloid cascade hypothesis, particularly as it has historically driven biologically bottom-up investigations of ADAD-informed transgenic rodent models (e.g., 5xFAD, 3xTg). These have unfortunately proven limited in their capacity to suggest durable and actionable translational inferences directly transferrable with clinical benefit to vulnerably aging human populations [105]. Acknowledging these limitations, recent efforts to develop more-representative rodent models of LOAD are ongoing and have substantially involved systems biological experimentation and "-omics" [106, 107].

The human participants emphasis of NIA-AA, in contrast, attenuates many such threats to reproducibility and translational potential, where ongoing investigation within the field continues to clarify the specific applicability of these limitedly invasive research methods in practice. Even where specifically human neuropathology has been considered in AD research, the historical absence of minimally invasive measures of antemortem AD pathology (i.e., amyloid, tau) has limited the evaluation of cognitive-pathologic clinical correlations to the autopsy setting [108, 109]. The integration of antemortem biomarker

findings with detailed clinical phenotyping (including cognitive assessment) in in aging adults with AD thus poses a powerful, emerging research paradigm necessary to study the full, dynamic complexity of this multifactorial disease.

The development of the NIA-AA model has also suggested gaps in our knowledge surrounding even the amyloid and tau pathologies definitionally characterizing the disease postmortem. Despite substantial recent efforts, antemortem studies in AD have demonstrated minimal evidence for a uniformly and ordinally fixed sequence of cortical amyloid, tau, and neurodegenerative biomarker findings as advanced by the pathobiologically linear amyloid cascade [39, 40, 110-113]. Whether this itself exists subject to further biological dissociation according to moderating demographic variables (specific predisposing etiologies, sex, estimated polygenic risk) remains uncertain within the field. In contrast, ordinally agnostic scoring of amyloid, tau, and neurodegenerative (A/T/N) biomarker findings in the presence of ongoing or anticipated cognitive instability has become frequently employed [39, 40]. Whether this exists sufficient to capture relevant antemortem biological change in AD leading to translationally effective biomarkers and therapies remains unclear [114]. It remains similarly unclear whether the proteopathic focus of A/T/N in defining AD facilitates novel translational insights into disease biology, where this may instead conceptually recapitulate limitations of the amyloid cascade hypothesis itself [109]. Although all such antemortem biological and cognitive changes codified in NIA-AA represent continuous trajectories preceding ultimately frank decline [40], this temporally evolving pathological sequence can be approximately discretized into three clinical phenotypic stages shared across clinically distinct populations (LOAD, DSAD, ADAD) at elevated risk of eventual dementia. This fixed sequence thus contributes a shared

phenotypic space (i.e., a shared phenotypic criterion) useful for deconvolving peripherally evident, systems biological change in advancing AD conditional upon differentially predisposing sources of etiologic risk [35, 37, 90, 91, 96].

1.3 Clinical Phenotypic Staging of AD: *An Instrumentally Fixed Sequence for Pursuing Translational Systems Biology Aims in Vulnerably Aging Adults Themselves*

The process of advancing AD itself implicates patterns of complexly systemic biological change substantially preceding objective cognitive and ultimately functional decline, as has been demonstrated abundantly by the past decade of antemortem biomarker research in at-risk aging adults. The NIA-AA model thereby advances the stage of preclinical AD as the earliest point at which empirically assessable biological change (i.e., biomarkers) associated with ultimate dementia anticipates the occurrence of objective clinical impairment in disease progression [90, 91]. The preclinical phase of AD consists of a correspondingly unclear duration which may span multiple decades depending upon the specific pathobiology in question. In terms of dynamically evolving complex systems, preclinical AD represents a definitionally "metastable" state in that cognition remains objectively unimpaired concurrent with (and perhaps despite and because of) diverse underlying, disease-associated biological instability [60, 95, 98, 115-121].

Mild cognitive impairment (MCI) constitutes the ordinally subsequent phenotypic stage following preclinical disease in the NIA-AA model [96], where this definition substantially parallels prior phenotypic constructs of MCI [122, 123]. At this stage of progressing illness, at least one objective deficit compared to non-impaired, normative peers becomes apparent (i.e., ≥ 1.5 standard deviation discrepancies in relevant cognitive domains). Amnestic deficits in long-term verbal memory represent attributes of the AD

clinical phenotype which, while not necessary features of AD, possess specific relevance to the role of medial temporal lobe (MTL) structures within the disease (i.e., amnestic MCI, **aMCI**) [96, 124, 125]. It should be emphasized, however, that recently evolved, primate specific (if not human specific) cortical speciation and change (including the MTL) represents anatomy broadly and diversely susceptible in AD [52, 126-150]. Because of its transient role in the clinical sequence of AD, MCI represents a challenging component of the AD cognitive phenotype to assess, particularly in susceptible populations with co-occuring, premorbid, developmental cognitive deficits (i.e., Down syndrome) [151-153]. This difficulty is further complicated by the fact that MCI may genuinely present as either stable or rapidly transient, where the underlying biological correlates of these alternative profiles remain poorly understood [154, 155]. Importantly and definitionally, MCI does not imply decline in functional status and skills of daily living. In contrast, only clinically manifest AD suggests frank decline in at least two distinct cognitive domains where this status proves functionally limiting in daily life within the NIA-AA taxonomy [35, 37, 97].

The past decade of AD antemortem biomarker research has demonstrated the value of biologically stratifying the sequence of clinical phenotypic change associated with progressing dementia in vulnerably aging, human clinical populations themselves. With respect to this axis of phenotypic variability alone, ongoing efforts within the field continue to detail and refine clinical-pathobiological associations and dissociations of potentially substantial translational value [39, 40, 156]. It remains unclear, however, if the ordinal sequence of dementia progression in AD represents the sole or principal aspect of the broader clinical phenotype to be dissociated according to now-emerging translational systems biology approaches leveraging large “-omics”-scale data including metabolomics.

Because the amyloid cascade hypothesis has only limitedly considered this upstream etiologic heterogeneity involved in AD, the dissociation of these factors itself represents an understudied and under-systematized area of research in translational AD systems biology [46]. Despite and perhaps paradoxically because AD definitionally results in ultimately shared pathological features within the cortex (e.g., neuritic Aβ plaques, neurofibrillary tauopathy, synapse loss, neuronal death, cortical atrophy), systems biological perspectives on AD can propose novel methodological approaches to consider and deconvolve peripheral blood pathobiological correlates of these disparate etiologic drivers.

1.4 Causatively Upstream Etiopathological Heterogeneity in AD Susceptibility: *LOAD, DS-AD, and ADAD*

Core pathological attributes of AD (e.g., amyloid plaques, neurofibrillary tauopathy) definitionally become apparent in the ultimate progression of the disease, yet recent findings have suggested that the specific distribution of these features in neuroanatomical space and time may prove complexly dependent upon further dissociable, preceding etiologies (i.e., LOAD, DS-AD, ADAD). These potentially dissociable patterns of systems biological vulnerability in AD according to differing, predisposing etiologies has been only indirectly considered by prior research informed mainly by the amyloid cascade hypothesis. Even at the level of amyloid pathology itself, a lower density of Aβ plaques has been reported in the DS-AD cortex compared to LOAD, where those plaques in DS-AD may possess a comparatively more amorphous morphology and larger average size [46]. Emerging antemortem imaging findings have also suggested that alterations of amyloid (DS-AD, ADAD)[157-159] and glucose (DS-AD) [160] metabolism within the striatum may represent

early events in the onset of AD. Intriguingly, similar patterns have not been described in LOAD. Attributes of the AD clinical phenotype and co-occurring illness (e.g., metabolic/vascular burden, diverse seizure liability, affective/behavioral change) may also be subject to important and clarifying dissociations on the basis of differing etiologies and the extent of developing dementia [46]. Because AD cannot be considered as unidimensional in phenotypic or biological terms, the translationally informative deconvolution of this complexity according to differing, upstream etiologies has become a recent priority to facilitate precision medicine and personalized healthcare. There exist at least three clearly biologically and clinically distinct populations at shared, elevated risk of ultimate AD: sporadic, late-onset AD **(LOAD)**; Down syndrome AD **(DS-AD)**; and familial, autosomal dominant AD **(ADAD)**.

1.4.1 Sporadic, Late-Onset AD: LOAD

The specific investigation of LOAD in dedicated rodent model systems and clinical cohorts represents only a distinct and emerging subset of all AD research historically; yet, this belies the fact that LOAD encompasses greater than 95% of all abnormally aging adults who will develop AD [92]. In contrast to both DS-AD and ADAD, the genetic liability for LOAD presents as highly polygenic in a manner which has proven translationally challenging (i.e., many small-effect-size, yet cumulative contributions to risk distributed throughout the human genome) [161-166]. The ε4 allele of the apolipoprotein E gene (*APOE* ε4) represents the largest single common genetic source of LOAD risk in those otherwise chromosomally typical, with one copy elevating the odds of AD two to threefold and APOE ε4 homozygosity elevating this risk as much as twelvefold [167-169]. Advanced age itself constitutes the only other singular factor substantially predisposing individuals to LOAD.

Consistent with the focus of this dissertation, much emerging biomarker and therapeutic literature in LOAD has pursued the hypothesis that AD exists as a disorder demonstrating metabolic and bioenergetic dyshomeostasis. The description initially made by de la Monte and colleagues of AD as "Type III Diabetes" recasts many of the neuroendocrine, histopathological, molecular, and biochemical attributes of the disease as intrinsically metabolic in their limiting constraints precluding healthy trajectories of successful cognitive aging [16, 170-174]. This accords with findings suggesting that viral infection and/or resurgence [175-180] in addition to cancer history [181-188] may inform upon the primary pathogenic event or events at the earliest preclinical stage of AD. Although perhaps suggesting other relationships in human disease, all of these pathobiological processes demonstrate extensive alterations to systemic metabolism.

Critically, many intrinsically metabolic factors (e.g., sleep quality, diet, exercise) in addition to biopsychosocial wellness exist modifiable in aging, in many cases at relatively low cost and/or risk to participants [17, 34, 189-207]. Current research aims to better understand which attributes of the AD clinical phenotype exist modifiable by these integrative therapeutic approaches according to differing extents of cognitive decline and/or specific, upstream etiologies. As a similarly systemic and integrative biological phenomenon over unclear scales of systems biological organization, the intersection of adaptive molecular functioning and metabolic homeostasis in healthy cognitive aging likely mediates many of the systemically diffuse effects of health-promoting, modifiable lifestyle choices [208-211]. Here too "-omics" methodologies will prove instrumental in advancing specific and discrete biological correlates (if not candidate mechanisms) associated with these global benefits as

a function of individual-specific factors consistent with the aims of precision healthcare for AD.

1.4.2 Down Syndrome AD: DS-AD

As a common genetic disorder (approximately 1 in 700 births), Down syndrome is one strongly genetically penetrant source of both AD risk and premorbid intellectual deficits [75, 76, 78, 212]. Down syndrome is most often caused by trisomy of chromosome 21, which encodes the amyloid precursor protein (*APP*) gene linked to both DS-AD and ADAD through protein coding mutations within this same gene [71, 73]. Established perspectives suggest that overexpression of *APP* in DS neurons at the 3:2 trisomic gene dosage ratio effectively initiates pathological attributes of the amyloid cascade in vulnerable neurons and ultimately leads to cognitive decline [46, 74-76, 213]. More conservatively, gene dosage effects of *APP* in DS-AD (and A*PP* coding mutations in ADAD) explain the precocious and substantial cortical amyloid deposition in these populations. Recent findings have alternatively challenged whether overabundant APP production alone exists sufficient or necessary to drive the broader pathogenesis of DS-AD [214, 215], suggesting that its genetic basis in relation to trisomy 21 remains subject to necessary clarification. With noteworthy, exceptional individuals demonstrating minimal age-associated cognitive decline, approximately 70% of all individuals with DS will develop AD prior to age 65 following substantial cortical amyloid and tau pathology by age 40 [46, 74-76, 78]. Consistent with biologically systemic perspectives on AD, DS (as a syndrome) broadly reflects an accelerated aging phenotype not limited to, but including, the CNS and other metabolically vulnerable, aging tissues (e.g., due to elevated oxidative stress) [78, 216-220]. How these latter biochemical and metabolic factors result from or mutually drive the dementia-promoting

risk of trisomy 21 and *APP* triplication remain open questions addressable through the unique strengths of systems biology and "-omics" methods.

1.4.3 Familial, Autosomal Dominant AD: ADAD

Only 1-2% of all those who develop AD will do so because of autosomal dominant, Mendelian mutations heritable within family pedigrees [92, 221]. These protein coding mutations within amyloidogenic genes (e.g., *APP*, presenilin 1-2/ *PSEN1-2*) have, however, disproportionately informed both the amyloid cascade hypothesis and amyloid-centric therapeutic strategies in translational AD research for decades. Specifically, ADAD mutations transgenically incorporated into rodents (e.g., 5xFAD, 3xTg) represent some of the most established model systems in translational AD research, whereas LOAD-specific rodent models have only very recently been developed and initially characterized [105-107, 222]. Even in this population which has long directly informed genetic perspectives on the amyloidogenic basis of AD pathogenesis, dysmetabolism (of lipids and lipoprotein complexes) may moderate the sequential pathologies described by the amyloid cascade hypothesis [223, 224]. Like DS-AD, the relationship of these metabolic factors and cognitive decline to the cortical proteopathies more directly arising from amyloid-associated, protein-coding mutations remains uncertain, but very likely broader than the linear, molecular dysfunction described by the amyloid cascade hypothesis proper.

1.4.4 Proposed AD Etiopathogenic Dissociations: Summary and Significance

In all, the systematic pursuit of peripheral blood plasma metabolic dissociations based on distinct, predisposing AD etiologies has not been reported in the translational literature to date. The differential association of such distinct etiologies to cognitive staging and status in explicitly systems biological terms has been pursued even less so. This is

unfortunate, as stratification of this peripheral plasma metabolomic variability according to attributes of the AD clinical phenotype may suggest novel biomolecular inferences and insights into the illness. If these instrumental clinical phenotypic dissociations (i.e., based on cognitive staging, differing etiologies) can nominate and contextualize the discrete elements of wider biochemical networks, then these efforts will more systematically advance precision AD biomarkers and therapeutic targets in a rational, data-driven manner. This hypothesis presumes, however, that cognitive staging and antecedent etiologic status in AD exist sufficient to parametrize the disease process phenotypically and, thus, guide robust dissociations of implicated biological processes (at the molecular class and pathway levels) within peripheral blood plasma metabolism. While the present dissertation argues that this is effectively the case (particularly for specifically metabolic investigations of AD) [12, 13, 27-29], further qualifications have been proposed recently within the field. These bear further discussion and consideration regarding their implications for the effective advancement of much-needed translational research investigating AD.

1.5 Defining AD Clinico-Phenotypically for Systems Biology: *Which AD and Why?*

Substantial, productive efforts have been made by the AD field in the past decade to harmonize definitions of the disease nosologically. This has better facilitated its integrative understanding across differing scales of biological analysis, research methodological paradigms, early translational model systems, and sub-disciplinary vernaculars. In many cases, these distinct constructs in relation to abnormal trajectories of cognitive aging have jointly been described as "AD" in different capacities and to differing purposes in research [109]. This has substantially complicated their reconciliation into a durably unified clinical phenotype useful in directing focused translational systems biology efforts in AD [35, 37-40,

89]. The past decade of antemortem biomarker research in those abnormally aging has clearly demonstrated that not all such individuals strictly present with the cortical amyloid plaque and tau pathologies most clearly indicative of AD [39, 40, 108, 109, 114]. In this sense, these neuropathological findings suggestive of AD are sufficient to explain co-evident, age-associated cognitive deficits, although they are not necessary to do so. Mixed pathological findings in cognitively abnormal aging have proven more frequent than was previously thought [39, 40]. Strikingly in the phenomenon of asymptomatic AD (ASYMAD), substantial cortical neuritic amyloid and NFT pathology only becomes apparent postmortem following no documented history of objective, antemortem cognitive decline exceeding that of healthy same-aged peers [225-228]. Posterior cortical atrophy, logopenic primary progressive aphasia, age-associated dysexecutive syndrome, and often corticobasal syndrome share neuritic amyloid and tau pathologies with each other and AD. They do not, however, involve memory-centric, amnestic cognitive deficits colloquially associated with AD dementia. All the same, these neuropathological features satisfy Jack and colleagues' recent antemortem neuropathological definition of AD by definition—"no more and no less" [109]. These clinical and pathobiological descriptions of the disease as both A) age-associated cognitive decline and B) a distinctive pattern of associated amyloid and tau proteopathies clearly illustrates its elusive and multifactorial description in abnormal aging.

For these reasons, the present dissertation will instead prioritize the neuropsychological cognitive staging of AD as definitional of clinical phenotypic status, directing translational systems biological investigation independent of further qualification by amyloid or tau protein biomarkers [39, 40, 109, 151]. Although this poses some possible limitations to resulting translational inferences, the definition of AD in principally

proteopathic terms may prove equally problematic in AD systems biology. One aspect of the disease or the other is not clearly a sole priority for translational research, yet cognitive change itself suggests a multifactorial lens to contextualize AD and its associated biology. Modern and robust cognitive findings of probable AD indeed correspond to a non-zero (but small) probability of false positive diagnosis which has benefitted from modern psychometrics and clinical neuropsychology of aging [109, 151, 229, 230]. In the case of clinically and biologically distinctive populations (DS-AD and ADAD) experiencing genetically-driven risk specifically, any false positivity would likely prove even proportionally less than for LOAD. If only for these former populations at substantial genetic risk of AD, this would support the cognition-focused aims of the present dissertation as it considers the complex biology underpinning these diverse dementia etiologies.

In addition, other age-associated neurological diseases demonstrating characteristic proteopathies and co-occuring cognitive deficits (e.g., Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis/ frontotemporal dementa) may also be investigable by translational systems biology approaches. Future experiments may even productively advance comparisons of these conditions against attributes of the multifactorial AD clinical phenotype reviewed in this introduction. In the context of the present dissertation, however, deliberately focused dissociations of AD clinical phenotypic attributes through systems biology and "-omics" may alone prove translationally valuable and informative. For example, the ordinally ascending ranking of LOAD to DS-AD to ADAD approximately describes a pattern of escalating genetic burden for cortical amyloid deposition in aging [46]. Systems biological dissociations according to differing AD etiologies may thus metabolically identify correlates of this differentially incremental susceptibility to this pathologic burden. In doing

so, the systems biological investigation of metabolism in AD may also suggest further, novel relationships amongst these unique human clinical populations potentially unrelated to amyloid and tau pathologies themselves [12, 13].

Other current perspectives within the field do strongly emphasize hallmark proteopathies in their antemortem definition of AD. Jack and colleagues have recently proposed an amyloid, tau, and neurodegeneration (A/T/N) framework to score evident neuropathology and parametrize a biological definition of AD [39, 40]. It remains substantially unclear, however, if this taxonomy alone exists sufficient to describe the range of pathobiological change characterizing (if not driving) the disease [114, 231]. Both the amyloid cascade hypothesis and the A/T/N framework largely consider glucose dysmetabolism in AD to represent neurodegeneration across multiple biological scales (e.g., tissue atrophy, neuronal death, synapse loss) [6, 39, 40, 232-235]. This contrasts with emerging perspectives increasingly portraying AD as a temporally dynamic and systemic biological process with complex, multifactorial dependencies upon metabolic homeostasis disproportionately constraining (but not limited to) the aging brain in particular [16, 114, 132, 133, 231, 236-240]. In this sense, it remains currently unclear if the A/T/N taxonomy sufficiently reflects or contextualizes the specifically metabolic pathobiology apparent in AD of interest to this dissertation. This only reiterates the importance of employing AD cognitive staging (rather than amyloid and tau alone) to define the clinical dementia phenotype considered by this dissertation independent of further qualifications (e.g., A/T/N). Indeed, it may be the case that antemortem, neuropathologically qualified definitions of AD, in the worst case, introduce underappreciated bias into purportedly "unbiased" systems biological investigations of large "-omics" data. How the field semantically associates AD amyloid and

tau proteopathies in relation to the clinical disease phenotype clearly implies analytical consequences in translational AD systems biology.

Although only preliminarily addressed in this dissertation, these considerations have increasingly challenged the field to consider whether AD represents a condition of A) progressive proteopathies with dysmetabolic spectators or B) metabolic dyshomeostases with associated, progressive proteopathies. This contrasts with the amyloid cascade hypothesis and A/T/N taxonomy in their characterization of AD cortical glucose dysmetabolism as a principally neurodegenerative read-out [6, 35, 39, 40, 91, 96, 232-235]. In recapitulating these pathobiological attributions inherited from the amyloid cascade hypothesis (regarding amyloid, tau, glucose hypometabolism), it remains unclear if A/T/N will ultimately better describe AD in a manner which more successfully advances effective biomarkers and therapeutic targets. This is to say that how the field attributes AD pathobiology has critical bearing on definitions of clinical phenotype and how these definitions, in turn, statistically direct systems biological investigations pursuing translational deliverables (i.e., biomarkers and rationally targeted, precision therapies). The definition of AD clinical phenotype, in this context, remains highly non-trivial in relation to any derived, systems biological inferences including those pursued by experiments proposed here.

These considerations critically do not alter the observation central to this dissertation that an upstream diversity of biologically and clinically dissociable etiologic risk converges (with disease evolution) to pathobiology which is virtually identical in the ultimate progression of AD. If this cannot be taken for granted and remains poorly explained by models such as the amyloid cascade hypothesis and A/T/N, translational AD systems biology

must question what types of biological processes participate in these dynamics in the course of progressing cognitive decline. Of still unclear relationship to amyloid and tau pathologies, AD-associated dysmetabolism of the kind commonly attributed to neurodegeneration may instead suggest broader, organizing constraints upon the brain in abnormal aging. Specifically, its physiological progression accompanying cognitive decline may somehow both A) drive and B) proceed as a function of this eventual uniformity across clinically diverse, at-risk populations. Translational AD systems biology carried out empirically in this dissertation can peripherally dissociate these dysmetabolic constraints in terms of a well-parametrized AD clinical phenotype. If these dissociations in peripheral metabolism show differential metabolism (by etiology, degree of cognitive decline), then this may reflect a physiologically extended CNS-peripheral axis mediating a "final common metabolic pathway" in the etiopathogenesis of AD.

1.6 A "Final Common Pathway" in AD Etiopathogenesis? *Systemic Dysmetabolism as a Candidate Driving Process*

For graphical and heuristic purposes, one can consider some systems biological distribution of various biochemical states in time over the progression of incipient AD (i.e., from preclinical to MCI to finally AD). Empirically, this state space could be quantified and/or parametrized through "-omics" methods (i.e., metabolomics) **(Figure 1)**. This initially broad state space distribution in advancing disease must (by definition) narrow towards exactly the occurrence of neuritic Aβ plaques, neurofibrillary tauopathy, and neurodegeneration ultimately definitional of AD. This invites an etiologically central question: How do dissociably predisposing AD etiologies (LOAD, DS-AD, ADAD) map to the systems biological state space in disease progression at all points prior to the pathological ubiquity

characterizing fully manifest dementia? This dissertation aims to propose several such dissociative mappings to metabolic pathways, consistent with AD being increasingly recognized as a metabolic disorder.

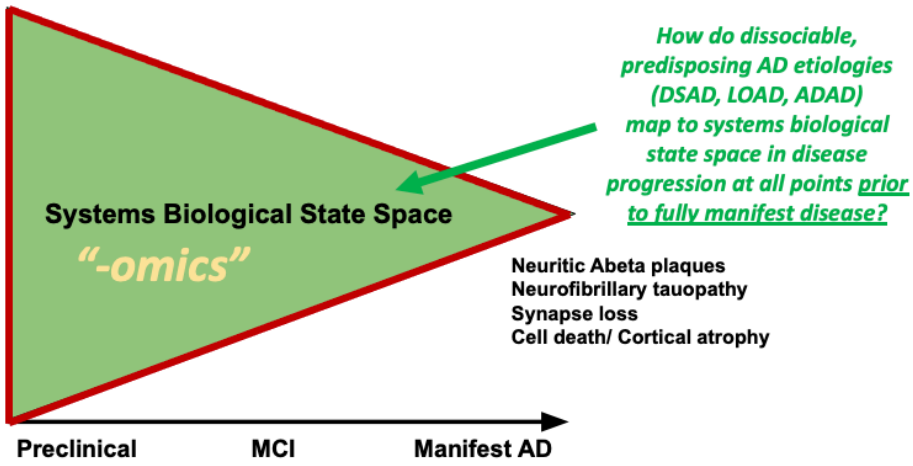

Figure 0.1 A schematic diagram depicts the narrowing distribution of systems biological state space in AD with the clinical phenotypic sequence of progressing dementia.

One possibility may be that precarious metabolic homeostasis in vulnerable cognitive aging reflects a biological system under dynamically increasing constraint in advancing disease, where many of these same constraints themselves implicate metabolism. Innovations in metabolomics and a resurgence of translationally biochemical/ metabolic perspectives over the past decade have indeed increasingly underscored that "the physiological state of cells and tissues reflects both the cell's regulatory systems and its state of intermediary metabolism." To this point, McKnight and others have discussed how the dominance of gene-centric, molecular models of complex, multifactorial human disease has historically obscured their frequently biological interdependencies with metabolism [241]. The amyloid cascade hypothesis of AD also represents a quintessentially molecular model of complex human disease and the molecules it prioritizes also represent metabolic products

and substrates. In this way, metabolic constraints involving (if not driven by) these molecules could also prove functionally and homeostatically limiting in biological systems associated with abnormal cognitive aging.

This pattern of unsustainably incurred "compensations for failure" in pathological aging emerging from untenable "failures of compensation" indeed resembles the NIA-AA model of antemortem change in AD defined in terms of incremental biological and cognitive change [35, 37, 40, 91, 96]. As speculated by McKnight in biology generally, AD may represent one such disease in translational biomedicine which, barring greater consideration of metabolism, proves "simply intractable" in translational, clinical phenotypic terms where the biological duality of molecular function and metabolic homeostasis therein remains under-investigated [241]. Observable independent of (but possibly related to) amyloid and tau pathologies, complex patterns of cortical glucose dysmetabolism inconsistent with frank neurodegeneration appear ubiquitously in early LOAD [132, 133, 233, 235, 242-244], DS-AD [160, 245-248], and ADAD [249].

Within the aging brain, these AD-associated changes instead implicate primate-specific (if not human-specific) neocortical metabolism via aerobic glycolysis [132, 133, 146, 147, 239, 243, 250], induce dynamically hypo and hyper-metabolic states in the sequence of advancing disease [160, 240, 251-253], spare (if not favor) ketone body fuel metabolism [231, 237, 238, 254], and exist in white matter tracts apart from neuronal cell bodies [236]. This suggests that AD involves substantial dysmetabolism within the brain. It does not, however, necessarily support the interpretation of these findings as a neurodegenerative read-out [40, 232]. Because of our better appreciation of its complexity in recent years, these

findings strongly challenge the prevailing interpretation of cortical FDG dysmetabolism in AD as an indicator principally of neurodegeneration or synaptic dyshomeostasis [40, 232].

Recent synaptic vesicle glycoprotein 2A (*SV2A*)-directed PET (i.e., synaptic density PET, [^{11}C] UCB-J PET) in euploid AD participants has indeed demonstrated reduced tracer retention consistent with FDG-PET hypometabolism in the medial temporal lobes. Within these regions, the correlated attenuation of glucose metabolism and synaptic density suggests degeneration-associated metabolic hypoactivity. This occurred, however, in contrast to weaker inter-tracer relationships in the metabolically avid neocortex, which demonstrated glucose hypometabolism consistent with prior findings, but in excess of that suggested by synaptic density loss alone [255]. Similar glucose hypermetabolism inconsistent with neurodegeneration, yet related to cortically global Aβ deposition, has also been recently described in the aging putamen in DS [160], which resembles prior findings of compensatory temporal lobe hypermetabolism and metabolically involved neurite sprouting in this same aging population [252, 256].

Intriguingly, this dysmetabolism (much like systems biological phenomena such as homeostatic adaptation to aerobic exercise, cancer, diet/ lifestyle choices, and the response to infectious pathogens) is evident in peripheral tissues including blood [11-13]. Furthermore, the extent of these disease effects includes developmentally mesodermal-lineage cells (i.e., peripheral blood mononuclear cells, **PBMCs**) altered in those with AD versus cognitively stable controls. This suggests that these systemic effects of AD are not limited to CNS (i.e., neuroectodermal and yolk sac/ myeloid origin) cells and tissues, despite apparently much biological symmetry between CNS and peripheral tissues as a function of the disease process [98]. Instead, the brain may experience disproportionate metabolic

constraints and failure due to AD only compounded and accelerated by systems biological network disconnection involving the periphery and proceeding with illness[19-23]. Many consequences and drivers of this pathophysiological disconnection in AD may fundamentally reflect metabolically and biochemically feedforward processes, the integrity of which can index trajectories of cognitive decline, stability, and resiliency.

Accumulating evidence increasingly suggests that dysmetabolism manifests in AD as systemically perturbed process where its manifestations or correlates within the periphery remain incompletely considered, particularly as these vary according to dissociable factors, namely differing predisposing etiologies and extents of disease development. In the past decade, resurgent metabolic perspectives surrounding the biological basis of disease and increasingly powerful "-omics" (i.e., metabolomics) approaches have provided the opportunity to characterize and evaluate the systemic extent of AD-associated metabolic change in peripheral blood [11-13, 28]. These platforms can inform basic disease research and deep putative-target investigation; however, they can also function as a discovery platform for the highly applied identification of rational, data-driven biomarker and therapeutic candidates in AD. These potentially dynamic and sensitive changes evident peripherally in the course of AD are well-matched to the strengths of modern, "-omics"-scale biochemical measurement platforms surveying fluid specimens such as peripheral blood plasma. Metabolomics contributes a key methodology well-suited to the field's recent biochemical and bioenergetic thinking. It affords complementarily systemic and metabolic readouts of complexly distributed, progressive disease processes such as those hypothesized by this dissertation to be dissociable according to attributes of the AD clinical phenotype.

1.7 Metabolomics: *Measuring the Precursors and Products of Metabolism at "-omics" Scale*

Metabolomics describes the emerging "-omics" approaches to quantifying and characterizing low-molecular-weight, biochemical species (< 1.5 kDa) present in easily accessed human biofluids such as peripheral blood plasma. It employs analytical chemistry instrumentation such as nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), the latter of which represents the platform used to empirically measure metabolite feature abundances considered in this dissertation. Metabolomics currently affords greater uncertainty in molecular annotation compared to proteomics or modern, RNA-seq transcriptomics. The assignment of MS-detected mass fragments to parent molecules of biological significance in metabolomics proves specifically challenging [14, 257].

Hundreds to thousands of metabolite features may be measured in a single metabolomics experiment, yet this large quantity of data exists practically limited by constraints upon metabolite annotation. In practice, substantial proportions (~30%) of MS candidate mass features will not be annotatable [14, 257]. To maximize knowledge derived from metabolomics experiments, alternative approaches to analysis employing genome-scale metabolic models and relying only upon un-annotated feature-wise mass-to-charge ratios have also been reported. Unique in their hybridization of empirical metabolomics with prior knowledge of biochemical pathway interrelationships, this dissertation relies substantially on these computational metabolomics methods (i.e., Mummichog) to interpret phenotypic contrasts in relation to associated biology at the level of pathways rather than putative, single molecules [258].

Peripheral blood plasma metabolomics enables the dynamic and sensitive measurement of systemically ongoing disease processes at the molecular level. In Crick's Central Dogma, these molecules represent some of the most functionally downstream from DNA and proximal to manifest disease biology. At the same time, this oversimplification neglects the many feedback loops interconnecting the metabolome with the proteome, transcriptome, genome, and epigenome [30]. Metabolomics can this directly inform upon peripherally systemic, biochemical changes related to active disease processes differentially according to attributes of the AD clinical phenotype (e.g., differing upstream etiologies) [25, 26]. In this way, a better understanding of AD-associated dysmetabolism may yield inordinate value for A) basic, mechanistic research in dementia and B) the development of biomarker and therapeutic candidates. From a clinical and practical perspective, peripheral blood metabolomics also serves as a relatively inexpensive, minimally invasive, routine, and serially tolerable methodology to better understand AD biology within vulnerably aging human populations themselves. This contributes substantial advantages compared to A) invasive studies using lumbar puncture to ascertain CSF specimens or B) costly neuroimaging approaches using specialized instrumentation/facilities which expose participants to ionizing radiation (in the case of positron emission tomography, **PET**) [27].

CNS-peripheral blood metabolomic relationships remain a productive area under current investigation in AD [228, 259]. It has previously been proposed that peripheral circulating cells act as "sentinels" of emerging disease or otherwise that peripheral circulation distally reflects the downstream metabolites of brain parenchymal A/T/N pathologies [41, 42, 45, 260-262]. This explanation, however, neglects decades of consistent findings suggesting that AD manifests as cell biological changes to peripheral tissues both

neuroectodermal and not in embryologic origin [263-271]. Peripheral blood mononuclear cells and skin-derived fibroblasts from those with AD demonstrate differential dysmetabolism according to predisposing etiologies (e.g., ADAD versus LOAD), where direct, mechanistic pathophysiological relationships between these tissues and AD remain unclear [272, 273]. This exists at least partially dissociable from similar findings implicating the age-associated dysfunction of peripheral, intensively metabolic organs and tissues in AD-associated cognitive decline (e.g., pancreas, liver). Strikingly, pathologies associated with abnormal aging and metabolic compromise within these non-CNS tissues strongly resemble AD amyloid and tau proteopathies in addition to recapitulating patterns of pathobiological liability observed in AD [19-23, 53, 57, 58, 274-280].

As with dysmetabolism overall, it remains unclear if specific peripheral biological perturbations in AD exist as either incidental, epiphenomenal systems biological "bugs" or "features" with respect to elements of the associated clinical phenotype [281, 282]. If the latter proves correct, the phenomenon of pathophysiological dysmetabolism over disparate biological scales exists as an organizing principle possibly informing multiple neurodegenerative diseases (e.g., AD, Parkinson's disease, Huntington's disease), the human host response to viral infection, and multiple cancers [98, 115, 283]. This directly intersects with existing theory surrounding the role of dynamical biological network dyshomeostasis in complex human disease [98]. It furthermore implicates dysmetabolism as a basis for considering the nosologic interrelationships amongst diseases of abnormal aging in terms of complex systems science [284-286]. Indeed, the empirically driven nomination of pathobiological disease correlates subject to these complex dynamics in the clinical phenotypic evolution of AD represents the primary aim of the present dissertation.

Metabolomics affords unique experimental designs in translational antemortem human-participants AD research which are not without caveats; yet, the systemic scope of peripheral plasma metabolomics may also afford insight into AD-associated pathobiology otherwise largely unconsidered in relation to advancing dementia. Exactly this possibility motivates the distinctly peripheral and systemic metabolomic scope of this dissertation. The following empirical chapters explore the hypothesis that these dynamics can be quantified within peripheral blood using metabolomics dissociated in terms of biochemical pathways and processes according to different predisposing etiologies and extents of disease development.

CHAPTER 1. Late-Onset, Sporadic Alzheimer's Disease: **LOAD**

More than 95% of individuals who ultimately experience AD develop its idiopathically and sporadically occurring, late-onset form (LOAD). Its only singular, substantial, and non-*APOE* predictor is advanced age itself [46, 92] . The peripheral biochemical changes associated with LOAD are highly heterogeneous and remain under-contextualized in terms of associated disease biology proximal to the clinical dementia phenotype. This is inadequate to identify disease-modifying individual differences vital to the implementation of precision healthcare, where this information will disproportionately inform care for most adults experiencing AD [24, 27, 30, 94]. Both increasing evidence and historical findings support the substantial role of dysmetabolism accompanying the evolution of LOAD both within and beyond the brain, as reviewed extensively in the previous introductory section. To underscore several examples, LOAD may prove particularly responsive to lifestyle, sleep, dietary, and exercise interventions where all these biologically systemic factors affect or are affected by metabolism [34, 196, 197, 205, 287-289]. Also, components of metabolic syndrome (i.e., insulin resistance/diabetes, dyslipidemia) confer modifiable LOAD risk associated with abnormal cognitive aging [170, 290]. Both dyslipidemia and insulin resistance have also been identified as CNS and peripheral correlates (if not potential drivers) of AD from the earliest stages of disease pathogenesis, even preceding objective psychometric deficits compared to healthy peers [171, 291].

These manifestations of AD as gross metabolic dyshomeostases accompanying abnormal cognitive aging are historically well-established and can be observed using conventional clinical laboratory measures and nuclear imaging [235, 242, 292-298]. Liquid chromatography-mass spectrometry (LC-MS) untargeted metabolomics profiling

approaches can, however, provide much greater molecular resolution to identify previously overlooked biology implicated according to the extent of developing AD (i.e., preclinical, MCI, clinical AD) across at-risk, aging adults [25, 258, 299]. This includes many of the approximately 13.7% of individuals worldwide who carry at least one copy of the *APOE* ε4 allele, the vast majority of whom possess this as their most significantly predisposing, singular genetic risk factor [168, 300]. Considering the recent approval of FDA-approved amyloid modifying therapies, these aims may be particularly important to better understand the early preclinical stage of AD where A) such compounds may be most efficacious and B) biomarker and therapeutic targeting may be of correspondingly high priority. Mapstone and colleagues have reported in LOAD both a 10-lipid (and subsequently a 24-metabolite) blood plasma panel. These demonstrated excellent classification performance (receiver operating characteristic-area under the curve, **ROC AUC** > .90) in detecting imminent cognitive decline (within 2.1 years on average) in individuals healthy at blood draw [28, 29].

These initial experiments in the Rochester-Orange County Aging Study (R/OCAS) cohort of genetically typical aging adults, however, focused primarily on the identification of predictive biomarker panels tracked for translational, clinical use. Subsequent studies have since replicated and biologically considered the substantial association of peripheral blood lipids with cognitive instability in early AD [301]. The pursuit of systemically elaborated metabolic relationships in preclinical AD within R/OCAS peripheral plasma, however, has not been undertaken. The extrapolation of any metabolic inferences from R/OCAS alone poses distinct pitfalls, namely that these plasma measurements might not be representative of individuals experiencing LOAD overall (i.e., that site-specific false findings could exist). Instead, some amount of measured metabolomic variation may also reflect background

spectrometer noise or confounding variability in the implementation of site-specific study protocols [14, 31]. To directly address these limitations, additional biobanked blood specimens from preclinical LOAD participants were obtained from the UCI Alzheimer's Disease Research Center (UCI ADRC). Specifically, these participants were selected to maximize demographic similarity (in years of education, sex, age, and ethnicity) with those individuals reported in R/OCAS. This proactive research design thus anticipates and mitigates the possibility of false positive associations resulting from measurement noise inherent in modern untargeted LC-MS metabolomics profiling. This is accomplished in part by projecting the results of empirical metabolomics experiments onto known biochemical pathways using computational approaches [258, 299].

These analyses can address several questions vital to better understanding the very early metabolic underpinnings of LOAD as evident within peripheral circulation. First, it remains unclear what metabolic pathways and processes beyond lipid metabolism differ between cognitively stable older adults and preclinical individual in R/OCAS. Recent computational approaches (i.e., Mummichog) which integrate prior knowledge of biochemical relationships with empirical metabolomics measurements can address exactly this question. Second, it remains unclear if highly-demographically-comparable plasma samples from UCI ADRC submitted to this same analysis pipeline would suggest similar biochemical perturbations as a function of their shared preclinical LOAD status relative to cognitively stable, matched control participants.

If a core consensus set of biochemical pathways, processes, or molecular classes are common across these independently ascertained R/OCAS and UCI ADRC LOAD cohorts, then these may suggest prioritized biomarker and therapeutic targets for further investigation. If

indeed observable in this largest, genetically typical population of older adults experiencing early AD, these peripheral changes could suggest evidence of a physiologically extended, CNS-peripheral metabolic axis dyshomeostatically altered early in disease pathogenesis. Implicated (and perhaps highly distributed) biological systems might become progressively incompatible with and constrained apart from neurobiological and cognitive trajectories of successful aging in preclinical LOAD.

METHODS

Rochester/ Orange County Aging Study (R/OCAS)

Participants and Cognitive Assessment. A total cohort of 525 aging adults participated in this five-year, longitudinal study of AD cognitive decline in individuals otherwise genetically typical. The University of Rochester Research Subjects Review Board and the University of California, Irvine Institutional Review Board each approved a common research protocol for this investigation. All participants were community-dwelling older adults from the broad Rochester, NY and Irvine, CA areas ascertained by local media, senior organizations, and word of mouth. Participants were included if they were age 70 or older, could proficiently read and write English, and had corrected vision and/or hearing sufficient to complete clinical assessment materials. Participants were excluded if they A) demonstrated other major neurological or psychiatric illness or B) had recent (< 1 month) usage of anticonvulsants, neuroleptics, HAART, antiemetics, and antipsychotics. All participants were followed yearly for the duration of the five-year study or until manifest cognitive impairment became evident.

A battery of cognitive tests was administered by a single investigator (MM), where this was intended to quantify major cognitive domains (attention, executive functions,

language, memory, and visuo-perceptual skills) impaired by emerging LOAD. These test-level assessment scores were aggregated into composite *z*-scores reflecting each domain, where a *z*-score less than 1.35 below the cohort median was considered impaired. These low-scoring individuals were considered to demonstrate incident amnestic mild cognitive impairment or early AD (aMCI/AD). Participants demonstrating stable (> 1 contiguous visits) impairment following initial cognitive health were retrospectively considered to demonstrate preclinical LOAD at this baseline timepoint. Older adults who remained cognitively stable during the duration of the study were considered as healthy controls.

Phlebotomy Protocol, Blood Processing, and Long-Term Storage. All participants underwent blood draws between 8:00 am and 10:00 am on a yearly basis. All morning blood draws were completed under fasting and medication withholding conditions. Whole blood specimens were initially placed on wet ice and fractionated into their components (i.e., plasma) according to standard procedures within 24 hrs. All specimens were stored at -80°C prior to metabolomics analysis at the Lombardi Cancer Center Metabolomics Shared Resource Facility at Georgetown University. All plasma specimens submitted to metabolomics analysis underwent no more than one freeze/thaw cycle. This ensured a minimum of metabolomics analysis artefact due to sample-age-related degradation.

UCI Alzheimer's Disease Research Center Cohort

Participants and Cognitive Assessment. Participant blood specimens were accessioned from the University of California, Irvine Alzheimer's Disease Research Center (UCI ADRC). Donors were at-risk, genetically typical adults enrolled in a longitudinal aging study in which participants completed recurring visits at approximately 9 to 12-month intervals. In addition

to routine phlebotomy and cognitive assessment, some participants at varying durations of follow-up also underwent neuroimaging and/or lumbar puncture for CSF proteomics. Implemented test batteries for profiling age-associated neuropsychological decline conformed to minimal standards established by the National Alzheimer's Coordinating Center Uniform Data Set (**NACC UDS**). Like measures in R/OCAS, the minimal NACC UDS neuropsychological battery intended to quantify major cognitive domains (attention, executive functions, language, memory, and visuo-perceptual skills) impaired by emerging LOAD. These tests included (but were not limited to) the Montreal Cognitive Assessment (MoCA), immediate and delayed story recall, trail making, forwards/backwards digit span, named category fluency, and complex figure recall in addition to overall clinical appraisal.

A single investigator (MM) reviewed longitudinal, participant-level cognitive findings to identify preclinical status. Specifically, test-level assessment scores were aggregated into composite *z*-scores reflecting each domain, where a *z*-score less than 1.35 below the cohort median was considered impaired. These low-scoring individuals were considered to demonstrate incident amnestic mild cognitive impairment or early AD (aMCI/AD). Participants demonstrating stable (> 1 contiguous visits) impairment following initial cognitive health were retrospectively considered to demonstrate preclinical LOAD at this baseline timepoint. Older adults who remained cognitively stable during the duration of the study were considered as healthy controls.

Phlebotomy Protocol, Blood Processing, and Long-Term Storage. Participants underwent phlebotomy according to standard procedures. Participants were not instructed to withhold medications prior to blood draw, nor were they instructed to fast. Collection occurred at participants' convenience and was not standardized with respect to daily time

of draws. Peripheral plasma was isolated at the UCI ADRC according to standard protocols for EDTA-treated whole blood within 24 hrs. All specimens were stored at -80°C prior to metabolomics analysis at the Lombardi Cancer Center Metabolomics Shared Resource Facility at Georgetown University.

Metabolomics Methods

Untargeted LC-MS Metabolomics. Ultra-performance liquid chromatography electro-spray ionization-quadrupole-time of flight-mass spectrometry (UPLC-ESI-QTOF-MS; Xevo-G2 QTOF, Waters Corporation) was used to conduct untargeted metabolomic profiling as described in previous work [12, 13, 28]. Briefly, plasma samples were prepared for MS by solvent extraction and resolved using reverse phase chromatography on an Acquity UPLC (Waters Corp.) online with a QTOF-MS in positive and negative electrospray modes with optimized run parameters. LC-MS peaks were determined from resulting raw instrument data using XCMS software [302]. XCMS processing of LC-MS data within R/OCAS resulted in a total of 4721 small-molecule (< 1.5 kDa) chemical features; **2738** in the negative mode (ESI-) and **1983** in the positive mode (ESI+). Similar analyses of plasma specimens from UCI ADRC identified a total of 5720 putative metabolites; negative mode *(ESI-)*: **2413**, positive mode *(ESI+)*: **3307**. These features resulting from LC-MS metabolomics were defined in terms of physicochemical properties (Mass-to-Charge Ratio: ***m/z***; chromatographic retention time: **RT**).

Statistical Methods

Differential Abundance Analysis and Integrative Modeling Pipeline. Considering only control participants and those individuals with preclinical LOAD, the final untargeted data matrix for R/OCAS included 71 unique participants by 4721 metabolic features. The

corresponding untargeted data matrix for UCI ADRC included 54 unique participants by 5720 metabolite features. This resulted from the exclusion of those features which did not vary in their abundances across participants. These were submitted to differential metabolite abundance (DA) analysis contrasting their relative abundances in the blood plasma of those experiencing preclinical LOAD versus those who remained cognitively healthy (**Figure 1.1**).

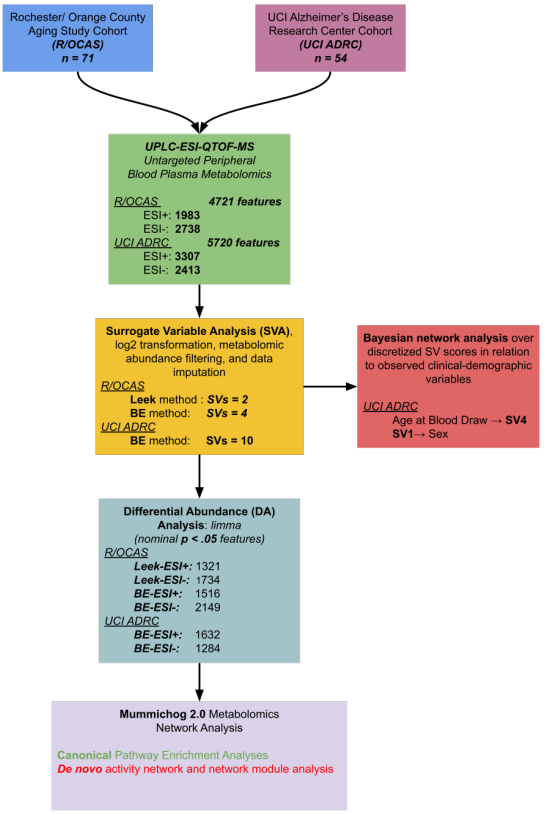

Figure 1.1 A schematic diagram details the untargeted LC-MS metabolomics pipeline and downstream statistical analyses to which R/OCAS and UCI ADRC preclinical LOAD blood plasma specimens were submitted.

Zero abundance and missing LC-MS measurements were replaced as "NAs." Features which survived variance thresholding were then submitted to *k*-nearest-neighbors imputation (K = 10) to generate a data matrix free of missing and artefactual values. These were subsequently base-2 logarithm transformed to improve symmetry and reduce positive

skewness of metabolite mass features. These data, however, almost certainly reflect biochemical variability in the blood plasma metabolome unrelated to that which stratifies control versus preclinical LOAD participants. Surrogate variable analysis (SVA) methods can parametrize this heterogeneous confounding variability into a modest number of latent sources of noise (i.e., the estimated surrogate variables). While SVA parametrizes true experimental signal as orthogonal to that represented by surrogate variables, multiple surrogate variables can correlate with each other. To assess the effects of differing surrogate variable estimation methods (Buja & Eyuboglu [303] versus Leek [304]) on resulting biochemical modeling and inferences, both methods were evaluated where they suggested a non-zero number of surrogate variables.

Overall, methods such as SVA can facilitate reproducibility in the analysis of high-dimensional "-omics" data including metabolomics [305]. More concretely, the initially unlabeled, latent variables discovered by SVA can and should be further contextualized in terms of observed demographic variables (e.g., sex, age, *APOE4+* genotype). To accomplish this, the discretized scores [306] of individual participants on each of the surrogate variables were submitted to Bayesian network modeling [307]. This allowed the relationships amongst these latent and observed variables to be visualized and considered in an integrated manner. Fitted surrogate variable scores for each participant were then included as covariates in linear models which estimated the abundance of each observed metabolite as a function of cognitive status (control versus preclinical LOAD) [308]. The nominal, unadjusted *p*-values associated with this phenotypic contrast for each metabolite feature (indexed by m/z ratio, RT) were then submitted to integrative pathway analysis using Mummichog 2.0 software [258]. For canonical pathway analyses in Mummichog, the

significance of the overlap across these DA metabolic pathways for both R/OCAS and UCI ADRC LOAD cohorts was determined using Tanimoto-Jaccard statistics and bootstrapped significance testing ($\alpha = .05$).

Software. Analyses employed R version 4.0.5. Imputation was completed using the *impute* package. SVA was carried out using the *sva* package. Empirical Bayes-moderated linear models and metabolite-wise phenotypic contrasts were evaluated using the *limma* package. Mummichog 2.0 was used to model systems-scale, coordinated changes in the peripheral metabolome due to either control or preclinical AD status: mummichog.org. The *bnlearn* package contributed functions for constructing Bayesian networks, which ingested features jointly discretized by the package *GridOnClusters*. Tanimoto-Jaccard statistics and significance testing were completed using the *jaccard* package.

RESULTS

R/OCAS and UCI ADRC: Participant Characteristics

Participant characteristics for R/OCAS control and preclinical participants are shown in Table **1.1**. Chi-squared tests of independence demonstrated that neither participant sex nor *APOE*4+ status proportionally differed to statistical significance in control versus preclinical aging adults with LOAD, *p*'s > .60. Participant age at blood draw also did not significantly differ by Mann-Whitney *U* test according to control or preclinical AD status in R/OCAS, $p > .40$. Participant characteristics for UCI ADRC control and preclinical participants are also shown in **Table 1.1.** In this cohort, the relative proportions of male and female participants did not differ due to control versus preclinical AD status, **chi-squared test of independence** $p = 1$. Carriers of the *APOE*4 LOAD risk allele, however, were overrepresented

amongst preclinical participants, **chi-squared test of independence** $p = .04$. As in R/OCAS, participant age at blood draw in UCI ADRC did not significantly differ due to preclinical AD status, $p > .42$. Across both cohorts, it was also possible that the interaction of cognitive status (control or preclinical AD) and cohort (R/OCAS or UCI ADRC) mediated age at blood draw, sex, *APOE*4+ genotype, and total years of education. Logistic generalized linear models found no such significant relationships except for *APOE*4+ genotype. Individuals possessing this risk allele were over three-fold overrepresented in preclinical participants from the UCI ADRC cohort, $p = .028$.

Table 1.1 Participant Characteristics for the R/OCAS and UCI ADRC LOAD Cohorts

	n (M/F)	***APOE4+ Individuals***	***Mean Years of Education (SD)***	***Mean Age at Blood Draw (SD)***
R/OCAS				
Control	**53** (19/34)	**14**	**15.6** (2.4)	**81.6** (3.5)
Preclinical AD	**18** (8/10)	**3**	**15.3** (3.1)	**80.7** (3.0)
UCI ADRC				
Control	**28** (11/17)	**3**	**15.9** (2.5)	**80.5** (6.8)
Preclinical AD	**26** (11/15)	**10**	**16.3** (2.0)	**81.9** (6.2)

R/OCAS: Differentially Abundant Peripheral Metabolite Features Differentiate Control and Preclinical LOAD Plasma

Following initial metabolomics data pre-treatment (including imputation and log*2*-transformation) (see **METHODS),** 4721 small-molecule LC-MS mass features (*ESI-* mode: **2738**, *ESI+* mode: **1983**) were subjected to surrogate variable analysis (SVA). This method addresses the often-substantial capacity of LC-MS to quantify nuisance variability unrelated to AD status in the plasma metabolome. This undesired experimental noise can, however, be

anonymously parametrized by a set of potentially correlated "surrogate variables" (SVs) in downstream analyses [304, 309]. These empirical estimation methods can improve reproducibility in large-data "-omics" experiments by specifically considering those molecules in the peripheral metabolome associated with the control versus preclinical AD contrast [305]. Estimation of the appropriate number of significant surrogate variables to include, however, depends upon the choice of SVA algorithm: Buja-Eyuboglu (BE) [303] or Leek [304]. To minimize bias in downstream analysis due to algorithm choice, both were used to fit respective sets of surrogate variables to R/OCAS metabolomics data (SVs_{Leek} = **2,** SVs_{BE} = **4**).

SVs are latent variables derived from high-dimensional "-omics" measurements and those observed clinical, demographic, and experimental variables which relate to them are not self-evident. To address this question in an integrative manner, participant sex and *APOE*4+ status in addition to discretized years of education and baseline age were submitted to Bayesian network modeling. These observed variables were considered with participant-level discretized BE and Leek SV scores, respectively [306, 307]. For Both BE and Leek-derived SVs, these models did not support significant associations between age at blood draw, *APOE*4 status, years of education, and sex with estimated SVs.

LC-MS mass feature abundances were then estimated from linear models as a function of **A)** AD status (control versus preclinical) and **B)** fitted, participant-level surrogate variable scores. Analysis of DA metabolite features was carried out in parallel using both Leek and BE methods of surrogate variable estimation. Of the 4721 features submitted to DA modeling using the Leek method, 1321 features in the ESI+ mode of detection and 1734 in the ESI- mode significantly differed due to AD status, nominal $p's < .05$. Using the BE

method of SV estimation, similar proportions of features were DA in peripheral plasma with preclinical status (ESI+: **1516**, ESI-: **2149**).

R/OCAS: Differentially Abundant Peripheral Metabolite Features between Preclinical and Control Plasma are Enriched within Known Metabolic Pathways

Mass features identified by *m/z* and RT were ranked according to nominal *p*-value and taken as input to integrative Mummichog 2.0 metabolomic network modeling. Peripheral metabolic change characterizing preclinical versus control LOAD participants significantly implicated multiple, known biochemical pathways (**Table 1.2**). Specifically, (*Leek-ESI+*)-identified processes including the metabolism of tyrosine, galactose, and folate (vitamin B9) were significantly altered in preclinical dementia plasma, as were the formation of both inflammation resolving (eicosapentaenoic-acid-derived, **EPA-derived**) and pro-inflammatory lipid (arachidonic acid-derived) signaling molecules, *p's* < .05. Analyses using *Leek-ESI-* parameters similarly found that the metabolism of several biogenic amines (e.g., aspartate, arginine, asparagine), vitamin B3 (nicotinate and nicotinamide), and nucleotide metabolism were altered in those with preclinical LOAD relative to controls, $p < .05$.

Analyses using *BE-ESI+* parameters suggested again that the metabolism of biogenic amines, vitamin B3, nucleotides, and folate was altered in preclinical versus control participant plasma. This accompanied significant pathway enrichments for sialic acid, glutathione, and glycerophospholipid metabolism, *p*'s < .05. Corresponding *BE-ESI-* analyses again indicated the significance of biogenic amine, vitamin B3, and galactose metabolism. These findings also suggested the importance of both signaling and fuel lipid metabolism, including saturated fatty acid beta-oxidation and the formation of inflammation-resolving (EPA-derived), polyunsaturated fatty signaling molecules, *p*'s < .05.

Table 1.2 Canonical Biochemical Pathways Differing between Control and Preclinical AD Plasma in R/OCAS by Mummichog 2.0 Analyses

Pathways	Overlap Size	Pathway Size	p-value	ESI Mode	SV Mode
Alanine and Aspartate Metabolism	4	6	0.01227	NEG	BE
Alanine and Aspartate Metabolism	4	4	0.00118	POS	BE
Alanine and Aspartate Metabolism	4	4	0.00218	POS	LEEK
Androgen and estrogen biosynthesis and metabolism	2	2	0.02067	POS	BE
Androgen and estrogen biosynthesis and metabolism	2	2	0.0268	POS	LEEK
Arginine and Proline Metabolism	9	10	8.00E-05	POS	BE
Arginine and Proline Metabolism	9	10	8.00E-05	POS	LEEK
Aspartate and asparagine metabolism	6	10	0.00781	NEG	BE
Aspartate and asparagine metabolism	8	8	8.00E-05	POS	BE
Aspartate and asparagine metabolism	8	8	8.00E-05	POS	LEEK
Beta-Alanine metabolism	4	4	0.00118	POS	BE
Beta-Alanine metabolism	4	4	0.00218	POS	LEEK
Carbon fixation	3	5	0.04495	NEG	Leek
Carbon fixation	4	5	0.00597	NEG	BE
Carbon fixation	2	2	0.02067	POS	BE
Carbon fixation	2	2	0.0268	POS	LEEK
Drug metabolism - other enzymes	4	6	0.01328	POS	BE
Galactose metabolism	3	4	0.01672	NEG	Leek
Galactose metabolism	3	4	0.01563	NEG	BE
Glutamate metabolism	4	4	0.00118	POS	BE
Glutamate metabolism	4	4	0.00218	POS	LEEK
Glutathione Metabolism	2	2	0.02067	POS	BE
Glutathione Metabolism	2	2	0.0268	POS	LEEK
Glycerophospholipid metabolism	6	13	0.04294	POS	BE
Glycine, serine, alanine and threonine metabolism	6	7	0.00076	POS	BE

Glycine, serine, alanine and threonine metabolism	6	7	0.00092	POS	LEEK
Histidine metabolism	3	4	0.01563	NEG	BE
Methionine and cysteine metabolism	4	6	0.01328	POS	BE
Methionine and cysteine metabolism	5	6	0.00294	POS	LEEK
Nitrogen metabolism	4	4	0.00118	POS	BE
Nitrogen metabolism	4	4	0.00218	POS	LEEK
Prostaglandin formation from arachidonate	3	5	0.04495	NEG	Leek
Purine metabolism	8	11	0.00084	POS	BE
Purine metabolism	8	11	0.00101	POS	LEEK
Putative anti-Inflammatory metabolites formation from EPA	2	2	0.02143	NEG	Leek
Putative anti-Inflammatory metabolites formation from EPA	2	2	0.01865	NEG	BE
Putative anti-Inflammatory metabolites formation from EPA	2	2	0.0268	POS	LEEK
Pyrimidine metabolism	6	7	0.00076	POS	BE
Pyrimidine metabolism	6	7	0.00092	POS	LEEK
Saturated fatty acids beta-oxidation	2	2	0.01865	NEG	BE
Sialic acid metabolism	3	5	0.04243	POS	BE
Tryptophan metabolism	3	5	0.04495	NEG	Leek
Tyrosine metabolism	8	17	0.01622	NEG	Leek
Tyrosine metabolism	7	12	0.00613	POS	BE
Tyrosine metabolism	7	12	0.0105	POS	LEEK
Urea cycle/amino group metabolism	5	10	0.03529	NEG	BE
Urea cycle/amino group metabolism	8	13	0.00302	POS	BE
Urea cycle/amino group metabolism	8	13	0.00513	POS	LEEK
Vitamin B3 (nicotinate and nicotinamide) metabolism	3	5	0.04378	NEG	BE
Vitamin B3 (nicotinate and nicotinamide) metabolism	7	8	0.00042	POS	BE

Vitamin B3 (nicotinate and nicotinamide) metabolism	7	8	0.00034	POS	LEEK
Vitamin B9 (folate) metabolism	3	4	0.01672	NEG	Leek
Vitamin B9 (folate) metabolism	2	2	0.02067	POS	BE
Vitamin B9 (folate) metabolism	2	2	0.0268	POS	LEEK

R/OCAS: Differentially Abundant Peripheral Metabolite Features between Preclinical and Control Plasma are Enriched within *De Novo* Metabolic Pathways

In addition to the evaluation of canonical metabolic pathways, Mummichog can reconstruct *de novo* metabolic pathway networks (spanning multiple *a priori* known individual pathways) specifically implicated by the control-preclinical LOAD comparison. Where these novel metabolic network "modules" and activity networks are relatively sparse, they can suggest highly specific biochemical signatures stratifying associated, clinical phenotypic comparisons. These may also indicate the biochemical rudiments of specific metabolic pathobiology in AD. Consistent with this, the ESI+ analyses employing either the BE or Leek procedures jointly implicated a *de novo* module centered on elaborated glutamate metabolism resembling glutaminolysis, module significance *p*'s < .05 (**Figure 1.2A**). This suggests that both BE and Leek methods capture plasma metabolomic variability truly associated with the control-preclinical phenotypic contrast versus model-specific artefact. Analyses using *BE-ESI-* parameters also identified a *de novo* module very suggestive of the preparatory phase of glycolysis, $p = .04$ (**Figure 1.2B**).

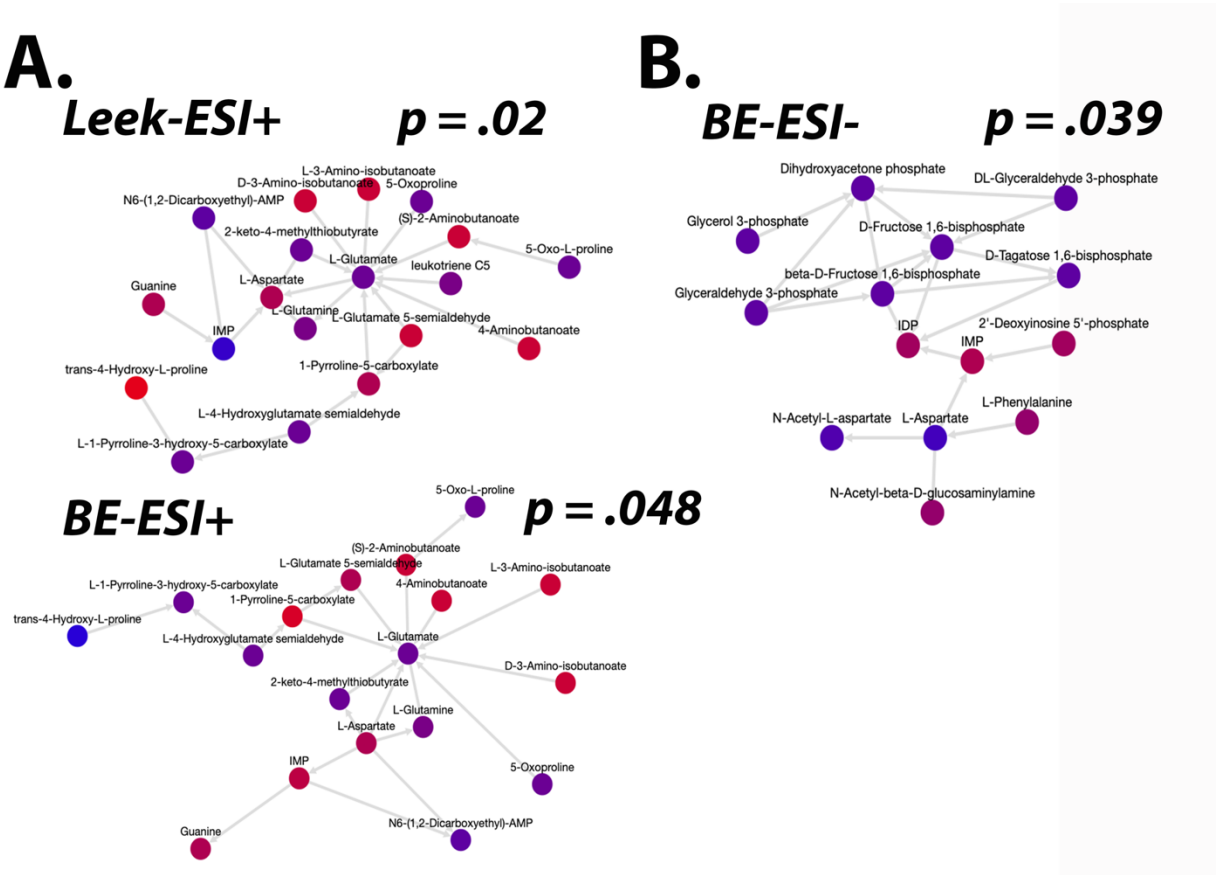

Figure 1.2 Significant Mummichog *de novo* metabolic modules correspond to control versus preclinical status statistical contrasts in R/OCAS. Reconstructed metabolic networks using both Leek (*top*) and BE (*bottom*) SVA methods in **Panel A** resemble glutaminolysis. **Panel B** includes hexose metabolic processes shared with the preparatory phase of glycolysis.

UCI ADRC: Differentially Abundant Peripheral Metabolite Features Distinguish Control and Preclinical LOAD Peripheral Plasma

Participant plasma specimens from UCI ADRC also underwent differential abundance analysis employing surrogate variable estimation methods (SVs_{BE} = 10). To contextualize these fitted surrogate variables in terms of observed clinical and demographic variables (e.g., sex, *APOE*4+ status, age), a Bayesian network model was estimated (**Figure 1.3**) [307]. This suggested probabilistic dependencies of A) SV4 on age and of B) sex on SV1. In total, 5720 LC-MS metabolite mass features (ESI-: **2413**, ESI+: **3307**) were submitted to downstream modeling as a function of estimated, participant-level surrogate variable scores and control

versus preclinical AD status. Of all 5720 UCI ADRC features submitted to DA analysis, linear modeling revealed that **1632** ESI+ and **1284** ESI- features significantly differed in their plasma abundances due to preclinical AD status, nominal *p's* < .05.

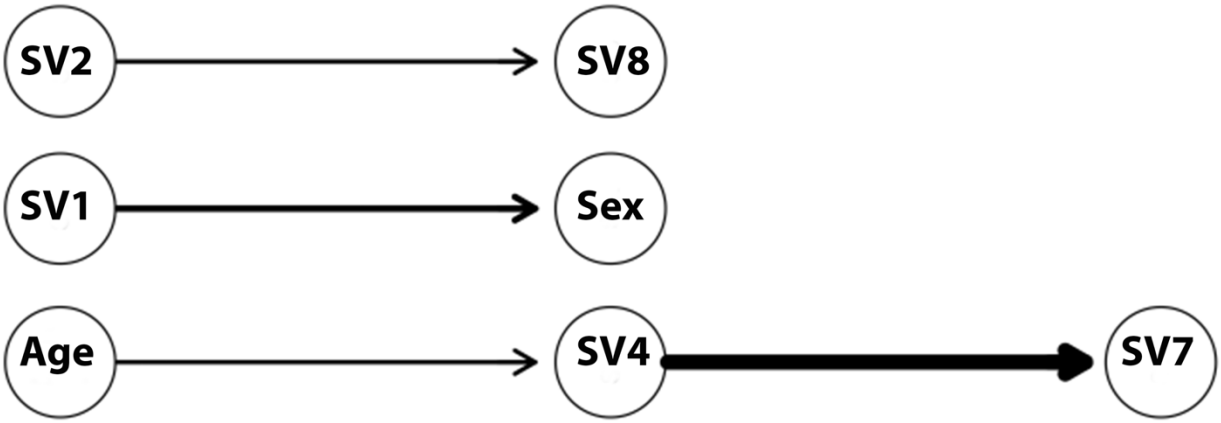

Figure 1.3 Bayesian network modeling associates surrogate variables to observed clinical-demographic factors. Age at blood draw and sex demonstrate respective probabilistic relationships with SV4 and SV1. Line boldness indicates confidence/strength of the estimated relationships between factors estimated from participant data and a fitted network structure [*bnlearn:* arc.strength()].

UCI ADRC: Peripheral Metabolite Features Differentially Abundant between Preclinical and Control Plasma are Enriched within Known Metabolic Pathways

Mass features (indexed by *m/z*, RT) were ranked according to nominal *p*-value corresponding to the control-preclinical contrast as evaluated for UCI ADRC participants. These values derived using BE surrogate variable estimates (Leek method identified no significant SVs in UCI ADRC) were then submitted to canonical pathway enrichment analysis using Mummichog 2.0 (**Table 1.3**). Specifically, the ESI+ detection mode demonstrated significant overrepresentation of DA metabolites involved in lipid metabolism, including *de novo* fatty acid biosynthesis, fatty acid activation, bile acid biosynthesis, and C21-steroid metabolism, *p*'s < .05. The ESI- mode of detection also strongly implicated lipid metabolism,

suggesting that the preclinical-control contrast involves plasma alterations to polyunsaturated fatty acid (PUFA) metabolism including that of linoleate and neuroprostanes, *p*'s < .03. This finding accompanied enrichment overall for *de novo* fatty acid biosynthesis and activation amongst preclinical-control DA features, *p*'s < .03.

Table 1.3 Canonical Biochemical Pathways Differing between Control and Preclinical AD Plasma in UCI ADRC

Pathways	*Overlap Size*	*Pathway Size*	p-*value*	*ESI Mode*
Bile acid biosynthesis	3	3	0.03949	POS
Biopterin metabolism	3	3	0.02924	POS
C21-steroid hormone biosynthesis and metabolism	3	3	0.03949	POS
D4&E4-neuroprostanes formation	2	2	0.02487	NEG
De novo fatty acid biosynthesis	3	4	0.02143	NEG
De novo fatty acid biosynthesis	4	6	0.00269	POS
Drug metabolism - other enzymes	2	2	0.02487	NEG
Fatty acid activation	3	4	0.02143	NEG
Fatty acid activation	4	4	0.01244	POS
Fatty Acid Metabolism	4	4	0.02361	POS
Limonene and pinene degradation	2	2	0.00849	POS

Linoleate metabolism	3	3	0.00664	NEG
Prostaglandin formation from arachidonate	2	2	0.00849	POS

R/OCAS and UCI ADRC: Significantly Shared, Enriched Metabolic Pathways Index Preclinical LOAD in Peripheral Blood Plasma

The canonical pathways indicated by Mummichog as altered in preclinical LOAD for both R/OCAS and UCI ADRC could indicate shared dysmetabolism. They could also, however, reflect an overlap in DA-enriched metabolic pathways due to chance alone. Tanimoto-Jaccard statistics and significance testing can evaluate if the former hypothesis is supported, taking the latter one as the null. All significantly enriched (*p*'s < .05**)** canonical Mummichog metabolic pathways for either R/OCAS or UCI ADRC were used to derive a Tanimoto-Jaccard coefficient quantifying this degree of similarity. The significance of the coefficient (i.e., the probability of achieving at least this extreme of a coefficient under the null hypothesis) was exactly estimated (*uncentered estimated coefficient:* **.057|** *centered coefficient:* **-.226)** and highly significant $p < .001$. This suggests that a shared "fingerprint" of altered canonical biochemical pathways (nominated across SVA methods and MS acquisition modes in peripheral plasma) characterizes the demographically-highly-similar R/OCAS and UCI ADRC preclinical LOAD cohorts beyond chance levels [310] (**Appendix 1.1**).

UCI ADRC: Differentially Abundant Peripheral Metabolite Features between Preclinical and Control Plasma are Enriched within *De Novo* Metabolic Pathways

As with the several significant R/OCAS control-preclinical AD contrasts, UCI ADRC also demonstrated *de novo* metabolic networks for these same clinical phenotypic relationships. Notably, the ESI+ mode analyses identified one significant module containing several saturated and unsaturated C16-20 fatty acids, p = .02 (**Figure 1.4A**). This involved an elaborated metabolic activity network suggesting the importance of Coenzyme A (CoA) and cholesterol in the metabolism of these lipids (**Figure 1.4B**). There was good concurrence in estimated Mummichog metabolic activity networks across ESI+ and ESI- MS analysis modes. Specifically, Mummichog analyses in this latter ESI- mode suggest the importance of C16-C20 acyl chain length saturated and unsaturated fatty acids to preclinical AD, where the metabolism of mono and triphosphate nucleosides may also intersect with that of these lipid molecules (**Figure 1.4C**).

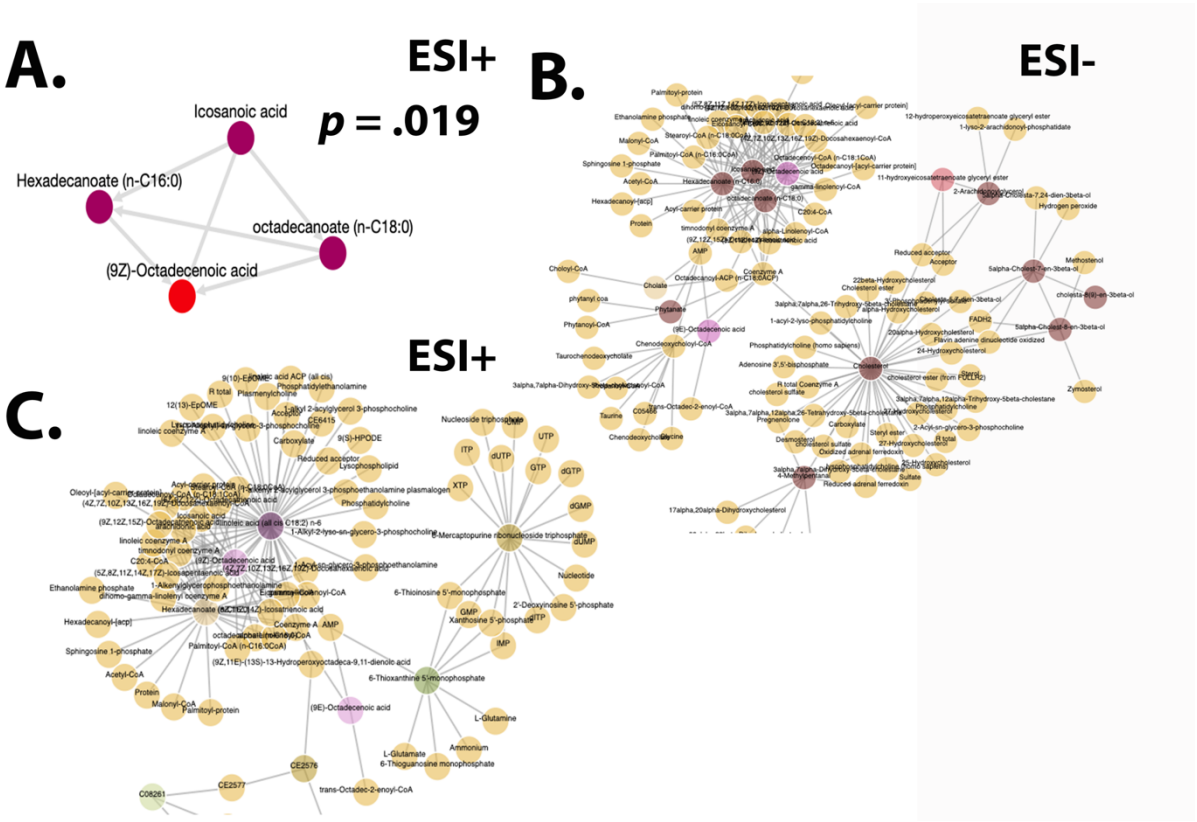

Figure 1.4 Metabolomics analyses of UCI ADRC preclinical LOAD plasma suggest evidence of abnormal lipid metabolism including that of saturated, monounsaturated, and polyunsaturated fatty acids.

DISCUSSION

This chapter sought to address whether systemic metabolism evident in peripheral circulation demonstrated pathway and network-scale alterations as a function of preclinical AD status in LOAD. Indeed, very large, recent studies of aging cohorts across multiple continents have suggested systemically metabolic declines in aging commencing during the sixth decade of life onwards [311]. If similar, early occurring changes could be identified reproducibly in the plasma metabolome across independent, abnormally aging cohorts, they might suggest metabolic and biochemical processes specifically reprogrammed as a function of early, preclinical LOAD, if not early AD overall. Because metabolomics quantifies many biological processes of unclear number and physiological extent in aging blood plasma, the

experiments and analyses pursued in this chapter employed several strategies to address this uncertainty. These aimed to both A) minimize the likelihood of false-positive, spurious associations and B) focus on plasma biochemistry specifically and robustly associated with the control versus preclinical AD status. First, surrogate variable analysis was employed (using multiple estimation methods) to parametrize sets of latent variables capturing metabolomic variability unrelated to AD status itself, but potentially relating to other, important clinical and demographic variables (i.e., age at blood draw, sex, *APOE*4+ risk genotype, years of education). The use of multiple surrogate variable estimation methods in the R/OCAS cohort (where these both suggested non-zero numbers of SVs) also minimized bias in the modeling of surrogate variables for downstream, feature-wise differential abundance analysis. Investigating these relationships further, integrative Bayesian network analyses suggested the relationship of several latent factors to sex and age at blood draw in UCI ADRC. The R/OCAS cohort, however, did not demonstrate significantly similar findings when submitted to this same analysis. Therefore, while such variables may impact the peripheral blood metabolome, this conclusion was not supported empirically based on R/OCAS and UCI ADRC preclinical LOAD participants.

Independent of statistical approaches, the design of these experiments in preclinical LOAD also permitted robust, independent comparisons. The deliberately high demographic and clinical similarity of the R/OCAS and UCI ADRC cohorts specifically allowed for highly harmonized cross-cohort comparisons, even when these samples were ascertained independently in terms of time and study site. Consensus definitions of “validation” and “replication” remain under-defined in translational metabolomics and biomarker research, but the examination of independently ascertained samples drawn from a substantially

overlapping clinical population agrees with other recent, "-omics" approaches for rigorously and reproducibly investigating AD (i.e., genome-wide association studies, GWAS) [312-314].

Even under these experimentally and statistically conservative premises, the experiments carried out in this chapter provide multiple lines of evidence suggesting shared, peripherally evident dysmetabolism at the preclinical stage of LOAD across both the R/OCAS and UCI ADRC cohorts. Integrative computational and statistical analyses through the integration of *sva*, *limma* and Mummichog software revealed these relationships at both the level of A) canonical metabolic pathways and B) *de novo* significant modules and broader activity networks. Most clearly, both the R/OCAS and UCI ADRC preclinical cohorts demonstrated enrichment for pathways involved in diverse aspects of lipid and fatty acid metabolism, including glycerophospholipid metabolism as reported by Mapstone and others [28, 29, 315-317], but also sterol, steroid, and bile acid metabolism [11, 318-320]. This also, however, included many biogenic amines (including amino acids); purine and pyrimidine nucleosides and their phosphorylated nucleotides derived from the pentose phosphate shunt; and small-molecule metabolites essential to redox homeostasis, cellular bioenergetics, and homeostatic biosynthesis [201, 207, 321].

Such constraints likely impact many tissues and organs in abnormal aging; however, these themselves may indirectly contribute to emerging brain dysfunction in preclinical LOAD through physiologically extended, brain-peripheral metabolic axes in early AD. These vicious, feedforward cycles of "compensation for failure" and resulting "failures of compensation" in the evolving disease process could be thought to result in a distribution of systems biological state distributions in disease (i.e., a dynamical distributional mixture model in evolving AD). Beyond some critical point in time and mapping to some subset of

dysfunctional and dyshomeostatic biological processes in abnormal aging, successful trajectories of age-associated cognitive change, resilience, and brain health may exist fundamentally incompatible with this pathobiologically distributional mixture developing in AD as a function of advancing preclinical LOAD itself.

The use of both R/OCAS and UCI ADRC also conferred advantages. The objective of employing the independent R/OCAS and UCI ADRC cohorts was to facilitate highly harmonized, but parallel comparisons in preclinical LOAD where participants were very clinically and demographically similar. Significant canonical pathway enrichments (i.e., Mummichog pathways) across these cohorts can suggest this overlap qualitatively; however, Tanimoto-Jaccard analyses can quantify and statistically test these putative associations relative to a null (i.e., random) distribution. When evaluated across pathways implicated in either R/OCAS or UCI ADRC, this suggested a modest degree of similarity in the canonical-pathway-wise "fingerprint" of preclinical LOAD across both. This overlap significantly deviated from expectations due to chance alone under the null distribution (*uncentered estimated coefficient:* **.057|** *centered coefficient:* **-.226,** $p < .001$).

Mummichog modeling across R/OCAS and UCI ADRC also identified several *de novo* metabolic networks associated with the preclinical LOAD versus control contrast. Interestingly, both Leek and BE surrogate variable methods in the ESI+ MS mode identified statistically significant network modules centered around glutamate. Glutamate is ubiquitous within the brain as an excitatory neurotransmitter, but it also functions as a biochemically vital intermediate participating in mitochondrial fuel metabolism, and orchestrated intermediary biosynthesis across neurons and astrocytes (i.e., the glutamate-glutamine metabolic cycle) [322, 323]. This resembles other hypothesized CNS inter-cellular,

metabolic shuttle systems (i.e., the astrocyte-neuron lactate shuttle) which may also be altered in AD [324]. Considered elsewhere in human disease metabolism as glutaminolysis [325], this fuel and biosynthesis-affording cellular biochemical pathway has been understudied relative to the CNS-specific functional roles of glutamate in neurotransmission and synaptic homeostasis. The way its broader metabolism could become homeostatically imbalanced against the functional demands of CNS-specific glutamatergic processes may suggest another early, discrete point of emerging "failures of compensation" in preclinical AD [326]. Bernier and colleagues have indeed recently reported that activated microglia can instead employ glutamate-metabolizing, glutaminolysis-like metabolic programs to sustain the chronic neuroinflammation often present in preclinical AD including preclinical LOAD [327].

This may be differentiable from associated glucose dysmetabolism in AD; however, the R/OCAS *BE-ESI-* mode *de novo* analyses suggest that components of the preparatory phase of glycolysis and glutaminolysis may be jointly implicated by and functionally intersect with each other in preclinical AD [328]. Interestingly, these networks include the molecule *n*-acetyl-aspartate (NAA), which is depleted in the AD brain as measured with proton magnetic resonance spectroscopy (^{1}H-MRS) [329-331]. The role(s) of NAA in AD pathobiology remain understudied, but NAA homeostasis may index mitochondrial integrity and the sufficiency of upstream aspartate and acetyl-coenzyme A metabolism. These biogenic-amine-involving processes themselves suggestively resemble the glutamate-containing *de novo* pathways identified here in preclinical LOAD peripheral blood plasma. While NAA is often considered a neuronal marker, it also contributes to lipid synthesis in oligodendrocytes [332]. It may be translationally productive to consider these NAA-

involving processes in AD involving the brain as components of an extended "glutaminolysis-like" network under metabolic jeopardy in early sporadic AD. Substantial opportunity clearly exists for the integration of minimally invasive metabolic imaging and blood biomarkers of AD to better demonstrate, contextualize, and dissociate these specific pathobiological relationships between metabolic brain molecules and those of the periphery. In recent years, much emerging work along these lines has been advanced by the emerging field of immunometabolism, which has most extensively studied the biochemical dynamics of activated microglia in AD [333, 334]. It has more recently also considered the dysmetabolic profiles of reactive astrocytes and even peripheral cells of the adaptive immune system [335-339]. Critically, all such processes involve intensive, functionally orchestrated metabolism (including that of lipids, glutamate, and glucose) much like the diverse metabolic processes altered in R/OCAS and UCI ADRC preclinical LOAD peripheral plasma here. Further integrative experiments employing CNS imaging and fluid biomarker data will be essential to clarify the therapeutically actionable biological processes and targets associated with these findings in early LOAD.

The present experiments were not without limitations. Most importantly, while the R/OCAS and UCI ADRC cohorts were overall highly similar, frequencies of the APOE4 risk allele were significantly more prevalent in UCI ADRC preclinical participants compared to R/OCAS. These findings prompted the exploration of parameterized surrogate variables in terms of clinical-demographic variables including *APOE* genotype. Across both preclinical cohorts and multiple methods of SV estimation, no fitted Bayesian networks suggested further moderating effects of *APOE*4+ status on the composition of the peripheral plasma metabolome in LOAD. Therefore, the disproportionality of this risk across R/OCAS and UCI

ADRC may not impact the identification of peripheral plasma metabolomic change characterizing preclinical participants with LOAD.

While Mummichog represents a powerful tool integrating empirical metabolomics experiments with prior biochemical pathway knowledge, it also demonstrates limitations in its current, user-facing format. Namely, Mummichog does not natively place implicated biochemical transformations in terms of associated enzymes and transporters where these may themselves suggest highly important inferences regarding the etiopathogenesis and early development of LOAD. Similarly, the identification of AD-associated metabolic activity networks using Mummichog neither guarantees human-interpretable, parsimonious findings nor associated, statistical significance testing. Chapter Four of this dissertation instead suggests how Mummichog activity network outputs (considered as a "natural" biochemical language and including preclinical LOAD contrasts considered in this chapter) can be assimilated into a small number of latent topics defined by individually associated biochemical processes and enzyme-encoding genes. Despite this substantial reduction in data dimensionality, an ideally high degree of semantic coherence can be tuned using contemporary topic modeling algorithms [340] . Similar strategies to identify associations between untargeted metabolomics chemical features and protein interaction networks have also been implemented by the Fraenkel lab using a prize-collecting Steiner forest algorithm (i.e., PIUMet) [341]: http://fraenkel-nsf.csbi.mit.edu/piumet2/. One aim of CHAPTER 3 will indeed implement this software to nominate (from untargeted plasma LC-MS metabolomic profiling experiments) specific proteins and thus gene expression associated with clinical dementia status for aging adults with Down syndrome (DS-AD).

In all, these converging peripheral blood biochemical findings in multiple preclinical LOAD cohorts suggest multifactorial brain-peripheral metabolic axes in abnormal cognitive aging which become systemically perturbed in the evolution of dementia, thereby affording AD-associated peripheral biomarker candidates. Crucially, these alterations were not limited to one biochemical pathway, but involved diverse, hub-like metabolites and pathways important for the dynamic integration and regulation of fuel metabolism, biosynthesis, and functional biological signaling in multiple CNS cells and peripheral tissues. In a vicious, feedforward process of metabolic "compensation from failure" precipitating an ultimate "failure of compensation" in AD, these incidental metabolic changes with advancing disease may become unsustainable and incompatible with healthy cognitive aging. More specifically, this biological precariousness could involve the substantial, activity-associated metabolic demands of chronically activated glia and immune cells beginning early in LOAD.

Aging individuals with LOAD, however, do not possess the amyloid-specific genetic risk experienced by those with DS-AD or ADAD. The following chapter aims to address whether DS-AD, as opposed to preclinical LOAD, demonstrates similarly diverse, peripheral blood metabolic correlates compared to cognitively stable, aging adults with DS. Unlike LOAD, those who experience DS-AD incur risk as a function of trisomy 21, genetically predisposed, early-onset amyloidosis, and oxidative-stress-associated accelerated aging [46]. Because of the strong ties of these biological processes to metabolism, DS-AD might also demonstrate alterations to the peripheral plasma metabolome resembling those observed in preclinical LOAD. The specific details of these similarities in DS-AD relative to LOAD (in addition to any differences) remain substantially unclear, as will be further examined in CHAPTER 2.

CHAPTER 2. Down Syndrome Alzheimer's Disease: **DS-AD**

In addition to his contributions towards discovering the trisomic basis of Down syndrome involving chromosome 21, Jerome Lejeune's late work established him as an early proponent of investigating DS and DS-AD cognition in metabolic terms [342, 343]. These intuitions suggested by Lejeune in DS thus resemble the historically metabolic work of researchers such as Raichle and Pettegrew, where these latter lines of inquiry, evidence and metabolic imaging methodologies have recently driven novel insights into the AD pathobiological process [132, 133, 344-346]. Several recent studies have, in fact, shown that core bioenergetic/metabolic deficits are a fundamental feature of AD neurodegeneration, including those pathobiological processes specifically precipitating DS-AD [12, 13, 347, 348].

Lejeune's observations also resemble a longstanding literature on the non-nutritional use of nutrients as potential metabolic therapeutics as was first pursued by Wurtman and others in AD [15, 16, 34, 201, 349]. Interest also grows in systemic, easily accessible, and metabolically intensive aerobic exercise interventions for DS-AD and AD [33]. More broadly in abnormal aging, this literature suggests that the catabolic, fuel-affording functions of metabolism often belie the anabolically vital biosynthetic and signaling roles often served by these same biomolecules. Critically, all such functional and homeostatic metabolic programs may become catastrophically and dyshomeostatically limited as a function of DS, advancing AD, or their combination in aging. This presents a particular challenge because the extent, scale, duration, and scope of these metabolic changes in the DS-AD pathobiological process remain unclear. The current limits on this knowledge, however, also suggest many opportunities to identify novel therapeutic targets and biomarkers in AD and DS-AD specifically.

The past decade of dementia research (enabled by burgeoning metabolomics technologies) has prompted the reconciliation of molecular and metabolic perspectives on complex, multifactorial diseases of abnormal aging including AD and DS-AD [241]. This accords with recently proposed translational policy and funding initiatives to leverage these and similar emerging, data-rich technologies to advance the study and treatment of hereto refractory human diseases demonstrating complex drivers and risk profiles [24]. "-Omics"-scale measurement approaches such as metabolomics have allowed researchers to explicitly pursue metabolic hypotheses of the kind suggested by Lejeune and others in DS and AD. These metabolic considerations have, however, only been recently explored in their specific contributions to the pathogenesis and dynamic course of DS-AD.

Amyloid-attenuating pharmaceutical therapies have recently been approved by the FDA; however, their efficacy to robustly halt, reverse, or stabilize emerging cognitive deficits in prodromal AD remains to be clarified in coming years beyond target engagement with cortical amyloid alone [350]. The time thus appears opportune to more completely interrogate Lejeune's original metabolic hypothesis in DS that: "[…] victory over the neural disturbances resulting from the genetic overdose of trisomy 21 would very likely also lead to a cure or to a prevention of Alzheimer [*sic*] dementia" [342]. For DS and other dissociable, AD-risk-imposing etiologies, these dynamics of biologically and metabolically "futile cycles" accompany (if not drive) dementia progression in which "compensations for failure" precipitate complex, biologically non-random "failures of compensation." These catastrophically feedforward, dyshomeostatic cascades during the emergence of age-associated decline may prove ultimately prohibitive of and incompatible with trajectories of healthy cognitive aging in individuals with DS.

It remains unclear, however, if the blood metabolome of those with DS meeting criteria for clinical AD resembles peripheral blood metabolomic changes observed in early LOAD as detailed in CHAPTER 2. This question remains poorly understood in terms of both specific, implicated metabolic pathways, but also in the diversity of DS-AD-associated biochemical processes involved. DS-AD initially proceeds from genetically driven, early cortical amyloidosis consequent to trisomy 21, unlike LOAD [46, 351, 352]. The principal aim of this chapter is to better characterize how the peripheral metabolome varies specifically due genetic amyloidosis risk in DS-AD. Systemic dysmetabolism characterizing individuals with DS throughout the lifespan appears to index (if not mediate) this aging-associated cognitive decline [12, 13, 160, 252, 353]. How these findings specifically relate to trisomy 21, consequent triplication of the APP gene, and elevated cortical amyloidosis remains less clear.

Generalizing from the examples outlined by Jerome Lejeune three decades ago [342, 343], these relationships may be substantially multifactorial, complex, and requiring homeostatic regulation orthogonal to the demands of functional physiological programs in individual tissues and cells. Critically, the possible configurations of those programs may become constrained in a manner incompatible with healthy cognitive aging and cognitive resiliency in emerging DS-AD. Befitting the densely interconnected biological networks and pathways which describe the intersection of metabolism and biological chemistry, multiple discrete foci of molecular and energetic compensations for failure may precipitate ultimate failures of compensation in the development of AD. These varying trajectories of illness driven by differing sources of genetic risk (including that specifically leading to amyloidosis) may, however, be dissociable within the peripheral blood plasma metabolome according to differing etiologies and clinical demographic variables [78]. Cortical amyloidosis in adults

with DS is a hallmark of these individuals as they age [352]. The triplication of the *APP* gene in trisomy 21 drives this pathology, yet it remains unclear if more complex, sporadic patterns of heritability also contribute to both the A) early embryonic development of DS, but also the B) emergence of systemic metabolic and bioenergetic constraints in later-life cognitive decline. The pursuit of this hypothesis should not miss the forest for the trees: amyloidosis and bioenergetics may both exist subject to metabolic processes and constraints in evolving DS-AD [347, 348, 354-356].

Some of this systems pathobiology in abnormal aging also demonstrates sporadic heritability, agrees with patterns of maternally driven DS and AD risk, and explains intergenerational patterns of LOAD in families also overrepresented with DS births [357-365] (but see also: [366]). This alone may suggest that metabolism represents a "final common pathway" mediating genetically conferred AD risk broadly across family pedigrees even independent of trisomy 21 gene dosage effects themselves. Understood more completely, these specific metabolomic perturbations in DS-AD peripheral blood plasma could further and more specifically suggest a physiologically extended, CNS-peripheral metabolic axis dyshomeostatically altered in aging individuals with DS also experiencing clinical AD. Implicated (and perhaps highly distributed) biological systems relating to trisomy 21 might thus become progressively incompatible with and constrained apart from neurobiological and cognitive trajectories of successful aging in DS-AD. If these dynamics can be measured at low cost and with minimal invasiveness in peripheral blood, they may also suggest novel plasma biomarkers and specifically DS-focused interventional targets. Critically motivating the experiments reported in this chapter, these pathobiological targets

may only incompletely overlap with those identified in LOAD (possibly as a unique function of amyloid-associated genetic burden due to trisomy 21 in DS-AD).

METHODS

Participants and Cognitive Assessment. Participants in this retrospective study were selected from individuals with DS who were enrolled in one of three longitudinal research studies at UC Irvine between December 2004 and March 2018. Collectively, these participants make up the Predicting Cognitive Decline in Adults with Down Syndrome (PCDA-DS) cohort. Of these aging participants with DS, 158 provided blood samples on 453 visits (range: 1–10 visits per subject). Blood samples were stored for future research as part of the individual study protocols and following informed consent from the study participants or assent of the participant and consent from the participant's legal guardian where required. All study protocols and informed consent procedures were approved by the UCI Institutional Review Board (UCI IRB HS#s: 2010-8008, 2004-3704, 1994-143, 2009-7244, 2002-2796).

Dementia status for all initial participants (n = 158) was determined by a single investigator (ED) at each blood draw visit (n = 453) using all available demographic, clinical, and cognitive data collected at the time of the blood draw. Cognitive assessment included the Rapid Assessment for Developmental Disabilities (RADD) [367], the Severe Impairment Battery (SIB) [368], and the Dementia Questionnaire for Mentally Retarded Persons (DMR) [369]. All DS participants were classified as meeting criteria for AD (DS-AD) or not meeting criteria for AD (DS-NAD). A total of 12 individuals were excluded from the analysis due to A) confounding medical or psychiatric comorbidities which obscured a clear dementia determination or B) inconsistent longitudinal clinical data indicating a reversal in state from

DS-AD to DS-NAD. Thus, a final group of 146 DS participants (78 DS-AD and 68 DS-NAD) were included in the present analyses. Anticipating that DS-AD participants would be older than DS-NAD participants, the blood specimen corresponding to the visit when the participant was oldest for DS-NAD participants was selected. For DS-AD participants, the blood sample corresponding to the youngest available age was submitted to metabolomics.

Phlebotomy Protocol, Blood Processing, and Long-Term Storage. Venous blood was collected using standard venipuncture technique into EDTA vacutainer collection tubes. Given general considerations of working with DS participants and their unique needs, the research team did not attempt to standardize blood collection procedures regarding medication administration, prandial state, or time of day. Not standardizing these collection protocols may have introduced biological noise limiting resolution to detect true differences in metabolite abundances between the groups. As for the LOAD analyses conducted in CHAPTER 1, rigorous statistical parametrization of this potentially confounding metabolomic variability minimized the likelihood of false-positive associations associated with these statistical risks [304, 309].

Following venipuncture, collection tubes were gently inverted several times and centrifuged to separate the plasma component. Plasma was transferred to individual 500-µl siliconized cryovials and stored long-term at the University of California Irvine Alzheimer's Disease Research Center (UCI-ADRC) biorepository at −80°C. Plasma samples were shipped via overnight courier to the Lombardi Cancer Center Shared Resource Facility Metabolomics Core at Georgetown University for mass spectrometry analyses. The average plasma storage duration at −80°C was 9.8 years (range 0.4–14.1).

Metabolomics Methods

Untargeted LC-MS Metabolomics. Ultra-performance liquid chromatography electro-spray ionization-quadrupole-time of flight-mass spectrometry (UPLC-ESI-QTOF-MS; Xevo-G2 QTOF, Waters Corporation) was used to conduct untargeted metabolomic profiling as described in previous work [12, 13, 28]. Briefly, plasma samples were prepared for MS by solvent extraction and resolved using reverse phase chromatography on an Acquity UPLC (Waters Corp.) online with a QTOF-MS in positive and negative electrospray ionization (ESI) modes with optimized run parameters. LC-MS peaks were determined from resulting raw instrument data using XCMS software [302]. XCMS processing of LC-MS data within PCDA-DS resulted in a total of **4962** small-molecule (< 1.5 kDa) chemical features; **978** in the negative mode (ESI-) and **3984** in the positive mode (ESI+). These features resulting from LC-MS metabolomics were defined in terms of physicochemical properties (Mass-to-Charge Ratio: ***m/z***; chromatographic retention time: **RT**).

Untargeted Gas Chromatography-MS Metabolomics. Gas Chromatography-MS (GC-MS) analyses took as input 50 µl volumes of isolated blood plasma extracted with 250 µl of water/methanol/chloroform solvent containing 4-nitrobenzoic acid. The resulting solvent fraction was then dried under vacuum. Sample derivatization was completed using 20 µl of methoxyamine added to dry samples in an agitator at 60°C for 30 min. This was followed by 100 µl of MSTFA. A 1.5 µl volume of the derivatized solution was injected in (1:5) split mode into an Agilent 7890B GC system (Santa Clara, CA, USA) coupled with a Pegasus HT TOF-MS (LECO Corporation, St. Joseph, MI, USA). Separation was achieved on an Rtx-5 w/Integra-Guard capillary column (30 m × 0.25 mm ID, 0.25 µm film thickness; Restek Corporation, Bellefonte, PA, USA), with helium as the carrier gas at a constant flow rate of 1.0 ml/min.

Electron impact ionization (70 eV) at full scan mode (40–600 m/z) was used, with an acquisition rate of 20 spectra per second in the TOF/MS setting. These GC-MS analyses provided a final dataset of 67 annotated species.

Targeted Tandem (LC-MS/MS) Metabolomics. Multiple reaction monitoring mass spectrometry-based targeted analysis of free amino acids and metabolites associated with energy metabolism (i.e., biogenic acids) was performed as developed by Waters cooperation [370]. Target molecules for consideration in peripheral plasma were identified based on existing knowledge of biochemical alterations in DS [371]. Briefly, plasma samples (25 µl) were mixed with 300 µl of methanol: chloroform (2:1). To this, 100 µl of water and chloroform were added, separately. The samples were A) vortexed and incubated on ice for 10 min and then B) centrifuged at 13,000 rpm at 60°C for 15 min. The upper aqueous layer was carefully transferred to a separate vial and dried under a gentle stream of nitrogen. The samples were reconstituted in ACN:Water (50:50) containing 1 µg/ml of internal standard (tyrosine-^{15}N). The supernatant was transferred to an MS vial and 5 µl of sample was used for analysis.

Targeted quantifiaction of lactic acid was performed using multiple reaction monitoring mass spectrometry. The samples were resolved on an Acquity UPLC CSH Phenyl-Hexyl column, 2.1 × 100-mm column online with a triple quadrupole mass spectrometer (Xevo-TQ-S, Waters Corporation, USA) operating in the multiple reaction monitoring (MRM) mode. Signal intensities from all MRM Q1/Q3 ion pair for lactic acid were ranked to ensure selection of the most intense precursor and fragment ion pair for MRM-based quantitation. This approach resulted in selection of cone voltages and collision energies that maximized the generation of each fragment ion species. The metabolite ratios were calculated by

normalizing the peak area of endogenous metabolites within participant plasma samples normalized to the internal standard (i.e., tyrosine- ^{15}N). The sample queue was randomized, and solvent blanks were injected to assess sample carryover using four biological replicates each for both DS-AD and DS-NAD plasma specimens.

Statistical Methods

Univariate Group-Wise Comparisons. To examine group differences which might influence metabolite expression, DS-AD and DS-NAD participants were compared on several demographic and clinical and cognitive variables using Student's independent-sample *t*-tests (participant age at blood draw, plasma storage duration) or chi-square tests of independence (participant sex, premorbid degree of intellectual disability, current medications). Student's independent-samples *t*-tests were also used to examine DS-AD and DS-NAD groups on cognitive outcomes (RADD, SIB, DMR-Sum of Cognitive Scores). The statistical significance threshold for all *t* and χ^2 statistics was $\alpha = .05$. GC-MS and LC-MS/MS analyses used Mann-Whitney *U* test false discovery rate (FDR)-adjusted *p*-values < .05. This non-parametric test of two independent groups guarded against the skewness of metabolite distributions across DS-AD and DS-NAD participant specimens. Log-base-2 ratios of groupwise median expression values (per metabolite feature) quantified the direction and magnitude of these differences. In all comparisons, DS-AD participants were considered relative to aging individuals with DS who did not meet criteria for clinical AD.

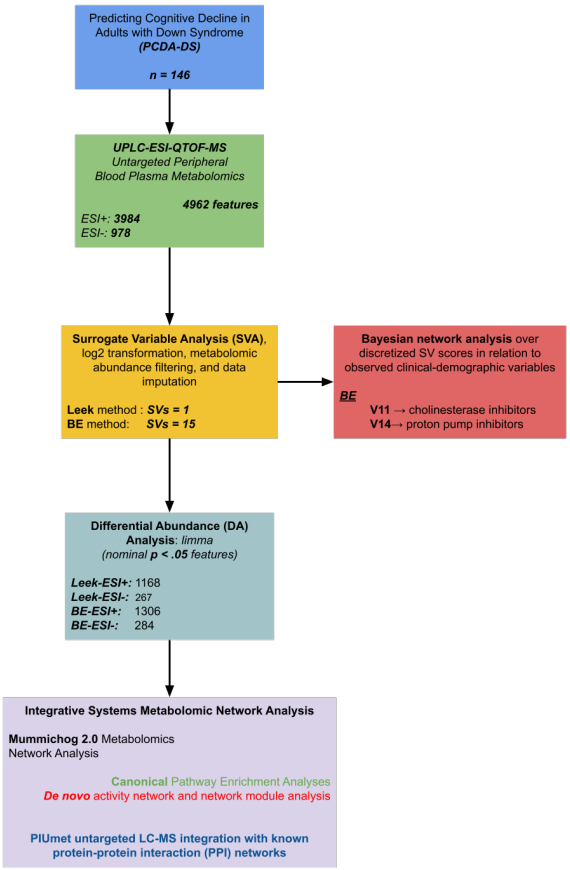

Figure 2.1 LC-MS untargeted metabolomics pipeline for profiling of DS-AD versus DS-NAD peripheral plasma specimens.

Untargeted LC-MS Differential Abundance Analysis and Modeling Pipeline. The statistical pipeline used in metabolomics experiments of DS-AD peripheral plasma are reported in **Figure 2.1**. Briefly, zero abundance and missing LC-MS measurements were replaced as "NAs." Features which survived variance thresholding were then submitted to *k*-nearest-neighbors imputation (K = 10) to generate a data matrix free of missing and artefactual values. These were subsequently base-2 logarithm transformed to improve symmetry and reduce positive skewness of metabolite mass features. These data, however, almost certainly reflect biochemical variability in the blood plasma metabolome unrelated to that in DS which stratifies participants with and without evidence of clinical AD. As with LOAD analyses in

CHAPTER 1, the aging plasma metabolome of those with DS likely reflects biological processes unrelated to cognitive status in DS-AD itself. This again motivated the use of surrogate variable analysis (SVA) to parametrize sources of potentially confounding variability unrelated to clinical, cognitive status in the plasma of aging individuals with DS. To minimize algorithmic bias, sets of significant surrogate variables were estimated using both Leek [304, 309] and Buja-Eyuboglu (BE) [303] methods (SVs_{Leek} = **1,** SVs_{BE} = **15**).

To better characterize observed participant variables (e.g., age at blood draw, *APOE*4+ risk genotype, sex) in relation to estimated SVs, Bayesian network models [307, 372] were constructed using participant-level, discretized BE and Leek SV scores for a subset of participants with known values for all clinical-demographic variables (n_{subset} = 127 participants) [306]. This allowed the relationships amongst these latent and observed variables to be visualized and considered in an integrated manner. Fitted surrogate variable scores for each participant were then included as covariates in linear models which estimated the abundance of each observed metabolite as a function of cognitive status (DS-NAD versus DS-AD) [308]. The nominal, unadjusted *p*-values associated with this phenotypic contrast for each metabolite feature (indexed by m/z ratio, RT) were then submitted to integrative pathway analysis using Mummichog 2.0 software [258]. Using both BE and Leek SV estimation methods, unadjusted *p*-values from feature-wise linear modeling were also transformed as the $-\log_{10}$(*unadjusted* ***p****-values*) to construct a set of empirical "prizes" to be modeled as prize-collecting Steiner trees (implemented in PIUmet software) [341]. This allowed for the inferred mapping of unannotated metabolomic mass features to latent dysregulated proteins differentiating DS-AD and DS-NAD blood plasma through known protein-protein interaction (PPI) networks. The mRNA expression of consensus genes

identified through PIUmet analyses were characterized at the tissue and CNS-cell level using GTEx and the Barres Lab Brain RNA-Seq database, respectively [373].

Software. Analyses employed R version 4.0.5. Imputation was completed using the *impute* package. SVA was carried out using the *sva* package. Empirical Bayes-moderated linear models and metabolite-wise phenotypic contrasts were evaluated using the *limma* package. Mummichog 2.0 was used to model systems-scale, coordinated changes in the peripheral metabolome due to either control or preclinical AD status: mummichog.org. The *bnlearn* package contributed functions for constructing Bayesian networks, which ingested features jointly discretized by the package *GridOnClusters*. PIUmet was used to estimate latent dysregulated protein networks from untargeted LC-MS profiling experiments: http://fraenkel-nsf.csbi.mit.edu/piumet2/.

RESULTS

Demographic, Clinical, and Cognitive Group Differences: DS-AD versus DS-NAD

Participant characteristics stratified by DS-AD versus DS-NAD status are reported in **Table 2.1**. The DS-AD and DS-NAD groups differed significantly on several relevant variables. The DS-AD group was significantly older ($p < .001$), had greater premorbid intellectual disability ($p < .001$) and DS-AD plasma was stored at −80°C significantly longer ($p < .001$) than the DS-NAD group. In addition, there were more females than males in the DS-AD group and more males than females in the DS-NAD group ($p < .001$). Finally, the DS-AD group had a higher proportion of individuals taking cholinesterase inhibitors ($p < .001$), anticonvulsants ($p < .001$), and the antioxidant supplement vitamins A, C, and E *(alpha-lipoic acid)* ($p < .001$) than the DS-NAD participants. These significant group differences were considered further using Bayesian networks. Specifically, these networks integrated A)

known clinical-demographic variables with B) participant-level SV scores computed in the differential abundance (DA) analysis of metabolite features in DS-AD versus DS-NAD plasma. Significant differences between the groups on cognitive outcomes were also observed. As expected, DS-AD participants demonstrated greater impairment on the SIB ($p < .001$), the RADD ($p < .001$), and the cognitive section of the DMR ($p < .001$).

Table 2.1 Participant Characteristics: Means and Standard Error of the Mean (*SEM*)

	n *(M/F)*	*Participant age at blood draw in years*	*Plasma storage duration in years*	*Premorbid degree of intellectual disability*	*SIB (max = 100)*	*RADD (max = 76)*	*DMR-SOC (max = 44)*
Down syndrome Alzheimer's disease ***(DS-AD)***	78 (36/42)	53.7 (.7)	11.8 (.3)	**32%** Mild **39%** Moderate **25%** Severe **4%** Profound	54.5 (3.9) *n* = 58	26.4 (2.3) *n* = 58	29.5 (1.3) *n* = 75
Down syndrome No Alzheimer's disease ***(DS-NAD)***	68 (44/24)	46.1 (1.3)	7.5 (.5)	**49%** Mild **32%** Moderate **10%** Severe **9%** Profound	82.5 (2.5) *n* = 64	46.4 (2.3) *n* = 65	10.3 (1.4) *n* = 67

Differentially Abundant Peripheral Metabolite Features Distinguish DS-NAD and DS-AD Plasma

Following initial metabolomics data pre-treatment (including imputation and log*2*-transformation) (see **METHODS),** 4962 putative metabolite features identified by LC-MS were submitted to surrogate variable analysis (SVA). These empirical estimation methods can improve reproducibility in large-data "-omics" experiments by specifically considering the molecular variability in the peripheral metabolome associated with the DS-AD versus DS-NAD comparison (e.g., as opposed to fasting and/or medication-withholding status). To minimize bias in downstream analysis due to algorithm choice, both Buja-Eyuboglu (BE) [303] and Leek [304] algorithms were used to fit respective sets of surrogate variables to PCDA-DS metabolomics data (SVs_{Leek} = **1,** SVs_{BE} = **15**).

Bayesian network analyses relating participant-wise surrogate variables to observed clinical-demographic factors did not observe associations between SVs and age at blood draw, sex, or *APOE*4+ genotype in the *APOE*-genotyped subset of the PCDA-DS cohort (n_{subset} = **127**; DS-AD $_{APOE4+}$ = **24**, DS-NAD $_{APOE4+}$ = **11**, chi-square test of independence p = .074). Using Leek-method SVs, anticipated associations were observed between cognitive and social subscales of the DMR, in addition to relationships between the RADD, SIB, and premorbid levels of intellectual disability (**Figure 2.2A**). BE-method SVs recapitulated these associations across cognitive measures, while also associating SV11 to cholinesterase inhibitor use (SV scores $_{taking}$ < SV scores $_{not\ taking}$). Similarly, participant scores on SV14 were observed to moderate status for taking/not taking proton pump inhibitors (SV scores $_{taking}$ < SV scores $_{not\ taking}$) (**Figure 2.2B**).

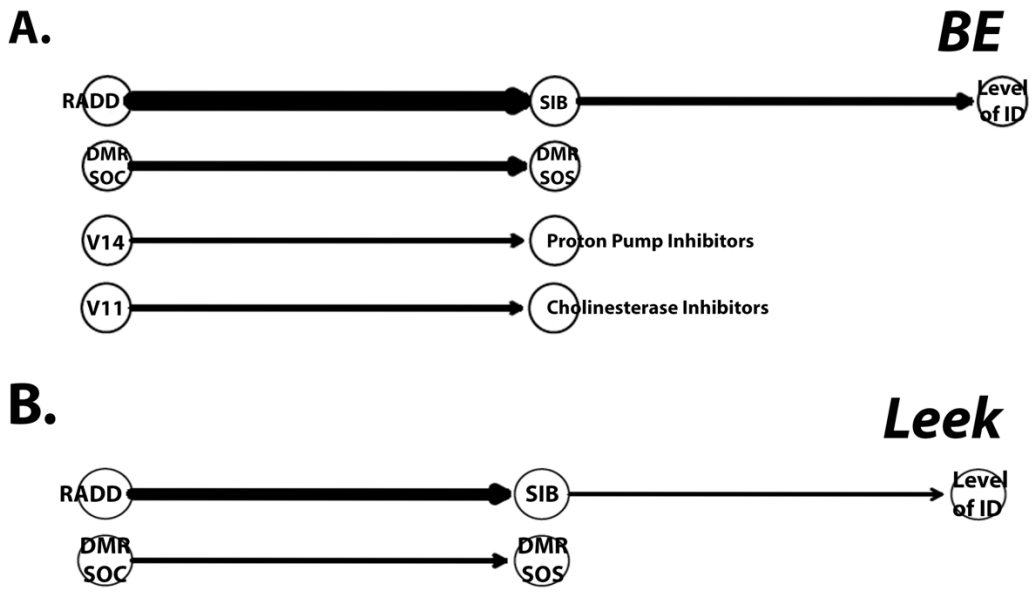

Figure 2.2 Bayesian network models relate **A)** BE and **B)** Leek estimated surrogate variables to observed clinical-demographic factors. In both cases, anticipated relationships between cognitive assessments were recapitulated (e.g., RADD, SIB, DMR). In the BE method network, significant surrogate variables related to cholinesterase and proton pump inhibitor use. Line boldness indicates confidence/strength of the estimated relationships between factors estimated from participant data and a fitted network structure [*bnlearn:* arc.strength()].

LC-MS mass feature abundances were then estimated from linear models as a function of **A)** AD status (DS-AD versus DS-NAD) and **B)** fitted, participant-level surrogate variable scores. Analysis of DA metabolite features was carried out in parallel using both BE and Leek methods of surrogate variable estimation. Of the **1590** features submitted to DA modeling using the BE method, **1306** features in the ESI+ mode of detection and **284** in the ESI- mode significantly differed due to AD status, nominal *p's* < .05. Using the Leek method of SV estimation, similar proportions of features were DA in peripheral plasma of those with clinical AD (ESI+: **1168**, ESI-: **267**).

Differentially Abundant Peripheral Metabolite Features between DS-AD and DS-NAD Plasma are Enriched within Known Metabolic Pathways

Mass features identified by *m/z* and RT were ranked according to nominal *p*-value and taken as input to integrative Mummichog 2.0 metabolomic network modeling. Peripheral metabolic change characterizing clinical AD in aging DS implicated multiple known biochemical pathways significantly associated with and enriched in relation to the DS-AD to DS-NAD comparison (**Table 1.2**). In particular, (*Leek-ESI+*)-identified processes including vitamin E ($p < .001$), porphyrin ring ($p < .01$), and glycerophospholipid metabolism ($p = .066$). These accompanied alterations to components of the fatty acid/ mitochondrial carnitine shuttle system ($p < .0001$), all of which were recapitulated in (*BE-ESI+*)-mode analyses, *p*'s < .05. The modeling of *Leek-ESI-* metabolite features also identified significant alterations to lipid metabolism, but further identified alterations to the central carbon metabolism of hexose sugars including glycolysis. Similar findings were noted in *BE-ESI-* analyses, in addition to differences in biogenic amine and pyrimidine nucleic acid metabolism, all *p*'s < .05.

Table 2.2 Canonical Biochemical Pathways Differing between DS-AD and DS-NAD Plasma by Mummichog 2.0 Analyses

Pathways	Overlap Size	Pathway Size	p-value	ESI Mode	SV Mode
Aminosugars metabolism	2	3	0.03185	NEG	BE
Arachidonic acid metabolism	2	2	0.01151	NEG	BE
Arginine and Proline Metabolism	3	5	0.01605	NEG	BE
Ascorbate (Vitamin C) and Aldarate Metabolism	1	1	0.09991	POS	BE
Beta-Alanine metabolism	3	4	0.00874	NEG	BE
Carnitine shuttle	13	23	8.00E-05	POS	LEEK
Carnitine shuttle	12	23	0.00025	POS	BE
Fructose and mannose metabolism	2	2	0.00832	NEG	LEEK
Fructose and mannose metabolism	2	2	0.01151	NEG	BE
Galactose metabolism	2	2	0.00832	NEG	LEEK
Galactose metabolism	2	2	0.01151	NEG	BE
Glutamate metabolism	2	3	0.03185	NEG	BE
Glutathione Metabolism	2	2	0.01151	NEG	BE
Glycerophospholipid metabolism	10	29	0.0663	POS	LEEK
Glycerophospholipid metabolism	2	4	0.0358	NEG	LEEK
Glycerophospholipid metabolism	3	4	0.00874	NEG	BE
Glycine, serine, alanine and threonine metabolism	3	4	0.00874	NEG	BE
Glycolysis and Gluconeogenesis	3	6	0.01546	NEG	LEEK
Glycolysis and Gluconeogenesis	4	6	0.00462	NEG	BE
Histidine metabolism	2	2	0.01151	NEG	BE
Leukotriene metabolism	2	3	0.02075	NEG	LEEK
Leukotriene metabolism	2	3	0.03185	NEG	BE
N-Glycan biosynthesis	1	1	0.1	POS	LEEK
Porphyrin metabolism	4	5	0.00403	POS	LEEK
Porphyrin metabolism	4	5	0.00143	POS	BE
Propanoate metabolism	2	3	0.03185	NEG	BE
Prostaglandin formation from arachidonate	3	4	0.00403	NEG	LEEK
Prostaglandin formation from arachidonate	3	4	0.00874	NEG	BE
Pyrimidine metabolism	3	7	0.04554	NEG	BE
Pyruvate Metabolism	2	3	0.02075	NEG	LEEK
Pyruvate Metabolism	3	3	0.00244	NEG	BE
TCA cycle	1	1	0.09991	POS	BE

Vitamin E metabolism	9	15	0.00084	POS	LEEK
Vitamin E metabolism	8	15	0.00109	POS	BE

Differentially Abundant Peripheral Metabolite Features between DS-AD and DS-NAD Plasma are Enriched within *De Novo* Metabolic Pathways

Analyses using Mummichog software also discovered several *de novo* metabolic pathways associated with the DS-AD versus DS-NAD comparison. The *Leek-ESI+* comparison particularly implicated components of the mitochondrial fatty acid carnitine shuttle system (**Figure 1.3A**), $p = .0027$. This finding was also observed in *BE-ESI+* with the further inclusion of coenzyme A (a further component of the carnitine fatty acid shuttle). This accords with additional findings from these analyses in *Leek-ESI+ and BE-ESI+* linking the metabolism of lipophilic, fatty vitamin E (alpha-tocopherol) and ubiquinone/ Coenzyme Q metabolites, which both serve to attenuate oxidative stress and promote redox homeostasis (**Figure 1.3B**). This corroborated analyses in *Leek-ESI-* which implicated glutathione redox metabolism in relation to components of glycolysis, pyruvate fermentation to lactate, the citric acid cycle, and immuno-bioactive PUFA signaling lipids, $p = .0087$. This also included glutathione conjugates of these latter polyunsaturated lipids and elements of the mitochondrial pyruvate/ malate shuttle (**Figure 1.3C**).

Analyses in *BE-ESI-* implicated very similar cytosolic-mitochondrial metabolism involving this same shuttle in addition to pyruvate fermentation and glutamate metabolism. Interestingly this *BE-ESI- de novo* network also included oxygenated biogenic amine (5-oxoproline) and short-chain fatty acid (SCFA) derivatives associated with microbiome status, $p = .00372$ (**Figure 1.3D)**, particularly as these relate to glutamate metabolism as in

glutaminolysis. Strikingly, *BE-ESI+* modeling recapitulated the intersection of phospholipid membrane remodeling and mitochondrial acyl-carnitine metabolism better known as Lands' cycle, p = .012 (**Figure 1.3E**). At a more macroscopic metabolic scale, this same process contributes substantially to physiologically extended hepatic lipoprotein and cholesterol metabolism which has been associated with AD and other metabolic disorders of abnormal aging. Through coenzyme A, this network also incorporated peroxisomally dependent ether lipid metabolism and hepatic bile acid biosynthesis/conjugation.

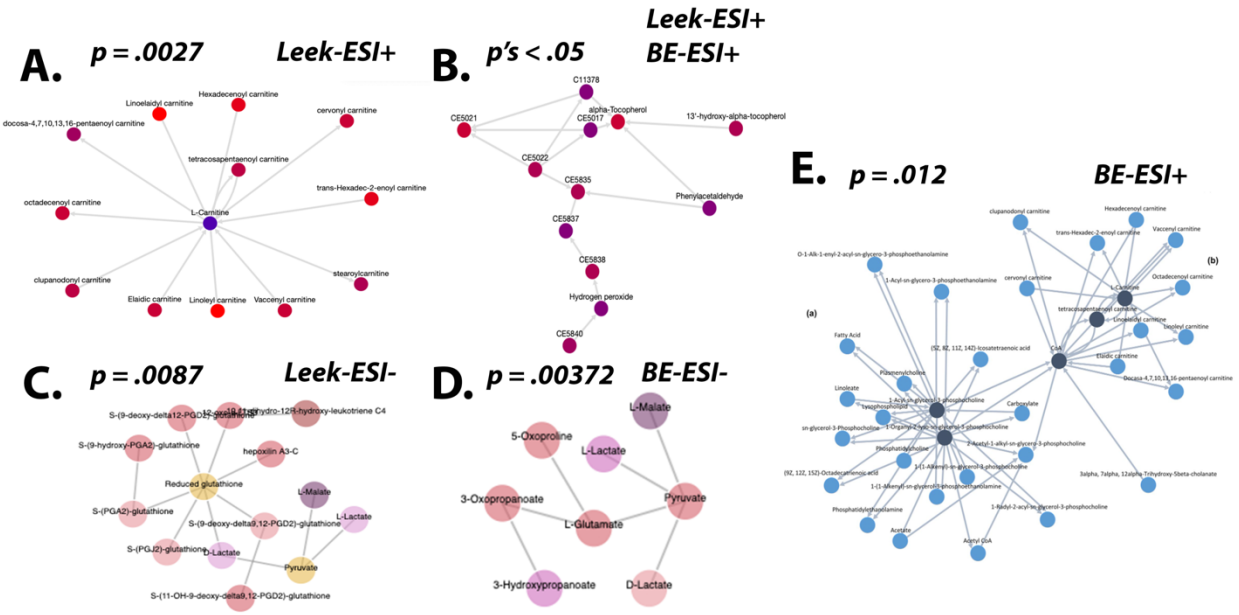

Figure 2.3 Significant Mummichog *de novo* metabolic modules are overrepresented with metabolite features nominated by the DS-AD versus DS-NAD statistical comparison.

While *de novo* Mummichog modeling can powerfully suggest pathway-level biochemical associations corresponding to clinical and cognitive status in DS aging, it does not directly map these processes to corresponding human genes. It also does not leverage prior biological knowledge regarding the associations between potentially implicated genes as described by protein-protein interaction (PPI) networks. In contrast, this alternative *de*

novo pathway reconstruction method (taking unannotated *m/z* features and -$\log_{10}$*(nominal **p**-values)* as input) has been implemented in the software PIUmet [374]. These analyses undertaken in parallel for both Leek and BE SVA methods in the DS-AD versus DS-NAD comparison demonstrated concordance in PCDA-DS blood plasma specimens. This included multiple metabolically relevant genes encoding enzymes such as branched chain amino acid transaminase 1 (*BCAT1*), prosaposin (*PSAP*), and ethanolamine phosphotransferase I (*SELENOI/ EPT1*) (**Figure 2.4**).

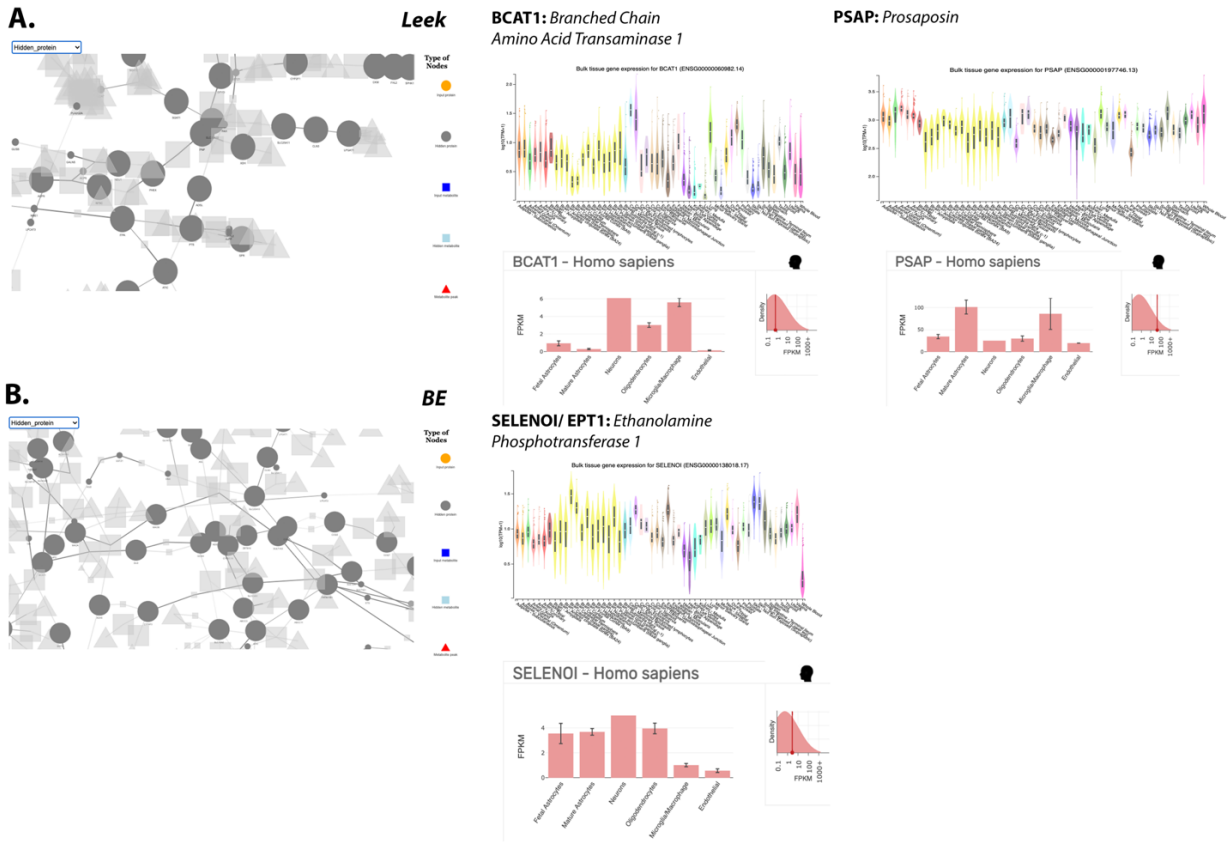

Figure 2.4 PIUmet de novo untargeted metabolomics analyses using **A)** Leek and **B)** BE SVA algorithms identify latent proteins implicated in metabolic differences characterizing DS-AD versus DS-NAD peripheral blood plasma. Global tissue-level expression in humans was accessioned using *GTEx*. Human, CNS-cell-level profiling was accessioned from the Barres lab Brain RNA-Seq database [373].

GC-MS and Targeted LC-MS/MS Confirm Peripheral Bioenergetic and Biosynthetic Metabolites are Altered in DS-AD Plasma

To follow-up on and further extend the findings from the untargeted LC-MS profiling, subsequent GC-MS and targeted LC-MS/MS experiments were conducted. These corroborated integrative LC-MS pathway analyses and provided further evidence of altered bioenergetic and biosynthetic metabolism in DS-AD. Specifically, GC-MS analyses identified lactic acid (the end product of lactic acid fermentation following glycolysis) as upregulated in DS-AD plasma (FDR = .0007, *Log2FC* = 1.2) (**Figure 2.5A**). Interestingly, a structural isomer of lactic acid—dihydroxyacetone—trended toward upregulation in DS-AD versus DS-NAD plasma (FDR = .062, *Log2FC* = .47). Although this isomer is not directly involved in glycolysis, its triose phosphate derivative—dihydroxyacetone phosphate (DHAP)—is itself a glycolysis intermediate. The significance of this finding remains uncertain, but it may indicate a shift toward metabolically ineffective, cytotoxic, and pro-inflammatory advanced glycation end-product (AGE) formation [375, 376]. Confirming *de novo* LC-MS analyses, GC-MS-analyzed plasma alpha-tocopherol/ vitamin E levels were elevated in those with DS-AD compared to DS-NAD (FDR = .028, *log2FC* = .275).

Findings from untargeted LC-MS and targeted GC-MS experiments implicated energy metabolism, specifically lactic acid in the case of the latter GC-MS platform. Further targeted experiments using LC-MS/MS were undertaken. This permitted the unambiguous identification and quantification of several organic acid abundances to better understand the relationship of this biochemical class to DS-AD in peripheral plasma (**Table 2.5**). Consistent with bioenergetic alterations, upregulation of pyruvic acid (FDR = .028, *Log2FC* = 1.1) in DS-AD participants was observed (**Figure 2.5B**). Unrelated to glycolysis itself, but perhaps

associated with bioenergetic functions, there was also a significant upregulation of methyladipic acid (FDR = .028, *Log2FC* = .64) in DS-AD relative to DS-NAD participants (**Figure 2.5C**). Importantly, quantitative LC-MS/MS analyses of lactic acid unambiguously confirmed its upregulation in DS-AD versus DS-NAD patients (FDR = .0001, *Log2FC* = .86; Median $_{DS\text{-}AD}$: 712.5 µg/ml, Median $_{DS\text{-}NAD}$: 392.7 µg/ml) (**Figure 2.5D**).

It has been previously suggested that nutrient availability may impact synaptic integrity [321]. It is also increasingly regarded that synaptic loss and derangement may be highly proximal to cognitive impairment [377]. For this reason, plasma levels of folic acid and uridine were evaluated in patient blood plasma. As proposed by Wurtman and others [201, 321], uridine levels may be rate limiting for the synthesis of membrane components such as phosphatidylcholine lipids (PCs). These are the same molecules Mapstone and others have previously found to be depleted in the blood plasma of older adults with preclinical AD [28, 29]. Additionally, deficiencies in folic acid may lead to limited regeneration of methionine methyl groups, impaired hepatic choline synthesis, and consequent reduction of available docosahexaenoic acid (DHA) to be used for membrane lipid synthesis [321]. Uridine levels were found to be significantly depleted in the plasma of DS-AD participants relative to the DS-NAD participants (Figure 2.5E) (FDR = .007, *Log2FC* = −1.31); however, levels of folic acid were not different between the groups (FDR > .5).

Recent studies (and PIUmet *de novo* analyses here) have also suggested that free amino acid levels (e.g., *branched chain amino acids:* isoleucine, leucine, valine) are depleted in the peripheral blood of those at increased risk of developing dementia and AD [378]. It is also possible that amino acid oxidation in the dementing or demented brain may serve as an alternative bioenergetic resource in the face of impaired glucose metabolism [326, 327].

Employing LC-MS/MS methods, no free amino acids significantly differed between DS-AD and DS-NAD participant bloods (all FDR > .05). This included the branched chain amino acids leucine and isoleucine, although valine was not measured due to abundances below the lower limit of reliable quantification in most samples. This null finding is challenging to interpret in the context of *de novo,* untargeted PIUmet analyses of PCDA-DS participant plasma, which associated branched-chain amino acid metabolic genes (*BCAT1*) to the DS-AD versus DS-NAD comparison across both Leek and BE method analyses.

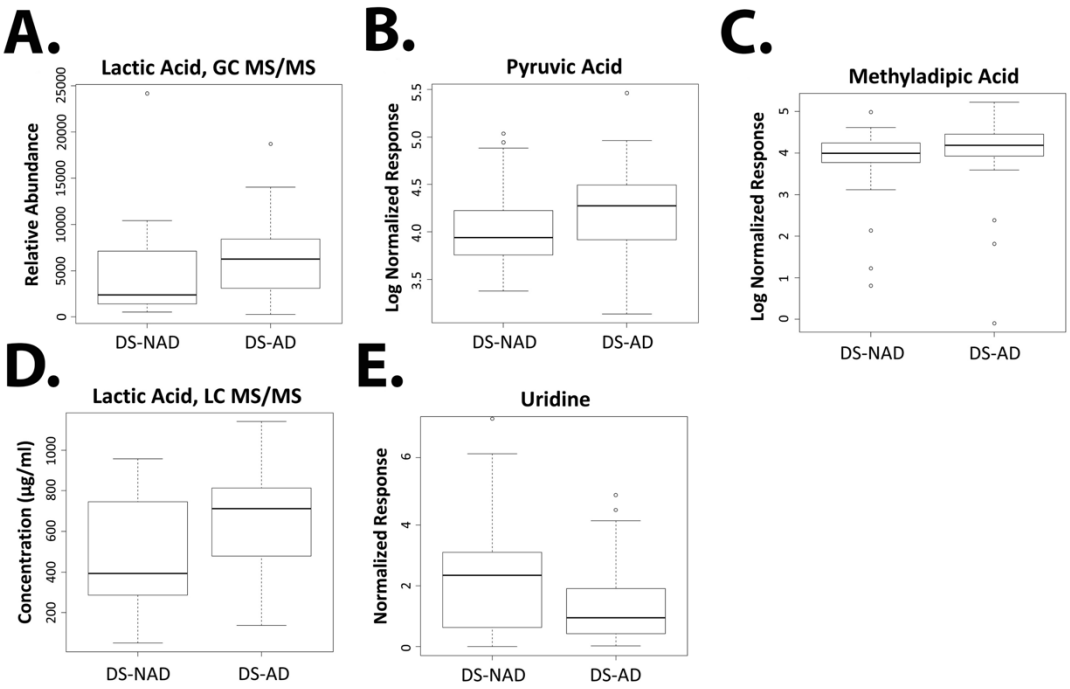

Figure 2.5 Targeted metabolomics experiments quantifying several biogenic acids which demonstrated FDR < .05 significant differences between DS-AD and DS-NAD plasma. This specifically included significant differences in lactic acid across GC-MS (**2.5A**) and LC-MS/MS (**2.5D**). Additionally, membrane biosynthetic cofactors (i.e., uridine) were significantly depleted in DS-AD blood (**2.5E**).

DISCUSSION

The present experiments in DS-AD and DS-NAD blood plasma using multiple metabolomics platforms and analysis approaches strongly support the hypothesis that

cognitive decline in this vulnerably aging population occurs concomitantly with emerging dysmetabolism. This specifically includes the peripherally apparent dysmetabolism of several biogenic acids including lactate important for bioenergetic processes. As observed in CHAPTER 1 for LOAD, DS-AD peripheral metabolic associations were diverse but included components of lipid, central carbon/glycolytic, and "glutaminolysis-like" metabolism identified across both canonical and *de novo* metabolic pathway analyses. Furthermore, PIUmet analyses of these same metabolite features stratified by clinical, cognitive status nominated several inferred genes encoding diverse metabolic enzymes. These enzymes were complexly distributed in their transcription across human tissues (including the brain) and heterogeneously expressed across CNS cell types including neurons and glia.

Critically, it is unclear if all metabolic models generated to describe the DS-AD versus DS-NAD comparison strictly represent disease-associated biochemical processes and/or pathways. This is because those cognitively impaired individuals with DS were also significantly more often prescribed anticholinergic medications in addition to antioxidant supplements (i.e., vitamins A, C, and E/*alpha-lipoic acid*). Bayesian network analyses of BE-estimated surrogate variables in relation to clinical covariates identified SV11 as covarying with use of cholinesterase inhibitor drugs. Because cholinergic dysmetabolism has long been described as a component of AD pathobiology [379, 380], the parametrization of cholinesterase inhibitor use by SV11 likely improved statistical power to identify AD-associated peripheral metabolic change itself unrelated to these pharmaceutical interventions in aging DS.

Similarly, vitamin E metabolism was associated with dementia status in aging individuals with DS. Because participants with DS-AD more frequently consumed dietary

supplements of this metabolite, additional experiments will be necessary to determine if this finding suggests an A) true pathobiological change in DS-AD remediated by the supplement or simply a B) readout of biological target engagement by therapeutics (e.g., vitamin E, cholinesterase drugs). This latter possibility may not be supported by the metabolomics experiments conducted in this chapter, which nominated vitamin E metabolism specifically despite DS-AD participants disproportionately supplementing with vitamins A and C also. Neither of these latter, small-molecule cofactors (nor their metabolic pathways) were mapped to the comparison of DS-AD and DS-NAD plasma. This importance of vitamin E is intriguing, as it represents one of the few, key lipophilic antioxidants in human tissues and cells, including the brain [381]. Its specific roles in DS aging, however, remain unclear [382]. Consistent with the hypothesis that clinical AD accompanies changes to a physiologically extended, CNS-peripheral metabolic axis, vitamin E metabolism is complexly distributed across human tissues and organ systems [383]. Regardless of the possible mechanistic roles of vitamin E or choline metabolism in AD pathobiology, metabolomic analyses of peripheral blood appear to sensitively index biological target engagement associated with these compounds in aging adults with DS to whom these therapies have been prescribed. Although beyond the scope of this dissertation, such peripheral blood biomarkers could be highly useful for minimally invasive therapeutic response monitoring and should be further studied.

Untargeted LC-MS Profiling and Modeling Experiments

Multiple, untargeted metabolomic modeling experiments and analyses in DS-AD plasma suggested a diversity of metabolic perturbations involving both central carbon and lipid metabolism. Very similar to findings reported A) by Mapstone and colleagues

previously and B) in CHAPTER 1 for LOAD, these included *de novo,* minimally biased reconstructions of phospholipid and acyl-carnitine intermetabolism (i.e., Lands' cycle) (**Figure 2.3E**). This included metabolite glycerophosphocholine (GPC) (i.e., phosphatidylcholine absent its fatty acid chains), which is elevated in the cerebrospinal fluid of euploid Alzheimer's patients [384]. Further metabolism of GPC is facilitated by the gene glycerophosphocholine phosphodiesterase 1 (*EDI3*), which controls the cleavage of GPC and, thus, augments glycerol-3-phosphate (G3P) and choline availability [385]. In addition to its role in glycerolipid synthesis, G3P contributes to redox balance and oxidative phosphorylation in the brain by oxidizing cytosolic NADH generated through glycolysis (i.e., the glycerol phosphate shuttle) and permitting further glycolytic flux. Although it remains to be evaluated empirically, such a ratio of GPC/PC or the abundances of its downstream metabolites may index lipid dysmetabolism in DS-AD and potentially AD broadly.

Mummichog *de novo* modeling also identified a second node highly suggestive of acyl-carnitine metabolism. Module membership for acyl-carnitines was heterogeneous with respect to acyl chain length and degree of unsaturation, indicating that the process captured by this node may be relatively non-specific across fatty acid species. Fatty acid composition differs between DS patients and non-affected sibling controls [386-389]; however, the mechanistic investigation of lipid dysmetabolism in aging DS and AD remains at early stages. In euploid AD patients, alterations of fatty acid metabolism are increasingly understood to impact cognitive and neuropathological outcomes [227].

It is interesting that a key metabolite bridging phospholipid and fatty acid pathways is Coenzyme A (CoA), which is conjugated to fatty acids to facilitate their transport into the mitochondria to undergo oxidation and fuel ATP synthesis. It is also required for the

remodeling of PC acyl chains via Lands' cycle, a process increasingly thought to exist in a complex equilibrium with the Kennedy pathway (i.e., glycerolipid biosynthesis) and the cytosolic free fatty acid pool [390]. Moreover, these lipid shutting dynamics may disproportionately implicate hepatic and lipoprotein metabolism in abnormal aging [22, 168, 318, 391, 392], although *APOE*4+ status was not significantly modeled in relation to estimated surrogate variables within PCDA-DS data. Alterations of coenzyme A-acetylating enzymes have long been appreciated as a component of AD cholinergic deficits. Coenzyme A also serves as a purinergic negative modulator of neuronal acetylcholine release [393], which is itself impaired in AD [394, 395]. Similarly, alteration of CoA metabolism and its flux through oxidative phosphorylation may reflect alternative and/or compensatory bioenergetic strategies to metabolic stress and insult [396].

Confirmatory, Targeted Metabolomics Experiments

Initial untargeted metabolomics profiling motivated confirmatory, targeted experiments quantifying several biogenic acids in peripheral blood related to cellular bioenergetic metabolism. Across both GC-MS and LC-MS/MS analysis platforms, lactate levels were elevated in the blood plasma of aging individuals with Down syndrome who met clinical and cognitive criteria for AD. Importantly, this elevated plasma lactate could indicate an elevated burden of epileptic events in these frankly dementing individuals with DS [397], for whom seizures occur more frequently and earlier compared to LOAD. Most individuals with DS-AD will develop seizures and their sudden onset in aging people with DS strongly suggests AD [46]. Indeed, aging participants with DS and AD were significantly more frequently prescribed anticonvulsant medications in the PCDA-DS aging cohort. No evidence

examined here or noted clinically, however, suggested that these participants experienced more epileptic events compared to those not meeting criteria for clinical AD.

Alternatively, the fermentative metabolism of pyruvate to lactate in the cellular cytosol suggests bioenergetic and biosynthetic strategies in DS tissues potentially both constrained by and in circumvention of emerging AD pathobiology. Lactate is the terminal product of lactic acid fermentation, in which pyruvate generated through glycolysis is diverted from complete, bioenergetically optimal oxidation in the mitochondria. Compared to oxidative phosphorylation, it is bioenergetically inefficient and favored under anoxic or hypoxic conditions as well as in pathological metabolic states (i.e., the Warburg effect in cancer) [398, 399]. Lactic acid fermentation yields a net two molecules of ATP for each molecule of glucose entering glycolysis. This is in stark contrast to the 30+ ATP afforded by complete oxidation of one glucose molecule by the citric acid cycle and electron transport chain. Critically, no significantly greater frequency of overtly hypoxic or anoxic events was noted for DS-AD relative to DS-NAD participants in the PCDA-DS cohort, which could otherwise explain mitochondrially independent, alternative pathways of glucose metabolism favoring lactate formation.

This biochemical reprogramming involving lactate could bypass dysfunctional and dyshomeostatic mitochondrial metabolism occurring in pathological aging, possibly as an ultimately futile compensatory strategy associated with AD cognitive decline as observed for the experiments included within this chapter. Clarifying these mechanistic details to improve translational outcomes will require further investigation of specific pathobiology, cells, and/or tissues implicated in DS-AD in addition to their associated oxygen-dependent, mitochondrial metabolism. Lactate metabolic reprogramming in aging DS evident within

peripheral circulation, however, suggests a distinct, but nonetheless physiologically extended, CNS-peripheral metabolic axis in aging. Its compromise and pathological alteration by evolving AD could thus systemically mediate trajectories of cognitive decline in DS.

This hypothesis, particularly as it relates to lactate and glycolytic dysmetabolism, has been previously considered in AD. Relatively inefficient glycolytic glucose metabolism independent of mitochondria and occurring despite sufficient oxygen to completely oxidize glucose (i.e., aerobic glycolysis, *the Warburg effect*) has been observed in LOAD both in the CNS and periphery [132, 133, 400, 401] . In the CNS specifically, aging-related alterations to aerobic glycolysis spatially correlate with amyloid deposition in the highly speciated human neocortex, particularly structures within the default mode network (DMN) [132, 133, 243, 402, 403]. Aerobic glycolysis may also mediate the co-occurrence of amyloid and tau pathologies in these same regions [133]. Much like complex, multicellular CNS metabolic processes including the neuroglial glutamate-glutamine cycle, lactate metabolism as proposed in the astrocyte-neuron lactate shuttle (ANLSH) hypothesis could also be altered in DS-AD [324, 404, 405].

Future studies of DS-AD should include more sensitive measures of cortical oxygen consumption to further clarify whether elevated lactate in DS-AD plasma suggests true reprogramming towards aerobic glycolysis specifically [406]. Due to the unstandardized blood draws used in the present study, it also remains a possibility that elevations in lactic acid in DS-AD versus DS-NAD patients reflect differences in these groups unrelated to core disease pathophysiology (e.g., due to dementia-associated comorbidities, disproportionate motion/agitation demonstrated by DS-AD patients during blood draw) [407]. Where

established and clarified further, elevation of peripheral plasma lactate in aging adults with DS-AD could provide another, biochemically discrete example of complex, multifactorial systems metabolic "compensations for emerging failure" driving emerging "failures of compensation" in evolving dementia.

Alterations to peripheral biogenic acids in those with DS-AD were, however, not limited to lactate, suggesting potentially broader metabolic dyshomeostases in this aging population. Targeted acidomics experiments further indicated a statistically significant increase in pyruvic and methyladipic acid within the plasma metabolome of participants with DS-AD. The significant elevation in pyruvic acid is interesting in the context of simultaneously elevated peripheral lactate in this group, as it indicates that DS-AD is not only marked by increased lactic acid fermentation, but either A) increased glycolytic flux itself or B) decreased entry of pyruvate into mitochondrial metabolism as acetyl-CoA. This could instead suggest the importance of glycolysis-adjacent pathways (e.g., the pentose phosphate pathway) vital for anabolic biosynthesis and redox homeostasis [408-410]. Rather than as fuel affording metabolism, this exactly describes how Raichle and colleagues have increasingly considered the role of aerobic glycolysis in LOAD, where DS-AD could involve similar processes [132, 411, 412].

Findings in DS-AD plasma regarding methyladipic acid may indicate elevated levels of phytanic acid ω-oxidation, in which methyladipic acid is the terminal metabolite of this process [413, 414]. Phytanic acid and its metabolites serve as ligands of the lipometabolic transcription factors PPARα and the retinoid X receptor (RXR) [413]. Together, the expression and activation of these transcription factors is indicative of energy deprivation and supportive of compensatory bioenergetic mechanisms such as ketogenesis [415].

Interestingly, phytanic acid is not a metabolite of human origin, but is rather consumed through the meat and dairy products of grazing animals as a metabolite of chlorophyll [416]. This may indicate that dysregulation in methyladipic acid in DS-AD individuals indexes dietary factors A) relevant to disease progression and B) potentially interacting with metabolic dysfunction consequent to trisomy of chromosome 21 and aging. Furthermore, phytanic acid metabolism depends upon peroxisomal integrity, where pathological alterations of this organelle have been associated with highly deleterious, genetically driven phytanic acid accumulation (i.e., Refsum disease) [416]. Peroxisomal metabolic dysfunction has also been previously associated with AD and neurodegeneration overall. In this way, its altered metabolism may reflect the integrity of these organelles in DS-AD and should be further explored in relation to associated fatty acid and lipid metabolic dyshomeostases [417].

Uridine was also differentially abundant in the plasma of DS-AD versus DS-NAD participants (i.e., depleted in DS-AD). Other than its role in phospholipid synthesis via the Kennedy pathway, it is also essential for the entry of alternative carbohydrate sources (e.g., galactose) into glycolysis and subsequent ATP generation [418]. It is thus possible that membrane lipid synthesis and glycolysis may compete for uridine under circumstances when glucose is unabundant relative to other, alternative carbohydrate sources such as galactose. The administration of supra-physiological doses of D-galactose to rodents is indeed a potent, accepted model of accelerated aging not unlike descriptions of DS aging as a “segmental progeroid phenotype” [216, 419, 420]. Although this has mechanistically been understood to result from the formation of toxic advanced glycation end products (AGEs) and consequent oxidative stress [421-423], it may also be consequent to D-galactose's effects

on bioenergetic tone and its dependence on uridine metabolites as metabolic cofactors. As evidenced by targeted GC-MS findings that the methylglyoxal-forming, triose phosphate derivative dihydroxyacetone trended toward upregulation in DS-AD versus DS-NAD patients (FDR = .062), these two phenomena may not be mutually exclusive [376].

The present experiments were not without limitations. Most clearly, cognitive assessment of PCDA-DS participants only distinguished the presence or absence of manifest, clinical AD, rather than mild cognitive impairment (MCI) or preclinical disease. This partially resulted from the retrospective nature of PCDA-DS analyses reported in this chapter, but also speaks to theoretical and methodological challenges inherent to studying DS-AD. Because of premorbid, lifelong intellectual disability characterizing these participants (to an often moderate or greater degree) [212, 351], robust, early cognitive change appreciable in LOAD or ADAD is not as easily observed for DS-AD due to psychometric assessment floor effects. Complicating translational studies in aging DS, this does not necessarily suggest that these stages of advancing AD do not occur in this population, nor that metabolic change does not characterize these progressive transitions. Indeed, because DS has been described as an oxidative-stress-induced condition of accelerated aging (e.g., cataract formation, diabetes, hair graying, loss of auditory/visual acuity) [216, 419], the rate of underlying pathobiological and metabolic change in advancing DS-AD relative to changes in clinical status could be highly complex and subject to individual differences key to improved, personalized therapies [78]. Better parametrizing this uncertainty surrounding true cognitive change in aging individuals with DS due to AD will be of key importance to mapping this early decline to systems metabolic correlates (if not drivers) quantifiable within peripheral circulation.

Conclusion

In all, biochemical alterations of blood plasma in aging individuals with DS and AD demonstrate increased levels of pyruvic acid, lactic acidosis, depleted uridine, and possibly lipid metabolic reprogramming compared to that of non-demented DS controls. These effects appear to occur in the absence of evident hypoxia or increased incidence of anoxic events in DS-AD as compared to DS-NAD. Although the drivers and purpose of this metabolic shift remain unclear and require further inquiry, DS-AD appears to be characterized by bioenergetically relevant alterations evident in peripheral blood plasma which could be leveraged as potential markers of this pathophysiology. Although requiring further validation, a minimally invasive, inexpensive plasma lactic acid assay may prove useful for clinical trials patient stratification and the staging of dementia progression in aging adults with DS. That this may serve similar functions in in dementing, euploid older adults is a possibility that should be evaluated in future work. Based on findings reported in CHAPTER 1 for preclinical LOAD, however, such biological similarities are highly plausible. Although incompletely overlapping, peripheral metabolic changes characterizing the development of both LOAD and DS-AD may convergently demonstrate how clinical AD can ultimately result from partially distinct (but dyshomeostatically equivalent) failures of metabolic compensation resulting from metabolically involved compensations for emerging failure.

CHAPTER 3. Familial, Autosomal Dominant Alzheimer's Disease: **ADAD**

Familial, autosomal dominant Alzheimer's disease (ADAD) has most strongly informed mechanistic hypotheses of AD pathogenesis and progression. These efforts were greatly advanced in molecular genetic terms by initial reports in the 1980s and 90s of large-effect, amyloidogenic, protein-coding mutations within amyloid precursor protein (*APP*) and presenilin (*PSEN1-2*) genes. Subsequent investigations for more than three decades of affected family pedigrees within which these mutations dominantly segregate have firmly established them as causative, mechanistic drivers of early-onset dementia and extensive cortical amyloidosis in aging [73]. These findings thus contributed substantial empirical support for the amyloid cascade hypothesis proposed first by Hardy, Selkoe, Higgins, and colleagues [5, 6], which consequently drove the generation and adoption of *APP* and presenilin transgenic mutant rodent models of AD. Such models (most faithfully recapitulating CNS amyloidosis) have unfortunately proven limited in their ability to advance therapeutics in humans [105]; however, this is perhaps also a function of ADAD composing less than 1% of all aging adults who will develop AD [221].

In addition, the amyloidogenic and molecular-genetic perspectives historically considered by much ADAD research (including specific investigations of the amyloid cascade hypothesis) have seldom considered amyloidogenesis and its catabolism as metabolic processes, nor AD as broadly and systemically dysmetabolic overall [354, 356, 424, 425]. It is thus not surprising that metabolomics studies themselves have been scarce in ADAD, including studies of peripheral biofluids such as blood plasma [426, 427]. Consistent with the amyloid cascade hypothesis, both peripheral blood and CSF research in ADAD have instead more frequently examined amyloid beta and tau abundances [428].

These investigations are not unlike similar, recent studies examining amyloid and tau proteopathies in in DS-AD, which (like ADAD) presents with early-onset and substantial, genetically driven cortical amyloidosis [429]. Consistent with findings in LOAD and DS-AD, Mosconi and colleagues have reported that pre-symptomatic adults with dominantly inherited *PSEN1* coding mutations demonstrate decreases in cerebral glucose metabolism. Critically, reductions exceeded those consistent with atrophy alone, as observed through structural MRI of these same regions [249]. This pattern resembles recent findings identified in LOAD using multi-tracer PET imaging, where clinically impaired individuals demonstrated cerebral glucose metabolic deficits (attenuated FDG-PET tracer retention) exceeding those explained by synaptic density loss alone in the neocortex, but not the medial temporal lobes (i.e., quantified using [^{11}C] UCB-J PET) [255].

Investigations of the amyloid cascade hypothesis in ADAD have often defined it as a linearly feedforward biological process proceeding from amyloid deposition to progressive tauopathy, neurodegeneration, and ultimately cognitive decline [5, 6, 430] (but also see: [431]). This contrasts with recent reports of a *PSEN1* coding mutation carrier in the seventh decade of life who, only in the past decade, has met criteria for mild cognitive impairment (MCI) [223]. Cognitive resilience persisted for this person despite prolific cortical amyloidosis, which was minimally accompanied by tauopathy or posterior cingulate glucose hypometabolism usually typical of advancing ADAD [249]. These exceptional outcomes were attributed to the participant's homozygosity for the rare Christchurch allele of the *APOE* gene, which poorly binds heparin sulfate proteoglycans similar to the AD-protective *APOE* ε2 allele [223, 224]. Other studies examining preclinical ADAD participants have failed to identify the Christchurch allele as a protective factor [432,

433], but suffered from small sample size. Nonetheless, they do suggest that poorly understood genetic variation in ADAD could explain individual differences in cognitive decline relative to family-wise-average ages of onset [434-436]. Although expressed APOE serves physiological roles related to amyloid metabolism and clearance [437, 438], it is also a critical component of lipoprotein complexes and intercellular lipid trafficking within the CNS in particular [168, 439-441]. This is highly consistent with the increasingly understood vulnerability of lipo-homeostasis in AD across multiple systems biological scales of organization in abnormal physiological and cognitive aging [11, 318].

Joe, Ringman and colleagues have also recently conducted proton magnetic resonance spectroscopy (^{1}H-MRS) neurochemical imaging for several preclinical ADAD mutation carriers within clinically identified, affected family cohorts [442]. These experiments indicated that *N*-acetyl-aspartate (NAA) and glutamate/glutamine (Glx) levels were depleted in the anterior cingulate cortices of preclinical mutation carriers. In contrast, the precuneus/ posterior cingulate demonstrated depleted NAA in addition to elevated myo-inositol (mI) and choline (Cho). Although cortical NAA levels have been thought to indicate neuronal integrity relative to its depletion in AD [329], this brain metabolite also serves as an anabolic reserve for oligodendroglial myelin lipid synthesis, perhaps in a manner related to the elevation of AD risk though broader cardiovascular compromise [443]. Increased levels of mI in these ADAD-affected neocortical regions also associated with estimated, family-wise ages of dementia onset. The metabolite mI is known to indicate pro-inflammatory, reactive glial activation in the AD cortex [329, 331], but also increasing amyloid beta burden in these affected structures [442].

Substantial opportunity exists to further investigate metabolic changes characterizing the development of ADAD, particularly as peripheral metabolomics approaches can resolve potentially many more disease-related compounds in blood plasma compared to current metabolic imaging methods. Considered in terms of prior biochemical knowledge, computational systems metabolomics approaches might have increased resolution to probe deeply into cellular metabolic pathways to better identify disease-related changes. In CHAPTER 1, analyses of blood plasma derived from two independent cohorts of preclinical LOAD participants (relative to cognitively stable controls) demonstrated converging evidence of shared, peripherally evident dysmetabolism at this early stage of disease. Interestingly, this included alterations to glucose/ hexose, lipid/ fatty acid, and "glutaminolysis-like" central carbon metabolism including that of NAA metabolites quantified in the AD cortex using ^{1}H-MRS.

Analogous to the preclinical phase of LOAD, aging adult participants carrying ADAD-causing mutations, but who remain cognitively stable, also demonstrate preclinical AD. Any associated alterations to the plasma metabolome in preclinical ADAD, however, remain unclear, particularly in relation to those changes identified in CHAPTER 1 for preclinical LOAD. Because LOAD is not driven by large-effect mutations in amyloid processing genes specifically linked to prolific amyloidosis in aging, the peripheral blood metabolic correlates of ADAD could differ from those identified in sporadic, late-onset disease. Even in such a case, these diverging patterns of compensation might nonetheless implicate vulnerable, tenuous metabolic programs situated in a final common biochemical pathway of AD etiopathogenesis. Despite these differences as a function of dissociable etiologic risk, emerging metabolic vulnerability and dyshomeostases in both preclinical LOAD and ADAD

could equivalently suggest evidence of physiologically extensive, CNS-peripheral metabolic networks mediating the effects of abnormal cognitive aging in AD.

Because CHAPTER 2 considered the PCDA-DS aging cohort (which dichotomized cognitive status in DS-AD based on presence or absence of clinical AD), it is not intuitive how these levels of cognitive status should be mapped to the continuum of objective impairment in aging adults without premorbid and lifelong neurodevelopmental differences (i.e., as in LOAD and ADAD). As detailed in the previous chapter, reaching consensus on this theoretical issue in coming years will be important for aligning systems biological disease staging across diverse and disparate clinical populations for precision healthcare aims. More immediately, peripheral plasma from neurodevelopmentally typical LOAD or ADAD participants demonstrating objective cognitive deficits of any severity could be compared to those peers who remained cognitively stable despite elevated, respective sources of risk. Similarly, in LOAD and ADAD, the plasma metabolome of preclinical participants could also be compared to respective peers demonstrating objective cognitive impairment if not decline (i.e., MCI/ AD). Family-wise average ages to AD diagnosis varied across affected mutation carriers and could be estimated for individual participants. This permits simple rank correlational analyses of metabolite features by anticipated years until clinical onset, which could suggest changes in peripherally indexed metabolic networks covarying with these chronic, progressive disease processes.

METHODS

Participants and Cognitive Assessment. Participants from the R/OCAS LOAD cohort demonstrating any degree of objective, amnestic cognitive decline (i.e., MCI/AD) and

cognitively stable, at-risk controls are described extensively in CHAPTER 1 METHODS. Briefly, this cognitive test battery quantified major cognitive domains (attention, executive functions, language, memory, and visuo-perceptual skills) impaired by emerging clinical AD. Test-level assessment scores were aggregated into composite *z*-scores reflecting each cognitive domain, where a *z*-score less than 1.35 below the cohort median was considered objectively impaired compared to healthy, same-age peers.

In addition, a total cohort of 80 individual participants from families possessing ADAD mutations were longitudinally followed by a single investigator (JR) in a comprehensive study of preclinical and manifest ADAD at a tertiary dementia referral center. The study was approved by the University of California Los Angeles (UCLA) Human Subjects Committee and informed consent was obtained from all individual participants included in the study (i.e., the UCLA ADAD cohort). All subjects were first-degree relatives of someone known to carry a pathogenic mutation in the *APP* or *PSEN1-2* genes, placing them at 50% risk for inheriting such a mutation and therefore developing ADAD. Genetic testing for the relevant mutation was performed as part of this study but subjects were not informed of the results. Revealing clinical genetic testing was offered outside of the research protocol for interested subjects.

The Clinical Dementia Rating Scale (CDR) was performed with an unrelated informant [444]. The CDR is a structured interview with input from both the participant and an informant who knows the subject well. In the CDR, asymptomatic persons are rated 0, and persons with questionable cognitive impairment are rated 0.5. Scores of 1, 2, and 3 represent mild, moderate, and severe stages of dementia, respectively. The Mini-Mental State Examination (MMSE) was also administered to each participant who contributed

peripheral blood specimens [445]. Each subject's age in relation to their estimated age of dementia diagnosis was further calculated. Because the age of onset of symptoms is consistent within a given family and mutation (but more variably so between families), an "adjusted age" can be calculated that estimates how many years from disease manifestation a given subject is [446]. In the single experimenter's experience, the age of clinical diagnosis of dementia is a more reproducible measure and therefore this adjusted age was calculated for each subject as his or her chronological age minus the median age of dementia diagnosis in his or her family.

Consistent with the 2011 National Institutes on Aging – Alzheimer's Association (NIA-AA) restatement of the McKhann criteria for the cognitive staging of emerging, clinical AD (i.e., preclinical AD to mild cognitive impairment (MCI) to clinical AD), each participant in the ADAD cohort was assigned to one of these groups based on cognitive scores, overall clinical appraisal, and genotyping relative to non-carrier family controls [35, 37, 91, 96]. Preclinical ADAD was defined as adult mutation carriers who scored CDR < 1. Objective cognitive impairment described those mutation-carrying individuals who demonstrated evidence of either MCI or clinical AD (CDR > 1). Non-carrier family controls accounted for shared, sporadically heritable variation unrelated to *APP* or *PSEN1-2* Mendelian mutations, but possibly related to cognitive decline in AD.

Phlebotomy Protocol, Blood Processing, and Long-Term Storage. Details for the collection and storage of R/OCAS plasma specimens are described in CHAPTER 1 METHODS. For ADAD participants, venous blood was collected using standard venipuncture technique into EDTA vacutainer collection tubes. Specimens were then centrifuged to separate the plasma component following venipuncture, which was aliquoted and stored long-term at −80°C. The

research team did not attempt to standardize blood collection procedures regarding medication administration, prandial state, or time of day. Not standardizing these collection protocols may have introduced biological noise limiting resolution to detect true differences in metabolite abundances between the groups. As for LOAD and DS-AD analyses conducted in CHAPTER 1 and 2 respectively, rigorous parametrization of this potentially confounding metabolomic variability minimized the likelihood of false-positive associations associated with these statistical risks [304, 309]. Plasma samples were shipped via overnight courier to the Lombardi Cancer Center Shared Resource Facility Metabolomics Core at Georgetown University for mass spectrometry analyses.

Metabolomics Methods

Untargeted LC-MS Metabolomics. Ultra-performance liquid chromatography electro-spray ionization-quadrupole-time of flight-mass spectrometry (UPLC-ESI-QTOF-MS; Xevo-G2 QTOF, Waters Corporation) was used to conduct untargeted metabolomic profiling as described in previous work [12, 13, 28]. Briefly, plasma samples were prepared for MS by solvent extraction and resolved using reverse phase chromatography on an Acquity UPLC (Waters Corp.) online with a QTOF-MS in positive and negative electrospray ionization (ESI) modes with optimized run parameters. LC-MS peaks were determined from resulting raw instrument data using XCMS software [302]. XCMS processing of LC-MS data resulted in a total of **3536** small-molecule (< 1.5 kDa) chemical features; **1569** in the negative mode (ESI-) and **1967** in the positive mode (ESI+). These features resulting from LC-MS metabolomics were defined in terms of physicochemical properties (Mass-to-Charge Ratio: ***m/z***; chromatographic retention time: **RT**).

Statistical Methods

Untargeted LC-MS Differential Abundance Analysis and Modeling Pipeline. The statistical pipeline used in metabolomics experiments of ADAD peripheral plasma is described in **Figure 3.1**. Briefly, zero abundance and missing LC-MS measurements were replaced as "NAs." Features which survived variance thresholding were then submitted to *k*-nearest-neighbors imputation (K = 10) to generate a data matrix free of missing and artefactual values. These were subsequently base-2 logarithm transformed to improve symmetry and reduce positive skewness of metabolite mass features. These data, however, almost certainly reflect biochemical variability in the blood plasma metabolome unrelated to that which stratifies specific statistical contrasts of interest in ADAD (e.g., preclinical disease versus non-carrier family controls). The aging plasma metabolome of those with ADAD likely reflects many biological processes unrelated to cognitive risk and decline itself. This again motivated the use of surrogate variable analysis (SVA) to parametrize sources of potentially confounding variability unrelated to clinical, cognitive status in the plasma of aging individuals with ADAD. To minimize algorithmic bias, sets of significant surrogate variables were estimated using both Leek [304, 309] and Buja-Eyuboglu (BE) [303] methods (SVs_{Leek} = **0,** SVs_{BE} = **13**). All subsequent modeling experiments employed the non-zero set of 13 BE-estimated SVs.

To better characterize observed participant clinical-demographic variables (e.g., age at blood draw, *APOE*4+ risk genotype, sex) in relation to estimated SVs, Bayesian network models [307, 372] were constructed using participant-level, discretized BE SV scores [306]. This allowed the relationships amongst these latent and observed variables to be visualized and considered in an integrated manner. Fitted surrogate variable scores for each

participant were then included as covariates in linear models which estimated the abundance of each observed metabolite as a function of cognitive status (*contrasts:* A) non-carrier family controls compared to preclinical disease, B) objective cognitive impairment (MCI/AD) compared to preclinical disease) [308]. The nominal, unadjusted *p*-values associated with these phenotypic contrasts for each metabolite feature (indexed by m/z ratio, RT) were then submitted to integrative pathway analysis using Mummichog 2.0 software [258]. For canonical pathway analyses in Mummichog, the significance of the overlap across these DA metabolic pathways was determined using Tanimoto-Jaccard statistics and bootstrapped significance testing (α = .05).

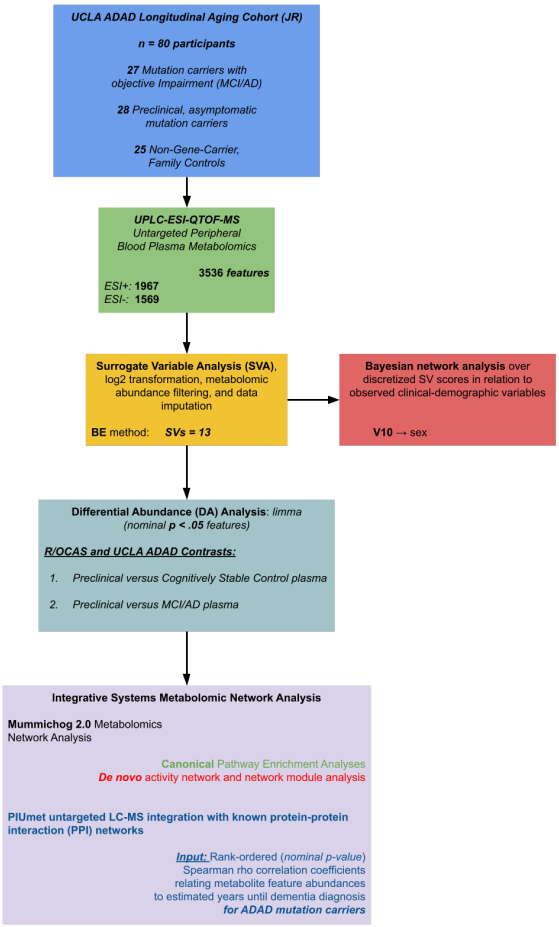

Figure 3.1 A schematic diagram details the untargeted LC-MS metabolomics pipeline and downstream statistical analyses to which R/OCAS and UCLA ADAD participant plasma specimens were submitted for integrative comparison.

Unadjusted *p*-values from feature-wise, Spearman correlation modeling were also transformed as the -$\log_{10}$(*unadjusted* ***p****-values*) to construct a set of empirical "prizes" to be modeled as prize-collecting Steiner trees (implemented in PIUmet software) [341]. This allowed for the inferred mapping of unannotated metabolomic mass features to latent dysregulated proteins correlated to estimated years until dementia diagnosis. Specifically, these relationships were computationally estimated through known protein-protein interaction (PPI) networks empirically evaluated within blood plasma using untargeted LC-MS metabolomic profiling. The mRNA expression of selected candidate genes identified through PIUmet analyses were characterized at the tissue and CNS-cell level using GTEx and the Barres Lab Brain RNA-Seq database, respectively [373].

Software. Analyses employed R version 4.0.5. Imputation was completed using the *impute* package. SVA was carried out using the *sva* package. Empirical Bayes-moderated linear models and metabolite-wise phenotypic contrasts were evaluated using the *limma* package. Mummichog 2.0 was used to model systems-scale, coordinated changes in the peripheral metabolome due to either control or preclinical AD status: mummichog.org. The *bnlearn* package contributed functions for constructing Bayesian networks, which ingested features jointly discretized by the package *GridOnClusters*. Tanimoto-Jaccard statistics and significance testing were completed using the *jaccard* package. PIUmet was used to estimate latent dysregulated protein networks from untargeted LC-MS profiling experiments: http://fraenkel-nsf.csbi.mit.edu/piumet2/.

RESULTS

R/OCAS and ADAD Cohorts: Participant Characteristics

To pursue comparisons between disease evolution across LOAD and ADAD, R/OCAS participants demonstrating any level of objective cognitive impairment (i.e., MCI/AD) compared to healthy peer controls were also considered (**Table 3.1**). Within the ADAD cohort and as anticipated, mutation carriers with MCI or AD scored significantly more impaired on the MMSE and CDR compared to preclinical mutation carriers, Mann-Whitney U *p*'s < .01. In contrast, MMSE scores and CDR sum of boxes between preclinical mutation carriers and non-carrier family controls did not significantly differ. The Global CDR score did, however, significantly differ between these two groups, Mann-Whitney U *p*-value < .01. In this genetically unaffected group of participants from families carrying ADAD-causing mutations, 24% of non-carriers themselves demonstrated evidence of preclinical or very mild dementia.

Neither sex nor *APOE*4+ genotype significantly differed in proportions across non-carrier, preclinical, and objectively impaired participants, chi-squared test of independence *p*-values > .40. Linear models were also fit (for mutation-carrying participants) to evaluate the impact of these same variables on estimated years until clinical dementia diagnosis. While sex did not significantly predict estimated years, APOE4+ genotype did demonstrate a trending main effect, $p = .072$. Specifically, *APOE*4+ individuals demonstrated median estimated years to diagnosis closer to zero (i.e., the estimated diagnosis event) compared to those without this risk genotype.

Table 3.1 Participant Characteristics for the R/OCAS and UCLA ADAD Cohorts

	n (M/ F)	*Mean age at blood draw in years (SD)*	*Mean MMSE (SD)*	*Mean CDR Sum of Boxes (SD)*	*Mean CDR Global (SD)*	*Mean estimated years to ADAD diagnosis **for mutation carriers** (SD)*	*Frequency of APOE4+ participants*	*Frequency of APP/ PSEN1-2 Mutations for Affected Carriers*
***R/OCAS:** MCI/AD Participants with LOAD*	52 (18/33)*	82.2 (4.2)					17	
***R/OCAS:** Cognitively Stable, At-Risk Controls (Non-Mutation Carriers)*	53 (19/34)	81.6 (3.5)					14	
***ADAD:** MCI/AD participants carrying amyloidogenic coding mutations*	27 (8/19)	43.4 (18.4)	21.6 (8.7)	4.26 (5.34)	.89 (.78)	-8.44 (10.7)	5	10 *APP*/ 17 *PSEN*
***ADAD:** Preclinical, asymptomatic participants carrying amyloidogenic coding mutations*	28 (10/18)	34.1 (8.81)	28.7 (1.05)	.143 (.23)	0.0 (0.0)	-13.11 (6.34)	4	1 *APP*/ 27 *PSEN*
***ADAD:** Non-Gene-Carrier, Family Controls*	25 (9/16)	39.2 (12.2)	28.4 (1.47)	.28 (.36)	.12 (.22)		7	

*Included one participant who did not complete further clinical-demographic screening (including sex), but was assigned as MCI/AD by a single investigator (MM)

Differentially Abundant Peripheral Plasma Metabolite Features Differentiate Control from Preclinical ADAD Plasma

Metabolic network modeling in CHAPTER 1 suggested that systems-scale dysmetabolism across metabolite classes and pathways can be observed in the peripheral

blood plasma of participants with preclinical LOAD. To evaluate this same possibility in preclinical ADAD, 3536 putative metabolite features identified by LC-MS were submitted to surrogate variable analysis (SVA) following initial metabolomics data pre-treatment (including imputation and log*2*- transformation) (see **METHODS).** Surrogate variables were fit across all available participant plasma specimens and improved statistical power to identify biochemical differences associated with preclinical, mutation-carrier status (compared to non-carrier, family controls). Because the Leek method of surrogate variable fitting did not estimate a non-zero number of SVs, downstream analyses exclusively used adjustment covariates derived from Buja-Eyuboglu (BE) computed values (SVs = 13).

Bayesian network analyses relating participant-wise surrogate variables to observed clinical-demographic factors identified few relationships between observed and surrogate variables, apart from a relationship between SV10 and sex (**Figure 3.2**). As expected, estimated age to dementia diagnosis demonstrated strong dependencies on age at blood draw. Estimated age until diagnosis itself impacted participant CDR Global scores, which corresponded to levels of cognitive functioning and Mendelian carrier status (i.e., cognitively stable non-carrier, preclinical carrier, and MCI/AD carrier) as described by the 2011 NIA-AA revised McKhann staging criteria for clinical AD [35, 37, 96]. Modeling of estimated age to dementia diagnosis also demonstrated unanticipated associations with participant-specific carrier genotypes (i.e., *APP* compared to *PSEN1-2* mutation carriers). Interestingly, but perhaps incidentally, presenilin gene mutation carriers were closer to time of estimated diagnosis compared to carriers of *APP* mutations, Mann-Whitney **U** p-value = .012. Those with *APP* gene mutations were, however, underrepresented and under-sampled (24% of all mutation carriers) relative to individuals with mutations in *PSEN1-2.* LC-MS mass feature

abundances were then estimated from linear models as a function of **A)** AD status (preclinical ADAD versus non-carrier control) and **B)** fitted, participant-level surrogate variable scores. Using BE method SV estimation, 140 features (ESI+: **88**, ESI-: **52**) of 3536 were differentially abundant in in the plasma of preclinical mutation carriers compared to non-carrier controls, all nominal Limma *p*'s < .05.

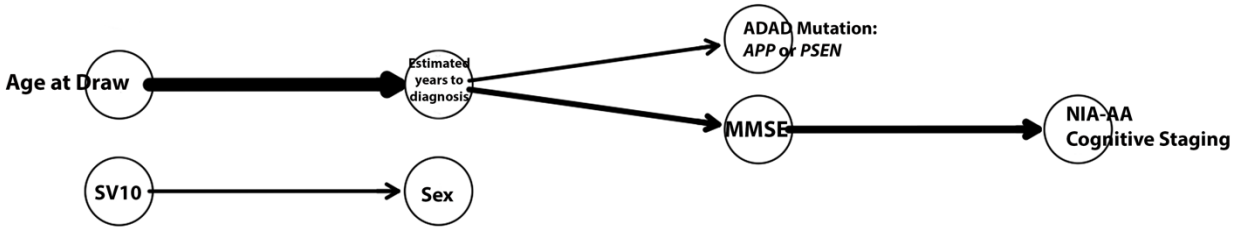

Figure 3.2 Bayesian network modeling associates surrogate variables to observed clinical-demographic factors. Participant sex demonstrated an association with SV10. Age at blood draw related to estimated years to dementia diagnosis, as anticipated. Line boldness indicates confidence/strength of the estimated relationships between factors estimated from participant data and a fitted network structure [*bnlearn:* arc.strength()].

Differentially Abundant Peripheral Metabolite Features between Preclinical ADAD and Control Plasma are Enriched within Known and *De Novo* Metabolic Pathways

Mass features identified by *m/z* and RT were ranked according to nominal *p*-value and taken as input to integrative Mummichog 2.0 metabolomic network modeling. Mass features identified by m/z and RT were ranked according to nominal *p*-value and taken as input to integrative Mummichog 2.0 metabolomic network modeling. Peripheral metabolic change characterizing preclinical versus control ADAD participants significantly implicated multiple, known biochemical pathways (**Table 3.2**). Specifically, ESI+ mode analyses identified the overrepresentation of DA metabolite features belonging to cysteine/methionine, porphyrin, and lipid metabolism (including that of bile acids and C21 steroid hormones), *p*'s < .05.

Table 3.2 Canonical Biochemical Pathways Differing between Preclinical ADAD and Non-Carrier, Family Control Plasma by Mummichog 2.0 Analyses

Pathways	Overlap Size	Pathway Size	Nominal *p*-value	ESI Mode
Drug metabolism - cytochrome P450	1	1	0.00723	POS
Methionine and cysteine metabolism	1	1	0.00723	POS
Porphyrin metabolism	1	4	0.02092	POS
C21-steroid hormone biosynthesis and metabolism	1	8	0.02554	POS
Bile acid biosynthesis	1	9	0.02622	POS
Leukotriene metabolism	3	4	0.00429	NEG
Glycine, serine, alanine and threonine metabolism	2	3	0.02126	NEG
Di-unsaturated fatty acid beta-oxidation	1	1	0.04016	NEG
Vitamin H (biotin) metabolism	1	1	0.04016	NEG
Urea cycle/amino group metabolism	1	1	0.04016	NEG
Arachidonic acid metabolism	1	1	0.04016	NEG
Prostaglandin formation from arachidonate	1	1	0.04016	NEG

Consistent with these findings, a unique empirical compound estimated by Mummichog and putatively identified as bilirubin was depleted in the plasma of participants with preclinical ADAD compared to controls (*Log2 Fold Change* = -.56). This was consistent with further, *de novo* metabolic network analyses of these samples, which identified an inferred metabolic activity network also centered on bilirubin metabolism. This included its *mono* and *di*-glucuronidation involving uridine phosphate co-factors and other heme metabolism intermediates (e.g., bilirubin) in the ESI+ detection mode (**Figure 3.3A**). Negative ionization mode analyses associated this preclinical ADAD statistical contrast instead with lipid metabolism, particularly polyunsaturated signaling lipid metabolism. Biogenic amine (e.g., glycine, serine, alanine), biotin, and urea cycle metabolites were also

overrepresented amongst those metabolomic features DA in preclinical ADAD blood plasma versus non-carrier controls, *p*'s < .05. Network, *de novo* metabolomic analyses in the ESI- mode for this contrast recapitulated the importance of biotin and polyunsaturated lipid metabolic hubs, particularly as these might be bridged by processes involving adenosine phosphate compounds (**Figure 3.2B**).

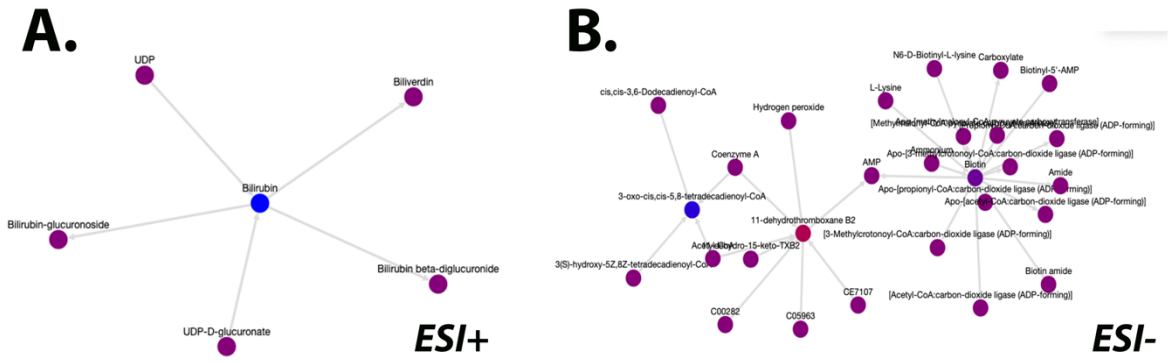

Figure 3.3 De novo metabolic activity networks in the A) **ESI+** and B) **ESI-** untargeted LC-MS detection modes inferred through the *preclinical ADAD-family control* statistical comparison. The discovered networks were unscored in terms of *p*-value significance.

CHAPTER 1 compared the statistical significance of the overlapping "fingerprint" between the R/OCAS and UCI ADRC preclinical LOAD cohorts in terms of shared, canonical metabolic pathways altered by emerging disease (quantified as Tanimoto-Jaccard coefficients). This same strategy can be employed to compare the overlap of canonical metabolic dyshomeostases jointly characterizing LOAD and ADAD preclinical participants. Defining all significantly enriched ($p < .05$) canonical pathways from R/OCAS and UCI ADRC cohorts as the preclinical LOAD signature (**Appendix 1.1**), this was compared to pathways overrepresented with DA metabolites in the ADAD preclinical plasma metabolome. Dysmetabolic, canonical fingerprints characterizing the ADAD and LOAD preclinical metabolomes demonstrated modest overlap to a statistically greater, significant degree than

would be anticipated due to chance alone (*uncentered estimated coefficient:* **.15 |** *centered coefficient:* **-0.11)**, $p = .001$ (**Appendix 1.2**).

Differentially Abundant Peripheral Metabolite Features between Preclinical and MCI/AD ADAD Plasma are Enriched within Known and *De Novo* Metabolic Pathways

Preclinical ADAD could suggest important insights regarding the pathobiology of emerging disease as indexed within the peripheral metabolome. It could also, however, suggest biochemical changes associated with the transition from A) cognitive resilience and compensation despite biological instability to B) concomitant, objective biological and cognitive deficits (i.e., MCI/AD). To evaluate this latter comparison in the ADAD plasma metabolome, peripheral blood from preclinical mutation carriers were compared relative to mutation carriers demonstrating objective cognitive decline (i.e., those meeting clinical criteria for MCI/AD). Untargeted LC-MS profiling experiments evaluating this comparison identified 3536 putative metabolite features which were submitted to surrogate variable analysis (SVA) following initial metabolomics data pre-treatment (including imputation and log*2*- transformation) (see **METHODS**).

Surrogate variables were fit across all available participant plasma specimens and improved statistical power to identify biochemical differences associated with preclinical, mutation-carrier status (compared to MCI/AD status) [304, 305]. As for the ADAD preclinical versus control comparison, downstream analyses exclusively used adjustment covariates derived from Buja-Eyuboglu (BE) computed values (SVs = 13), which were non-zero in number. Bayesian networks relating these SVs to observed, clinical-demographic covariates are reported in **Figure 3.1,** which suggested a relationship between SV10 and sex as

described for the ADAD *preclinical- family control* contrast. In total, **3536** LC-MS metabolite mass features (ESI-: **1569**, ESI+: **1967**) were submitted to downstream modeling as a function of A) estimated, participant-level surrogate variable scores and B) preclinical ADAD versus MCI/AD cognitive status. Differential abundance analysis in UCLA ADAD using linear modeling revealed that **173** ESI+ and **65** ESI- features significantly differed in their plasma abundances due to preclinical AD status compared to MCI/AD, nominal *p's* < .05.

Mass features (indexed by *m/z*, RT) were ranked according to nominal *p*-value corresponding to the preclinical- MCI/AD contrast as evaluated for participants from the UCLA ADAD cohort. These values derived using BE surrogate variable estimates were then submitted to canonical pathway enrichment analysis using Mummichog 2.0 (**Table 3.3**). Specifically, the ESI+ detection mode demonstrated significant enrichments (*p*'s < .05) for aminosugar, glycerophospholipid, fatty acid, porphyrin, galactose, and biogenic amine metabolism. Lipid dynamics were broadly affected, with specific impacts on steroid-backbone lipids including cholecalciferol, squalene, and cholesterol biosynthesis and metabolism (**Figure 3.4A**). Mummichog estimated several empirical compounds which corresponded to lipid metabolites and their metabolic co-factors. These included platelet-activating factor (PAF), dihydroxy-cholestenoate metabolites, 3-Methoxy-4-hydroxyphenylethyleneglycol (KEGG: **C05594**), bilirubin, and CDP-choline. *De novo* metabolomic network analyses recapitulated many of these as inferred dysmetabolic nodes characteristic of preclinical compared to MCI/AD ADAD mutation carriers.

Analyses in the LC-MS ESI- detection mode indicated possible dyshomeostases involving phosphatidylethanolamine (PE) glycerophospholipid metabolism (**Figure 3.4B**). Specifically, a Mummichog-estimated empirical compound putatively identified as PE was

upregulated in preclinical versus MCI/AD participants carrying ADAD-causing, coding mutations (*Log2 Fold Change* = .17). This compound could be involved in progressively disturbed lipid processes such as glycosylphosphatidylinositol (GPI)-anchor biosynthesis, vitamin A (retinol) metabolism, and glycerophospholipid metabolism, Mummichog canonical pathway enrichment *p*'s < .05.

Table 3.3 Canonical Biochemical Pathways Differing between ADAD Preclinical and MCI/AD Participants in Blood Plasma by Mummichog 2.0

Pathways	*Overlap Size*	*Pathway Size*	*Nominal p-value*	*ESI Mode*
Aminosugars metabolism	3	3	0.00101	POS
Glycerophospholipid metabolism	4	8	0.00681	POS
Galactose metabolism	2	3	0.01353	POS
Aspartate and asparagine metabolism	2	3	0.01353	POS
Glycine, serine, alanine and threonine metabolism	2	4	0.04504	POS
Porphyrin metabolism	2	4	0.04504	POS
Vitamin D3 (cholecalciferol) metabolism	1	1	0.04655	POS
Squalene and cholesterol biosynthesis	1	1	0.04655	POS
Fatty Acid Metabolism	1	1	0.04655	POS
Arginine and Proline Metabolism	1	1	0.04655	POS
Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	1	1	0.0021	NEG
Vitamin A (retinol) metabolism	1	3	0.00588	NEG
Glycerophospholipid metabolism	1	7	0.01622	NEG

In addition to UCLA ADAD cohort participants with MCI/AD, the R/OCAS cohort also included aging individuals with this same cognitive status. Using the same statistical pipeline as employed for previous Mummichog analyses in ADAD, significant canonical pathway enrichments (*p*'s < .05) were compiled into a fingerprint of metabolic dyshomeostases describing the preclinical versus MCI/AD LOAD comparison. Tanimoto-Jaccard coefficient

estimation and significance testing was then used to compare these two ADAD versus LOAD peripheral blood plasma metabolic fingerprints. These analyses demonstrated a modest overlap to a statistically greater, significant degree than would be anticipated due to chance alone (*uncentered estimated coefficient:* **.15 |** *centered coefficient:* **-0.18)**, p = .0015 (**APPENDIX 1.3**).

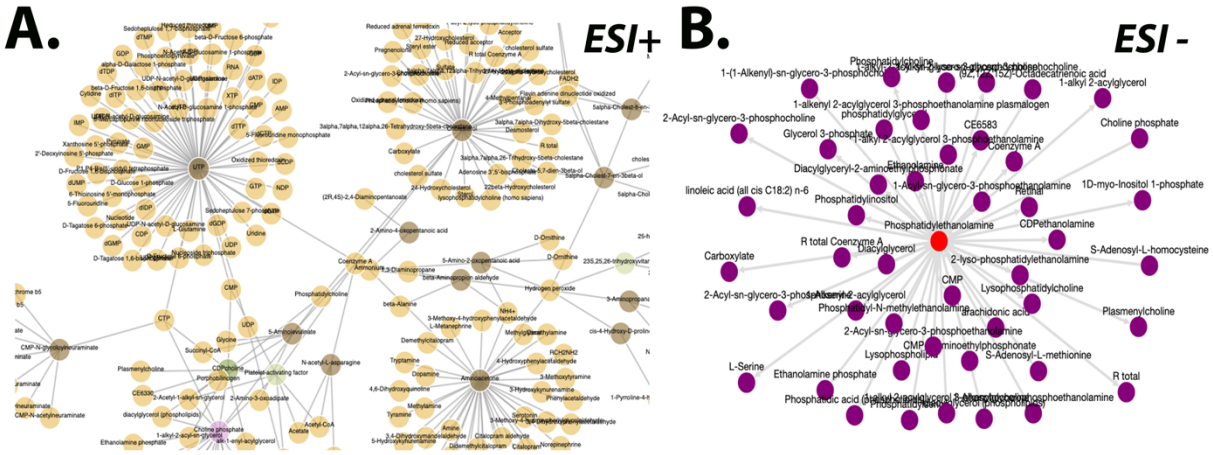

Figure 3.4 Mummichog *de novo* metabolomic activity networks in ADAD preclinical versus MCI/AD mutation carriers indicate alterations of lipid metabolism across both A) positive and B) negative ESI modes of LC-MS detection.

De Novo and Canonical Peripheral Metabolic Pathways Associate with Estimated Years to Diagnosis in ADAD Mutation Carrier Plasma

Compared to the sporadic occurrence of LOAD, asymptomatic adult individuals possessing ADAD mutations will in most cases eventually experience early-onset cognitive decline (but also see: [223]). This affords unique advantages for longitudinal research on abnormal human aging, particularly because estimated years until clinical diagnosis can be empirically approximated and vary predictably as a function of individual family cohorts [446]. It is, however, unclear how estimated age to diagnosis relates to the status of the peripheral plasma metabolome for mutation-carrying individuals independent of cognitive

status (preclinical or MCI/AD). Spearman correlations were calculated for 3536 untargeted LC-MS metabolomics features resulting in **470** which were significant at the nominal $p < .05$ level ($n_{\text{total mutation carriers}} = 55$). Mass features (indexed by *m/z*, RT) were ranked according to nominal *p*-value and submitted to canonical and *de novo* pathway analyses using Mummichog 2.0. A non-zero number of estimated empirical compounds were identified in the ESI+ mode analyses. These were enriched within pathways involved in mitochondrial fatty acid oxidation via the carnitine shuttle in addition to glycosphingolipid, purine, biopterin, and dihydroxy-cholestenoate metabolism (**Table 3.4**).

Table 3.4 Canonical Metabolic Pathways Significantly Enriched for Plasma Blood Biochemical Features Correlated with Estimated Years to Dementia Diagnosis

Pathways	Overlap Size	Pathway Size	Nominal *p*-value	ESI Mode
Biopterin metabolism	2	3	0.01731	POS
Purine metabolism	2	3	0.01731	POS
Glycosphingolipid metabolism	2	3	0.01731	POS
Vitamin D3 (cholecalciferol) metabolism	1	1	0.0389	POS
C5-Branched dibasic acid metabolism	1	1	0.0389	POS
TCA cycle	1	1	0.0389	POS
Prostaglandin formation from arachidonate	1	1	0.0389	POS
Fatty acid activation	1	1	0.0389	POS
Methionine and cysteine metabolism	1	1	0.0389	POS
Carnitine shuttle	2	4	0.04201	POS

Very interestingly, these analyses also identified an estimated empirical compound putatively identified as the bioactive, polyunsaturated signaling lipid 2-arachidonoylglycerol (2-AG), which demonstrated an inverse relationship in its plasma abundance compared to estimated years until dementia diagnosis. Similar findings suggested the depletion of the

polyunsaturated fatty acid dihomo-gamma-linoleic acid (FA C20:3; **DGLA,** *KEGG:* **C03242**) with advancing years to the estimated diagnosis event. DGLA is a desaturation and chain elongation derivative of the linoleic acid (FA C18:2), which must be consumed through diet in humans. DGLA can be metabolized into several eicosanoid (thromboxane and prostanoid) mediators with inflammation resolving effects inhibiting the production of arachidonic acid (AA)-derived, pro-inflammatory metabolites [318, 447]. Network-scale *de novo* analyses also identified DGLA as a dysmetabolic node, but also implicated its association to the mitochondrial fatty-acid carnitine shuttle and guanine phosphate (GTP) metabolism through coenzyme A (CoA)-involving processes (**Figure 3.5A**).

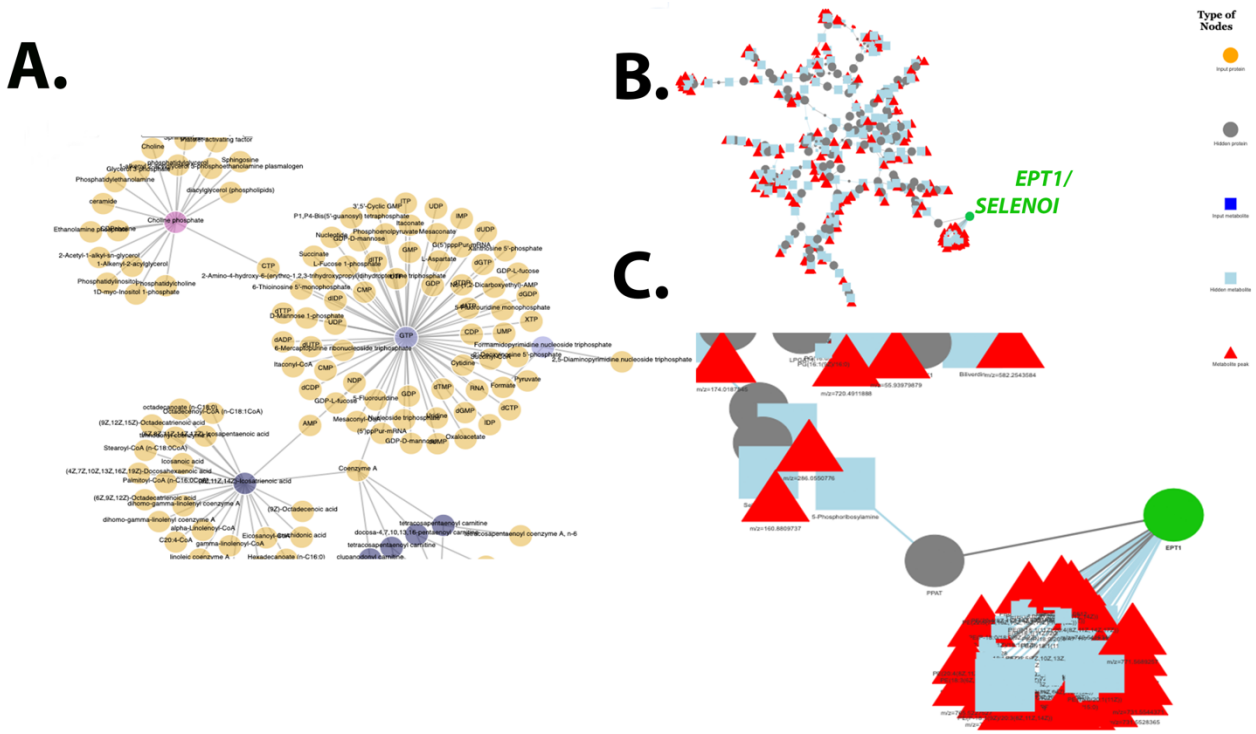

Figure 3.5 *De novo* metabolomic networks inferred from both Mummichog 2.0 and PIUmet software from the Spearman rho rank correlations of untargeted LC-MS metabolite features to participant-wise estimated years until dementia diagnosis. Correlation analyses were conducted for all ADAD mutation carriers regardless of NIA-AA cognitive staging (i.e., including participants meeting criteria for preclinical and MCI/AD.

Mummichog analyses characterizing estimated years to dementia diagnosis can suggest associated changes to metabolic pathways evident within the peripheral circulation of mutation-carrying individuals. These analyses do not, however, directly suggest associated, inferred metabolic proteins and enzymes. To address this shortcoming, PIUmet network analyses were also conducted over the set of metabolite-feature-wise Spearman correlations previously submitted to Mummichog analysis. Specifically, unadjusted *p*-values associated with respective Spearman correlations were transformed as the -$\log_{10}$(*unadjusted* ***p****-values*) to construct a set of empirical "prizes" to be modeled as prize-collecting Steiner trees (implemented in PIUmet software) [341]. This revealed a complexly distributed network of inferred protein-protein interactions (PPIs) and metabolites (**Figure 3.5B**). Like findings in CHAPTER 2 for DS-AD, the enzyme ethanolamine phosphotransferase 1 (EPT1/ SOLENOI) constituted a metabolic hub for metabolomic features correlated with estimated years to dementia onset (**Figure 3.5C**).

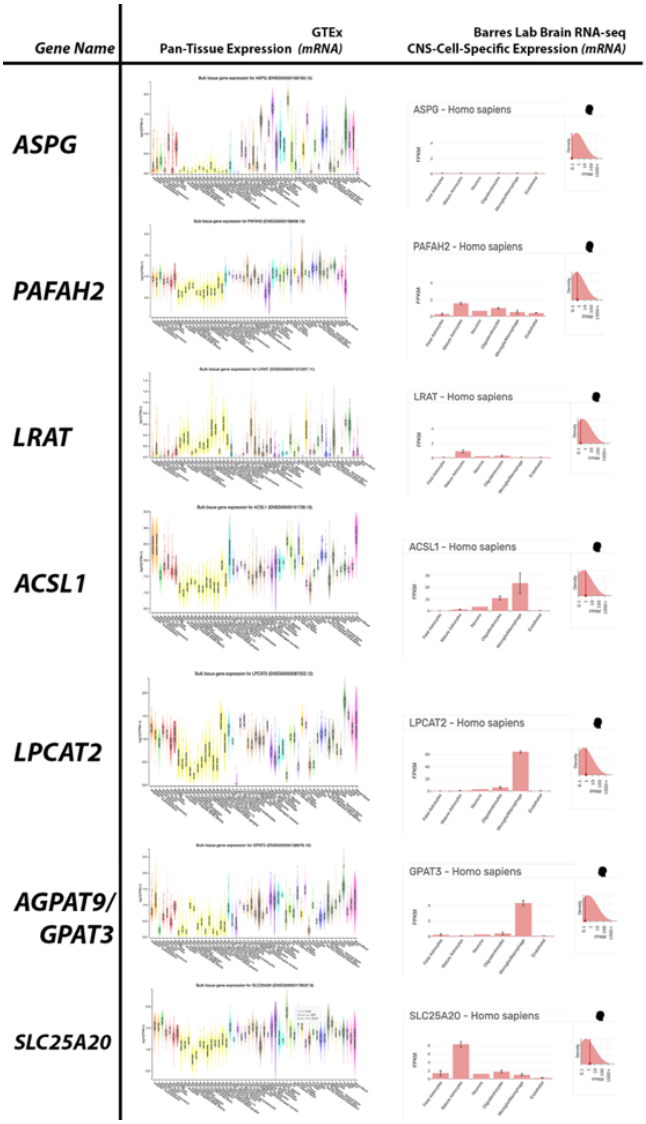

Figure 3.6 Enzyme-coding genes identified by PIUmet modeling of untargeted LC-MS metabolomics features as they correlated with estimated years until dementia diagnosis for all ADAD mutation carriers ($n_{\ total\ carriers} = 55$) independent of NIA-AA cognitive staging. Pan-tissue, bulk human gene expression and CNS-cell-specific expression were accessioned using GTEx and the Barres Lab Brain RNA-seq databases, respectively.

This also included the neurometabolic enzyme monoamine oxidase-B (*MAOB*), which is a putative marker of reactive astroglial activation associated with chronic, progressive neuroinflammation in AD [333, 335, 448-452]. Interestingly, Steiner-forest network modeling of these relationships also suggested several other, densely connected PPI hubs

related to lipid metabolism. These included the gene *ASPG* (encoding an enzyme which functions dually as a lysophospholipase and an asparaginase), platelet-activating factor acetylhydrolase 2 (*PAFAH2*), lecithin retinol acyltransferase (*LRAT*), acyl-CoA synthetase long chain family member 1 (*ACSL1*), LPC acyltransferase 2 (*LPCAT2*), glycerol-3-phosphate acyltransferase 3 (*AGPAT9/ GPAT3*), and the mitochondrial carnitine/acylcarnitine carrier protein translocase (*SLC25A20*) (**Figure 3.6**).

DISCUSSION

Previous chapters of this dissertation have provided evidence that, for both LOAD and DS-AD, emerging cognitive decline co-occurs with alterations to a diverse milieu of peripheral blood biochemicals and metabolic processes. The mechanistic details and significance of these findings in at-risk or abnormal aging remain to be further investigated. They could, however, indicate a peripherally extensive, CNS-peripheral metabolic axis subject to catastrophic, dyshomeostatic failure in AD following some duration of increasingly inadequate "compensations for failure." Beyond some critical point in systems biological organization, configuration, and functioning, these failures of compensation could be incompatible with adaptation and resilience required for successful processes of healthy cognitive aging.

Because metabolomic investigations of ADAD have been limited, it remained unclear if or how diverse any changes evident within the peripheral plasma metabolome would be as a function of AD staging. Understanding these relationships in terms of dissociable, predisposing AD etiologies, this question was particularly interesting as an extension of findings reported in CHAPTER 1 for LOAD and CHAPTER 2 for DS-AD. More specifically and

quantitatively, metabolomic "fingerprints" of the *preclinical-control* and *preclinical-objectively impaired* (MCI/AD) statistical contrasts were compared across etiologies (LOAD and ADAD) using Tanimoto-Jaccard significance testing. If metabolic change and dyshomeostases characterizing ADAD across its progression are different from those of LOAD as a function of non-shared, autosomal dominant mutation burden in ADAD, then LOAD and ADAD peripheral plasma metabolomes might similarly differ.

Very interestingly, both the *preclinical-control* and *preclinical-MCI/AD* contrasts across LOAD and ADAD cohorts demonstrated canonical metabolic fingerprints which significantly overlapped beyond chance alone. The degree of overlap was consistent with (if not exceeding) coefficients obtained in CHAPTER 1 for preclinical LOAD. Acknowledging this, the estimated degree of overlap in no analysis exceeded 20%. This suggests that, while some AD-associated dysmetabolic processes are shared between ADAD and LOAD, much peripheral metabolic variability significantly associated with clinical trajectories of decline in AD is not. In the very narrow sense of comparing ADAD and LOAD, these core peripheral fingerprints could themselves suggest elements of a "final common pathway" jointly impacted across these etiologically distinct populations in the development of cognitive decline. More broadly, metabolism also encapsulates many vital homeostatic and functional processes supporting healthy aging and cognitive resilience. In this sense, the significant (but modest) overlap between canonical biochemical pathways for equivalent clinical staging comparisons in ADAD and LOAD underscores metabolic processes in AD as conceptually integral and possibly final from an etiopathogenic perspective. Nonetheless, this also suggests a high degree of changing biochemical and dysmetabolic complexity emerging in AD overall. Distributed across differing mechanistic mediators, biomarkers, therapeutic

targets, and systems biological constraints, this heterogeneity of risk characterizing emerging dementia pathobiology agrees with the diversity of resulting pathologies and deficits observed in in abnormal aging.

Computational analyses employing Mummichog and the protein-interaction-centric PIUmet suggested systems biological change in ADAD highly consistent with this possibility for both the *preclinical-control* and *preclinical- MCI/AD* plasma comparisons. Specifically, these analyses implicated the hepatic metabolism of compounds such as heme and steroid derivatives of cholesterol metabolism including primary bile acids. Liver dysmetabolism in aging has been both A) previously associated with both elevated risk for AD and B) proposed as an active participant in the evolving disease process, particularly in relation to lipid metabolism [16, 19-23, 58, 274, 453, 454]. Particularly through the secondary metabolism of hepatic bile acids within the brain-gut axis [192], lipo-homeostasis and/or disordered biological signaling may systemically exacerbate biochemical constraints and insults ultimately driving cortical amyloid and tau pathologies [320, 455].

Independent of lipid metabolism itself, substantial literature now also suggests that metabolically intensive peripheral organs (substantially involving the liver) contribute to whole-body beta amyloid metabolism and clearance [23]. Because early-onset, substantial cortical amyloidosis is etiopathologically central to ADAD, it is not surprising that metabolic dyshomeostases worsened by the concomitant demands of amyloid clearance could themselves exacerbate and further ongoing cognitive decline [23, 354]. Substantial and early depositing cortical amyloid, however, also describes DS-AD, which is itself genetically predisposed albeit instead due to trisomy 21 [352]. Future research involving metabolically diverse readouts will be essential to better understand the comparative biological

circumstances and dynamics surrounding amyloid deposition in ADAD relative to DS-AD, but also LOAD.

Although highly similar in the end stages of neuropathological decline, very recent research by Ances and colleagues have indeed suggested that CNS amyloid burden differentially accumulates across ADAD and DS-AD populations over time. Only in DS-AD was this trajectory dynamic in time, proceeding sigmoidally from an initial sublinear rate of accumulation to a decompensatory overshoot. These findings are thus consistent with a pathophysiological, critical phase transition from conditions of tenuous metabolic compensation to those of rapidly emerging failure in evolving DS-AD. In contrast, the linearly increasing amyloid burden characterizing ADAD with age did not show evidence of acceleration or deceleration consistent with complex programs of systems biological compensation prior to frank clinical decline (Dr. Beau Ances lab 09/21, *unpublished talk*).

Both DS-AD and ADAD may be linked through the shared, early deposition of cortical amyloid because of large-effect genetic drivers. These apparent similarities could, however, belie important etiological distinctions important for understanding their respective pathogenic origins and dyshomeostatic development across affected biological systems. Whereas DS-AD may involve protective factors conferred through an elevated genetic dosage of chromosome 21 [456, 457], ADAD may instead represent a "purer" genetic lesion exerting unchangingly and uniformly deleterious effects on the aging brain which proceed unabated in these latter individuals. As discussed in CHAPTER 2, psychometric considerations of assessing cognitive change in adults with DS complicates straightforward comparisons with ADAD participants, particularly at early prodromal stages of disease in DS-AD possibly also subject to generalized accelerated aging processes [216, 419]. Better

clarifying these questions will assist in addressing how the pathobiological roles of dysmetabolism in AD correspond to potentially varied dynamic processes of cognitive and biological change which may differ across etiologies including DS-AD and ADAD. These could themselves proceed as a function of diverging, specific dysmetabolic limitations nonetheless driving functionally convergent, vicious cycles of increasingly untenable compensations for emerging failure. At some critical point in the development of clinical illness, this accrued vulnerability within biological systems could instead prohibit healthy trajectories of cognitive aging and resilience.

Because ADAD is highly genetically penetrant and demonstrates family-wise median ages of onset, mutation carriers can be longitudinally monitored as adults from preclinical status and objective cognitive impairment to the estimated time of clinical decline resulting in diagnosis. Independent of cognitive-staging-based comparisons of the peripheral plasma metabolome, it remained unclear how or if these estimated years until manifest clinical impairment related to biochemical abundances in blood. Network-scale modeling studies investigating these questions again identified *de novo* associations highly suggestive of hepatic metabolism. This specifically included PUFA (dihomo-gamma-linolenic acid, **DGLA**) dysmetabolism, where similarly impaired hepatic PUFA biosynthesis involving peroxisomes has been reported in aging adults with AD [19-21]. Further providing evidence of liver metabolic dyshomeostasis associated with years to estimated diagnosis in ADAD, several inferred enzymes nominated by PIUmet modeling (which were not generally expressed as mRNA in CNS cells) were highly expressed in the liver relative to all human tissues (e.g., *ASPG*).

Interestingly, these PIUmet analyses also identified several lipid metabolic enzymes (*ACSL1, LPCAT2, AGPAT9*) which are disproportionately expressed by microglia within the human CNS. This corresponds with proton MRS findings recently reported by Joe, Ringman and colleagues in which increasing levels of myo-inositol were observed in neocortical regions with increased proximity to estimated age of dementia onset [442]. Myo-inositol within the CNS has been advanced as a marker of reactive and activated glial activity characteristic with chronic neuroinflammation in AD [331]. Future investigations should integrate analyses of the blood metabolome and MRS neurochemistry to better understand these immunometabolic processes in aging human participants themselves through minimally invasive approaches.

Particularly as PIUmet network analyses related to estimated age of diagnosis implicated lipid and microglial metabolism, emerging literature has reported altered lipid metabolism and dyshomeostasis within these cells with their pathological activation in AD [452]. Consistent with reports that this proinflammatory state in AD also involves astrocyte reactivity [335], further PIUmet hubs (e.g., SLC25A20) also demonstrated disproportionate expression in these other glia at a mature stage of cellular development. Much as lipometabolic axes span tissues and organ systems in AD pathobiology at one scale, these likely also complexly span cell types and perhaps even affected, abnormally aging brain regions. Future research might productively explore these fundamentally metabolic relationships to pursue more precise biomarker and therapeutic targeting in AD consistent with personalized care and precision medicine objectives [24]. Incidentally, many of these efforts will ideally inform upon and specifically explore the diverse, etio-pathobiological bases of AD in terms of organizing, biochemical processes.

Semantically driven, inference generating pipelines such as those employed for this and previous chapters can suggest systems scale insights through the genome-scale metabolic modeling of network-scale, empirical metabolomics measurements from plasma specimens. These methods (including Mummichog and PIUmet) also demonstrated limitations in the integration and comparison of LOAD and ADAD metabolomes. Specifically, peripheral, metabolomic signatures of AD in these cohorts across cognitive staging comparisons often revealed only a modest core of shared dysmetabolic processes corresponding to canonical pathways. In contrast, many more estimated metabolic activity network relationships were estimated by Mummichog. Importantly, this scale of putative biochemical relationships indexed by cognitive-phenotypic contrasts explored in this dissertation greatly exceeded manual, segment-wise consideration.

It therefore remains unclear if the analysis pipelines employed thus far are sufficiently expressive to model and represent highly heterogeneous, metabolic change complexly distributed across LOAD, DS-AD, and ADAD at varying stages of developing cognitive decline. In this sense, the semantic consideration of changes to the peripheral plasma metabolome which covary with translationally important, clinical phenotypic dissociations could also describe the statistical investigation of AD metabolism (and dysmetabolism) as a natural language. More specifically, this could productively employ contemporary methods in unsupervised semantic-level topic mining applied to empirical metabolomics modeling results (e.g., from Mummichog) [340]. Contemporary topic and text mining methods could project contrast-level, phenotypic comparisons into a substantially lower-dimensional, conceptually abstracted peripheral metabolic basis-space summarizing interrelated perturbations to underlying biochemical classes and networks. In doing so, this

simultaneously and integratively compares many phenotypic contrasts across diverse degrees of cognitive staging and specific etiologies.

As a further substantial advantage, generative representational paradigms such as structural topic models (STMs) can estimate the optimal integer number of such "AD metabolic topics" (absent *a priori* expectations) empirically in the process of mapping these to Mummichog-level biomolecules and enzymes. Systematically exploring these relationships as elements of a semantically rich, natural metabolic language using explicitly synthetic statistical methods could better account for the distribution of non-shared peripheral metabolic change across AD etiologies. These efforts, in turn, could inform and advance more precise biomarker development and therapeutic targeting across these clinically diverse populations affected by abnormal aging. It could also more specifically address the distribution and variability amongst distributed, CNS-peripheral metabolic axes differentially impacting these individuals developing AD as a function of cognitive status and specific etiologies (LOAD, DS-AD, ADAD).

CHAPTER 4: Peripheral Systems Biochemistry in AD as Natural Language Processing

One of the central inferences from the preceding chapters has been that complexly distributed, systems metabolic and biochemical change accompanies the evolution of AD across differing risk etiologies. Furthermore, these distributed dyshomeostases might themselves complexly map to the compromise of various CNS-peripheral metabolic axes resulting from and furthering trajectories of abnormal AD cognitive decline. Although a modest core of metabolic deficits is statistically shared between ADAD and LOAD, a larger proportion of significantly enriched, metabolic pathways altered in disease remain unique. Ongoing metabolic investigations of DS-AD might suggest similar findings, although the relationship of A) DS-AD to B) ADAD and LOAD trajectories of cognitive decline remains to be further established for comparative purposes. Although necessarily pursued at a high level of conceptual abstraction, these high-level peripheral metabolic inferences could suggest translationally actionable biomarker and therapeutic target candidates of great practical importance.

Challengingly, the extent of potentially implicated metabolic processes indexed in peripheral plasma (as biological pathways and networks) is intractably large for manual inspection across such a substantial cross-section of systems biology. This substantial biochemical scope should itself be carefully considered as a non-trivial finding informing future translational efforts in AD metabolism. If AD pathobiology exists enriched within diverse metabolic network hubs made vulnerable by abnormal aging, the size and complexity of these extended biological networks alone could jeopardize the successful homeostatic functioning of extended CNS-peripheral physiological axes necessary for healthy cognitive resilience and stability. Across substantial diversity in AD etiopathogenesis

and development, specialized statistical methods may prove well-suited to organize this complexity in human-comprehensible terms for the targeted specification of testable/ mechanistic hypotheses involving metabolism in abnormal cognitive aging. More specifically, this diversity of observed peripheral metabolomic changes accompanying dementia could form the basis of an empirically derived, highly expressive, biochemical natural language describing the A) mapping of AD peripheral metabolic signatures to their B) respectively associated clinical phenotypic attributes. Although in its infancy, natural language processing (NLP) research specializing in scientific inputs (e.g., chemical structures, biological networks, compound/ gene IDs) has increasingly used large-corpus text mining methods to pursue translational biomedical objectives [458-462].

While text mining can conventionally summarize input terms extracted from records as individual tokens (e.g., by mean or mode values), its most powerful features include the abstractive mapping of tokens (e.g., chemical names, enzyme IDs) to a summarized, much-lower-dimensional concept space through the technique of "topic mining" [463]. Through this method, biochemical topics across AD could be described etiopathologically as a function of metabolite and enzyme term frequencies (i.e., estimated from Mummichog activity network modeling as carried out in prior chapters). Topic mining could thereby ingest and identify latent metabolic patterns in these Mummichog outputs by employing parsimonious, semantic-level generative NLP models to map these AD biochemical tokens to specific topics [258]. Similarly, various pairwise AD phenotypic contrasts across LOAD, DS-AD, and ADAD could further be described as a function of these discrete, fitted biochemical topics. To this end, topic modeling in peripheral AD biochemistry could serve to map the relationship of clinical phenotypic variance to semantic-level pathways and processes

through inferred topics in metabolism at *de novo*, network scale. Very importantly, these models would also be highly semantically compact and interpretable by human experts.

Much of this dissertation has employed supervised statistical methods (e.g., linear models) to evaluate relationships between the peripheral metabolome and multiple clinical comparisons across AD stages and etiologies. Unsupervised methods such as topic mining, however, may also be productive in suggesting the architecture of physiologically extended, peripheral-CNS metabolic axes in AD pathogenesis and progression with respect to clinical phenotypic diversity. These NLP-derived approaches could suggest many nodes of emerging dysfunction indexed within peripheral plasma which might be prioritized as precision AD biomarkers, therapeutic targets, and prior inferences informing targeted, mechanistic investigations. Unlike coarse-grained and minimally structured unsupervised learning methods (e.g., principal components analysis (PCA) [464], latent semantic analysis (LSA) [465], *k*-nearest neighbors clustering [466]), this very semantically dense mapping of biochemical to phenotypic information in AD plasma perhaps better resembles NLP and concept/topic mining in form, function, and intent. Semantic-level language models of AD peripheral dysmetabolism, however, have not been empirically explored in previous translational research.

In 2003, Blei, Ng and colleagues contributed a powerful, adaptive algorithm for the semantic topic modeling of text through unsupervised, hierarchical Bayesian generative models (i.e., latent Dirichlet allocation, **LDA**) [467]. Since then, these methods have iterated to support the modeling of correlated topics (e.g., overlapping or highly covarying biochemical pathways and processes) [468]. Recently described variants of these algorithms (structural topic modeling, **STM**) permit the modeling of structural covariates (i.e., clinical

phenotypic and metabolomic metadata) across individual, pairwise AD Mummichog contrasts [340]. This allows for the fitting of inference generating, well-indexed conceptual metabolic "libraries" which explicitly account for nuisance variance in the peripheral plasma metabolome (e.g., related to nothing more than SVA methods, ESI-MS modes). Finally, as a benefit of topic models descending from LDA, hard prior knowledge regarding the number of discrete topics to be modeled is not necessary. Instead, the content and number of topics can be empirically estimated through the mapping of structural covariates to some biochemical, *k*-topics through unsupervised variational inference. To maximize interpretability, a small range of peripheral metabolic and biochemical topics ($k < 10$) could be considered to represent Mummichog activity modeling of the plasma metabolome as described in previous chapters.

These analyses (with respect to both nuisance and clinical phenotypic contrast covariates) could estimate a small number of biochemical topics (i.e., peripherally indexed metabolic axes) as they jointly map distributions of A) molecules and enzymes estimated as textually defined Mummichog activity networks to B) specific, pairwise clinical-phenotypic AD contrasts. Doing so could better resolve and contextualize the highly diverse metabolic processes indexed in AD peripheral circulation and implicated by prior chapters' metabolomic differential abundance (DA) analyses conditional on clinical phenotype. This could map otherwise very diverse, networked biological processes in very translationally actionable and ideally concise terms. It will also almost certainly incorporate processes and molecules which have been limitedly studied (in addition to those only very recently studied) in AD [88]. In fact, this process of "semantic alignment" to potentially sparse, prior

literature is vital to heuristically validating the final conceptual fit across some modest number of biochemical topics (but also for optimal hypothesis generation).

More specifically, STM models could ingest and variationally model A) matrices of clinical phenotypic contrasts by B) tokenized Mummichog activity network terms where C) metabolic terms in these specific pairwise contrasts are indexed as raw counts of each term across phenotypic comparisons. This is not unlike contemporary transcriptomic methods for the statistical modeling of RNA-seq normalized read counts [469]. Importantly, structural covariates could further be included to deconvolve the statistical effects of nuisance factors (MS, SVA modes), but also to represent the linear interactional effects of how individual, fitted topics are variously "discussed" as biochemical processes in specific, pairwise phenotypic comparisons (**Figure 4.1**). Based on prior chapters' findings in LOAD, DS-AD, and ADAD, one can hypothesize that a core of shared dysmetabolism may span multiple or all etiologies (i.e., a strict, "final common pathway" through metabolism in evolving AD). A much broader number likely remain complexly distributed as a function of diverse clinical phenotypes, with these phenotypes only sparsely mapping across multiple biochemical topics as supported by findings of modest (but significant) canonical pathway overlap in prior chapters (e.g., of LOAD compared to ADAD). Dysmetabolism broadly could thus instead encapsulate a heterogeneous final common ***process*** in AD which complexly impacts many biological pathways where many of these dynamics remain to be empirically mapped to hopefully great translational success.

A. ***Tokenized Biochemical Term Counts***

	Clinical Comparison 1	Clinical Comparison 2	Clinical Comparison 3	Clinical Comparison 4	Clinical Comparison 5
AN.Molecule 1	5	10	0	4	9
AN.Enzyme 1	0	4	2	0	2
AN.Enzyme 2	7	4	0	6	7
AN.Molecule 2	1	1	4	1	0

B. ***Clinical Metadata Structural Covariates***

	SVA Algorithm	ESI-MS Mode
Clinical Comparison 1	BE	POS
Clinical Comparison 2	BE	NEG
Clinical Comparison 3	Leek	NEG
Clinical Comparison 4	Leek	POS
Clinical Comparison 5	BE	POS

Figure 4.1 Example toy matrices demonstrating the data structure of A) tokenized term counts modeled by STM in addition to B) corresponding comparison-wise structural covariate metadata. Matrices of tokenized counts are not statistically unlike tables of raw read counts obtained from RNA-seq experiments. Unlike RNA-seq read counts, however, token counts (i.e., the values of cells in **Panel A**) are submitted un-normalized to STM, as anticipated by this software.

Modern generative topic models such as STM aim to succinctly represent exactly these complex mappings inherent in semantically rich, metabolic/ biochemical relationships (as otherwise discussed at length in broader abstractive text mining and NLP). It remains to be empirically modeled based on Mummichog pairwise-comparison activity networks, however, A) how few topics can be coherently considered (for parsimony's sake), B) what individually dissociable biochemical enrichments occur across topics, and C) what distribution describes the mixture of phenotypic contrasts loading onto specific, identified biochemical topics. Doing so will ideally produce (from the peripheral plasma metabolome) a generative metabolic "library" highly enriched for and stratified by clinical diversity in AD cognitive staging and etiologies. In translational terms, these efforts might contribute

valuable maps charting the biological interrelationships of prioritized biomarker, therapeutic, and mechanistic follow-up targets disproportionately at risk of recurrent "failure of compensation" following from "compensations for failure" in evolving AD.

METHODS

Ingested Phenotypic Comparisons. Pairwise phenotypic comparisons were extensively evaluated (for LOAD, DS-AD, and ADAD participant specimens, respectively) using differential abundance (DA) analysis and downstream *de novo* Mummichog activity network modeling as described extensively in previous chapters (**Table 4.1**). To minimize artefact unrelated to the mapping of the peripheral metabolome to clinical phenotypic differences, all pairwise contrasts were considered across both the ESI+ and ESI- mass spectrometry analysis modes. Similarly, surrogate variable estimation was pursued across the Buja-Eyuboglu (BE) and Leek methods wherever either or both demonstrated non-zero SV count estimates. Study design and individual cohorts permitting, all pairwise phenotypic contrasts submitted to Mummichog activity modeling attempted to compare cognitively and clinically discrete groups (e.g., *preclinical-MCI* as opposed to *preclinical-MCI/AD*) wherever possible to minimally bias STM estimation by providing the algorithm the greatest feasible variance of clinically interpretable phenotypic comparisons to consider.

Table 4.1 Pairwise Phenotypic Contrasts Evaluated through Mummichog *De Novo* Modeling and Submitted to STM for Integrative Consideration

	CLINICAL POPULATION	COHORT	CONSOLIDATED AD CONTRAST	ESI-MS MODE	SVA ALGORITHM
1	ADAD	UCLA ADAD	*AD versus MCI*	NEG	BE
2	ADAD	UCLA ADAD	*AD versus MCI*	POS	BE
3	ADAD	UCLA ADAD	*preclinical mutation carrier versus family control*	NEG	BE

4	ADAD	UCLA ADAD	*preclinical mutation carrier versus family control*	POS	BE
5	ADAD	UCLA ADAD	*preclinical mutation carrier versus MCI*	NEG	BE
6	ADAD	UCLA ADAD	*preclinical mutation carrier versus MCI*	POS	BE
7	LOAD	UCI ADRC	*preclinical versus cognitively stable control*	NEG	BE
8	LOAD	UCI ADRC	*preclinical versus cognitively stable control*	POS	BE
9	DS-AD	PCDA-DS	*DS-AD versus DS-NAD*	NEG	BE
10	DS-AD	PCDA-DS	*DS-AD versus DS-NAD*	POS	BE
11	DS-AD	PCDA-DS	*DS-AD versus DS-NAD*	NEG	Leek
12	DS-AD	PCDA-DS	*DS-AD versus DS-NAD*	POS	Leek
13	LOAD	R/OCAS	*preclinical versus cognitively stable control*	NEG	BE
14	LOAD	R/OCAS	*preclincal versus cognitively stable control*	POS	BE
15	LOAD	R/OCAS	*preclinical versus cognitively stable control*	NEG	Leek
16	LOAD	R/OCAS	*preclincal versus cognitively stable control*	POS	Leek
17	LOAD	R/OCAS	*MCI/AD versus cognitively stable control*	NEG	BE
18	LOAD	R/OCAS	*MCI/AD versus cognitively stable control*	POS	BE
19	LOAD	R/OCAS	*MCI/AD versus cognitively stable control*	NEG	Leek
20	LOAD	R/OCAS	*MCI/AD versus cognitively stable control*	POS	Leek
21	LOAD	R/OCAS	*MCI/AD versus preclinical*	NEG	BE
22	LOAD	R/OCAS	*MCI/AD versus preclinical*	POS	BE
23	LOAD	R/OCAS	*MCI/AD versus preclinical*	NEG	Leek
24	LOAD	R/OCAS	*MCI/AD versus preclinical*	POS	Leek
25	LOAD	R/OCAS	*supernormal versus cognitively stable control*	NEG	BE
26	LOAD	R/OCAS	*supernormal versus cognitively stable control*	POS	BE
27	LOAD	R/OCAS	*supernormal versus cognitively stable control*	NEG	Leek
28	LOAD	R/OCAS	*supernormal versus cognitively stable control*	POS	Leek
29	LOAD	R/OCAS	*supernormal versus preclinical*	NEG	BE
30	LOAD	R/OCAS	*supernormal versus preclinical*	POS	BE
31	LOAD	R/OCAS	*supernormal versus preclinical*	NEG	Leek
32	LOAD	R/OCAS	*supernormal versus preclinical*	POS	Leek
33	LOAD	R/OCAS	*supernormal versus MCI/AD*	NEG	BE
34	LOAD	R/OCAS	*supernormal versus MCI/AD*	POS	BE

35	LOAD	R/OCAS	*supernormal versus MCI/AD*	NEG	Leek
36	LOAD	R/OCAS	*supernormal versus MCI/AD*	POS	Leek

Importantly for LOAD, the extensible and synthetic STM framework allowed for the consideration (through Mummichog) of a further R/OCAS sub cohort. These "supernormal" participants demonstrated exceptional cognitive resiliency inversely analogous to the deficits (< 1.35 standard deviations below the median) defining domain-specific, objective cognitive impairment (i.e., MCI/AD). Including those instead in the highest percentiles of aging outcomes (i.e., > 1.35 standard deviations above the median), supernormal participants alternatively contributed an interesting negative control for cognitive-decline-associated peripheral dysmetabolic processes. Instead, specimens contributed by these participants might instead suggest biochemical signatures of complexly distributed processes conferring or related to cognitive resilience.

Integrative Biochemical Text Mining and STM Concept Extraction Pipeline. Estimated, *de novo* activity networks included A) biochemicals coded as Kyoto Encyclopedia of Genes and Genomes (KEGG) terms and B) enzymes coded as BRENDA identifiers as defined in the MFN human, genome-scale metabolic model queried by Mummichog [258]. Implicated metabolic reactions per phenotypic comparison per cohort were parsed into countable tokens quantifying extracted metabolic term frequency counts (**Figure 4.2**). This resulted in a matrix of 2741 biochemical tokens by 36 individual phenotypic contrasts populated by term frequency counts. To ensure semantically adequate tokenization of the biochemical space spanned by the ingested terms, singleton metabolic terms in addition to all unnested biochemical bigrams, trigrams, and complete reactions were extracted to maximize relevant biochemical coverage across textual representations of inferred Mummichog activity

networks. Corresponding metabolomic metadata was paired to each clinical phenotypic contrast associated with a discrete activity network. Specifically, individual metabolic *network-phenotypic comparison* pair was coded according to its LC-MS detection mode (ESI+ or ESI-) and associated method of surrogate variable (SV) estimation.

In cases of multiple detection and SV estimation modes, all individual pairwise contrasts for a given clinical comparison were submitted to STM analysis for joint consideration as unique records. Doing so leveraged the ability of STM to fit these known, structural covariates to identified, estimated metabolic topics within the plasma metabolome. Individual tokens were filtered prior to modeling to exclude terms with likely minimal semantic significance and to speed the convergence of variational models. Specifically, tokens were filtered out if they proportionally occurred in fewer than 7.5% of all submitted pairwise contrasts (including all combinations of MS and SV modes). Tokens were also removed if they occurred in greater than 90% of these same pairwise contrasts by proportion. These latter biochemical terms were ubiquitous across most clinical phenotypic comparisons and, thus, would likely contribute minimal information relating to dissociable AD clinical phenotypes and pathobiological processes as reflected in the peripheral plasma metabolome.

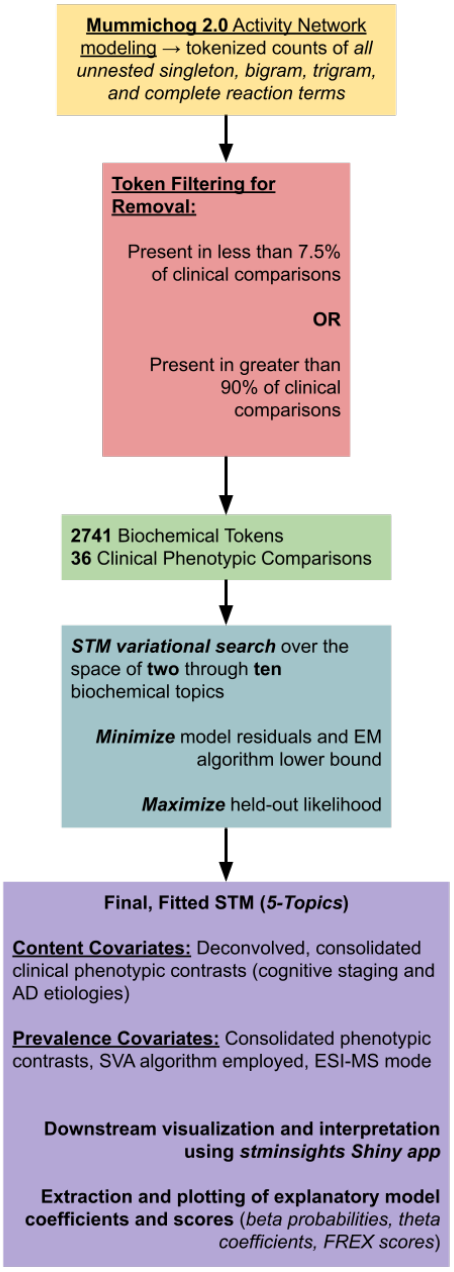

Figure 4.2 A schematic diagram details the computational modeling pipeline for structural topic modeling (STM) analyses of estimated AD peripheral plasma metabolic activity networks downstream of differential abundance (DA) analysis and Mummichog 2.0 *de novo* estimation. These latter statistical procedures are described at length in prior chapters.

A final 2741 metabolic token by 36 contrast matrix of term counts was then submitted to STM modeling. By resampling over a range of two through ten STM topics estimated using ingested metabolic activity network data, an empirical best fit was determined though A)

identifying those numbers of topics which maximized the held-out likelihood conditional on number of selected topics while also B) minimizing the expectation-maximization algorithm variational lower bound and associated, obtained model residuals. Final STM models were fit using both prevalence and content covariates. Prevalence covariates used here sought to explain overall base-rate topic frequencies as explained by a "null" model of nuisance explanators (e.g., ESI mode, SVA algorithm). Content covariates instead aimed to explain how specific phenotypic comparisons themselves (e.g., UCI ADRC preclinical LOAD participants versus controls) affected biochemical term usage describing certain metabolic topics. In this way, "consolidated" clinical and cognitive relationships to biochemical terms can be directly evaluated using STM independent of and accounting for possibly confounding ESI-MS and SVA covariates.

The final, fitted STM model considered topic prevalence as the fully crossed linear three-way interaction of these direct clinical and cognitive comparisons, ESI-MS analysis mode, and SVA algorithm. Consolidated, direct clinical-cognitive comparisons constituted the main effect submitted as a content covariate to STM. All resulting topics were then linearly modeled as the main effect of all pairwise phenotypic comparisons plus the fully crossed interaction across differing SVA algorithms and MS analysis modes. The significance threshold for inferential testing of these specified *clinical metadata-STM topic* relationships using linear models was set at the nominal $p < .05$ level. Generative model outputs were exported to the *stminsights* Shiny app for further inspection [470]. Specifically, loadings of biochemical terms on specific topics were quantified and ranked as the estimated probabilities *beta* of each biochemical term being drawn from the distribution of the k^{th} fitted metabolic topic. These were visualized as word clouds proportionately scaled by estimated

beta probabilities. Similarly, the proportional mappings of individual pairwise clinical comparisons across all biochemical topics were represented as arrays of *theta* coefficients. In characterizing biochemical topics through identified terms, *beta* probability coefficients from fitted STM models were supplemented with similar (and often overlapping) terms indexed by FREX scores [471]. Using these multiple, functionally comparable methods decreased the likelihood of methodological bias associated with pursuing further literature searches based on one or the other alone. In addition, FREX scoring advantageously nominates terms which are **FR**equent and **EX**clusive to a specific topic of interest. This heuristically represents many successful applications of topic identification and segmentation, which often guard against considering terms which are A) frequent, but ubiquitous and uninformative, but also those which are B) exclusive, but sometimes infrequent enough to occur through chance alone [471].

Software. Analyses employed R version 4.0.5. Differential expression analyses and Mummichog 2.0 modeling were implemented in software as described extensively in previous chapters to generate pairwise clinical comparisons: mummichog.org. Structural topic models (STMs) were fit and evaluated using the *stm* package [340]. The *stminsights* Shiny app permitted further, detailed inspection of the fitted model [470].

RESULTS

Tokenization of biochemical terms from upstream Mummichog activity network modeling resulted in a matrix of 2741 DA-analysis-implicated metabolic tokens across 36 pairwise phenotypic AD comparisons. These included combinations of clinical contrasts as determined from specific upstream SVA algorithms and ESI-MS analysis modes. To

specifically model the main effects of clinical status independent of any effects due to statistical preprocessing and chemistry alone, a structural topic model (STM) was implemented. This structural algorithm explicitly accounted for any potentially problematic, semantic noise variability associated with these covariates [340].

The 36 pairwise contrasts detailed in **Table 4.1** (and associated metabolomics metadata) were taken as input to metabolic topic modeling. Using STM, an initial variational search was conducted over the space of two through ten topics to estimate a low-dimensional basis of semantically dissociable, peripherally indexed dysmetabolic axes in AD. These search methods converged on an estimated optimal number of $k = 5$ topics. More specifically, the five-axis metabolic topic architecture was the one which most optimally A) minimized model residuals and Expectation-Maximization (EM) algorithm variationally estimated lower bounds while B) maximizing the likelihood of the fitted model conditional on biochemical terms held-out from individual clinical contrasts (**Figure 1.3**).

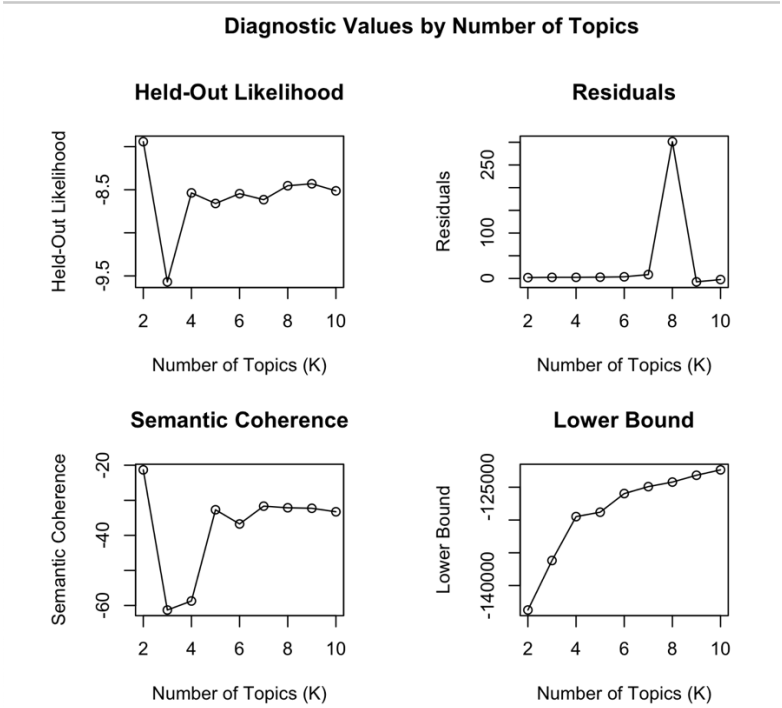

Figure 4.3 Model calibration curves resulting from STM variational searches plot several performance metrics conditional on the selection of between two and ten possible metabolic topics. The selection of $k = 5$ topics appears to balance the A) minimization of residuals and the lower bound with B) maximization of the held-out likelihood and parsimonious, stable semantic coherence. In practice, estimating *k*-topics is approximative and without a knowable, global optimum.

To better understand how upstream SVA algorithm and chemistry metadata corresponded to the five fitted metabolic topics, linear models were fit to each. To evaluate consolidated AD clinical comparisons, main effects due to these factors were evaluated plus the fully crossed interaction of SVA algorithms and ESI-MS instrument modes. Consistent with the hypothesis that peripherally indexed metabolic processes are complexly distributed in AD, no specific clinical contrasts nor metabolomic metadata covariates were significantly associated with any of the five fitted biochemical topics, all linear model coefficient nominal *p*'s > .05. Using the Shiny app *stminsights* [470] (**Figure 4.4A**), the five-topic fitted STM was further visualized and inspected. Because it remained unclear what specific biochemical terms mapped to specific topics (quantified through term-wise probabilities *beta*), initial

characterization visualized these proportional probabilities as word clouds for each. Cursory inspection of Topic 4 through probability word clouds (supplemented by FREX scoring of the same terms) suggested the disproportionate importance of N-acetyl-D-glucosamine (GlcNAc, *Kegg:* **C00140**) to this specific concept (**Figure 4.4B**). GlcNAc dysmetabolism has previously been associated with AD pathobiology, most interestingly through intracellular *O*-GlcNAcylation of target proteins as a regulatory post-translational modification, not unlike the hallmark pathological hyperphosphorylation of tau protein in the AD brain [472-475].

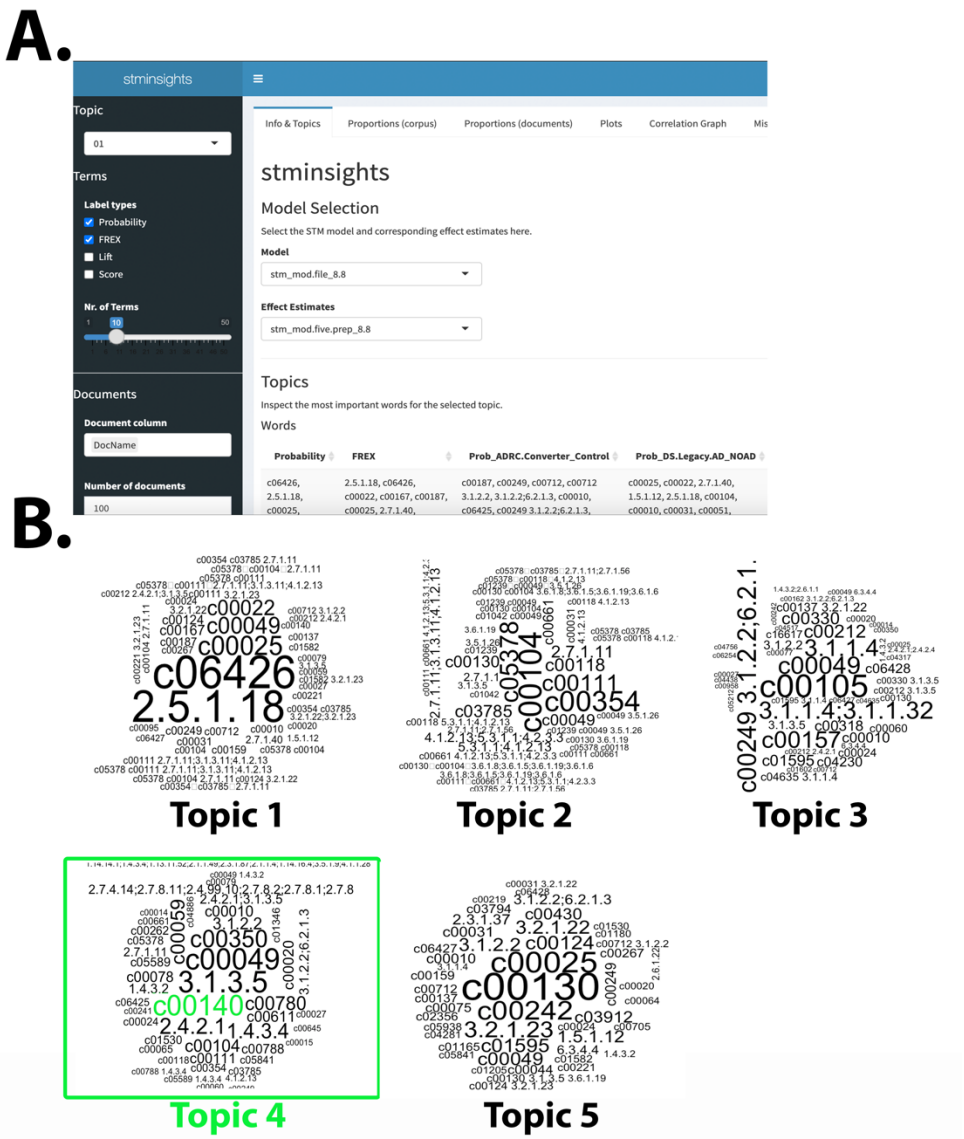

Figure 4.4 Panel A depicts the *stminsights* Shiny dashboard with the final, fitted AD topic model loaded for inspection. Biochemical terms (scaled proportional to *beta* probability loadings) suggest five putative, latent topics on metabolic topics in Panel B. Highlighted in green, preliminary analyses identified the highly-ranked metabolite N-Acetyl-D-glucosamine (GlcNAc, *Kegg:* **C00140**) within the discovered metabolic Topic 4.

To pursue this observation specifically and retain focused analytical scope in the discovered biochemical topic space, the inferred metabolic Topic 4 was exclusively considered in further comparisons. Due to its semantically rich, generative use factor, however, this STM model (or future extensions of it) afford substantially more opportunities

for additional exploration and characterization beyond this dissertation. Beyond GLcNAc itself, Topic 4 also demonstrated varied composition of biochemicals and enzymes as quantified through the top ten ordinally ranked terms by FREX score and term-wise *beta* probabilities (**Table 4.2**). These interestingly included a diverse set of molecules implicating the metabolic pathways of biogenic amine/catecholamine neurotransmitters, lipid metabolism, nucleic acid phosphates, and other complex aminosugars (e.g., *N*-acetyl-lactosamine). Terms relating to long-chain fatty acid activation by coenzyme A (necessary for mitochondrial oxidation as fuel) and saturated, long-chain fatty acids themselves clearly defined Topic 4 relative to all others identified (**Figure 4.5**). Because the fitted STM also mapped individual phenotypic comparisons to the semantic biochemical space, inspection of this matrix of *theta* coefficients can clarify which phenotypic comparisons were disproportionately described by Topic 4.

Table 4.2 Ordinally Ranked Biochemical, Enzyme IDs and Human Genes loading disproportionately on Metabolic Topic 4 as Quantified by *Beta* Probabilities and FREX Scores

RANK	*BETA*-PROBABILITY RANKING *(LARGEST TO SMALLEST)*	*FREX* RANKING *(LARGEST TO SMALLEST)*
1	3.1.3.5; **5'-nucleotidease**	1.4.3.4; **monoamine oxidase (*MAOA, MAOB)***
2	c00049; **Aspartic acid**	c00780; **serotonin**
3	c00140; **N-acetyl-D-glucosamine**	c00140; **N-acetyl-D-glucosamine**
4	c00350; ***Phosphatidylethanolamine***	2.4.2.1; **purine nucleoside phosphorylase *(PNP, LACC1)***
5	2.4.2.1; **purine nucleoside phosphorylase *(PNP, LACC1)***	c00788; **epinepherine**
6	1.4.3.4; **monoamine oxidase (*MAOA, MAOB)***	c00611; **N-Acetyllactosamine**
7	c00059; **sulfate**	c00350; ***Phosphatidylethanolamine***
8	c00780; **serotonin**	c00059; **sulfate**
9	c00104; **inosine diphosphate**	c05589; **L-Normetanephrine**
10	3.1.2.2; **acyl-CoA thioesterase *(ACOT1-13, THEM4-5, BAAT, PLA2G6, ACAA2, PPARG, PPT1)***	3.1.3.5; **5'-nucleotidease**

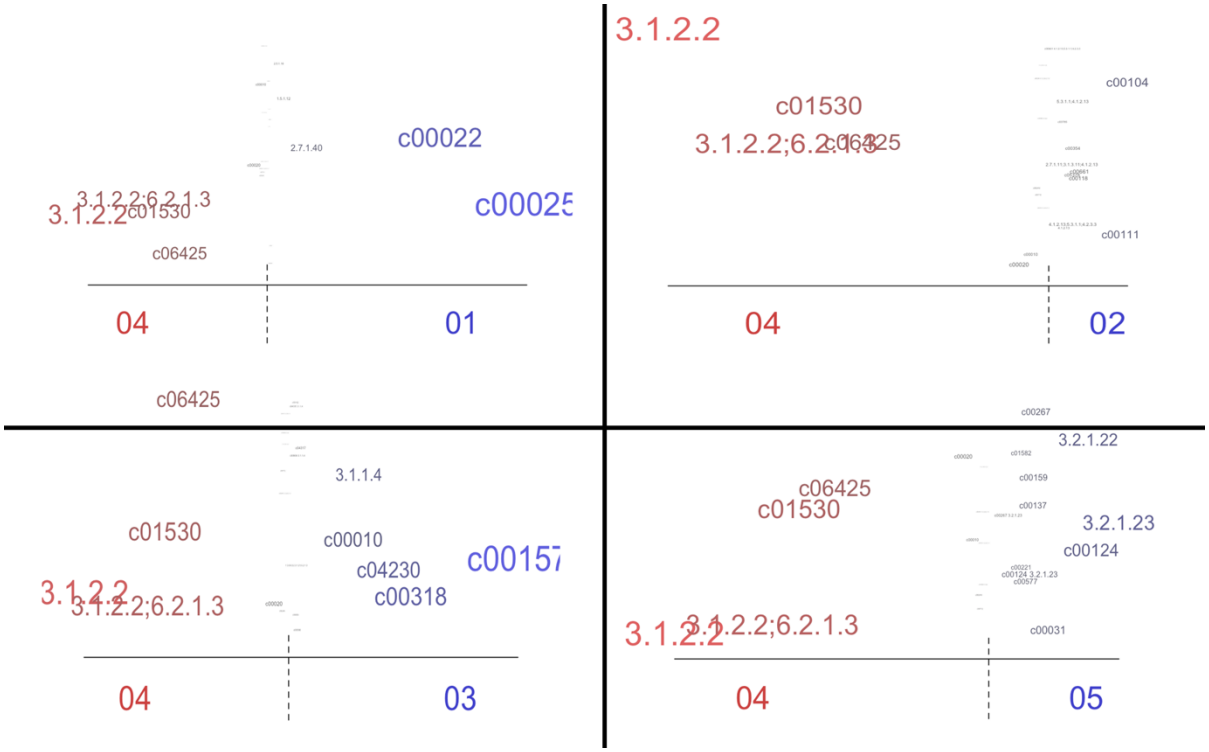

Figure 4.5 Pairwise comparisons of strongly loaded Topic 4 biochemicals relative to all other fitted topics. In all cases, terms related to mitochondrial fatty acid metabolism involving Coenzyme A clearly distinguished Topic 4 relative to all others.

Estimated *theta* values interestingly suggested that this metabolic axis was highly enriched within multiple comparisons involving R/OCAS cognitively supernormal participants. Non-trivial loadings were also, however, observed for several ADAD comparisons (**Table 4.3**). Because the fitted STM was estimated in relation to a main effect content covariate (i.e., consolidated cognitive and etiologic pairwise comparisons), Topic 4 was further defined through those terms uniquely used to describe its manifestation in comparisons involving supernormal participants and ADAD. Considering the top ten terms ranked ordinally by *beta* probabilities and FREX scores, this notably included many compounds substantially involving sialylated complex aminosugars and enzymes involved in their remodeling. It also included components of lipid, nucleic acid phosphate, and biogenic amine metabolism as previously identified through Term 4 loadings across all clinical comparisons (**Figure 4.6**). In contrast, enrichment for Coenzyme A and nucleic acid phosphate pathways characterized Topic 4 as it was referenced for the *ADAD mutation carrier- non-carrier controls* comparison (*ordinally top ranking KEGG and BRENDA terms by beta probability:* **c00020, c00010, c00024, c00241, c00060, 2.3.1.16, c00049, 1.4.3.2, 1.4.3.4, 3.1.3.5)**.

Table 4.3 Top Metabolic Topic 4 *Theta* Coefficient Values as Distributed across Consolidated, Main Effect Clinical Phenotypic Contrasts in AD

RANK	CLINICAL POPULATION	COHORT	CONSOLIDATED AD CONTRAST	ESI-MS MODE	SVA ALGORITHM	*THETA* COEFFICIENT
1	LOAD	R/OCAS	*supernormal versus MCI/AD*	POS	Leek	0.9994078
2	LOAD	R/OCAS	*supernormal versus cognitively stable control*	POS	Leek	0.9993403
3	LOAD	R/OCAS	*supernormal versus MCI/AD*	POS	BE	0.9986634

4	LOAD	R/OCAS	*supernormal versus preclinical*	NEG	Leek	0.9960087
5	LOAD	R/OCAS	*MCI/AD versus preclinical*	NEG	Leek	0.9701927
6	LOAD	R/OCAS	*preclinical versus cognitively stable control*	NEG	Leek	0.9537516
7	ADAD	UCLA ADAD	*preclinical mutation carrier versus family control*	NEG	BE	0.9514507
8	LOAD	R/OCAS	*MCI/AD versus cognitively stable control*	POS	Leek	0.7910050
9	LOAD	UCI ADRC	*preclinical versus cognitively stable control*	POS	BE	0.3377170
10	ADAD	UCLA ADAD	*AD versus MCI*	NEG	BE	0.2776085

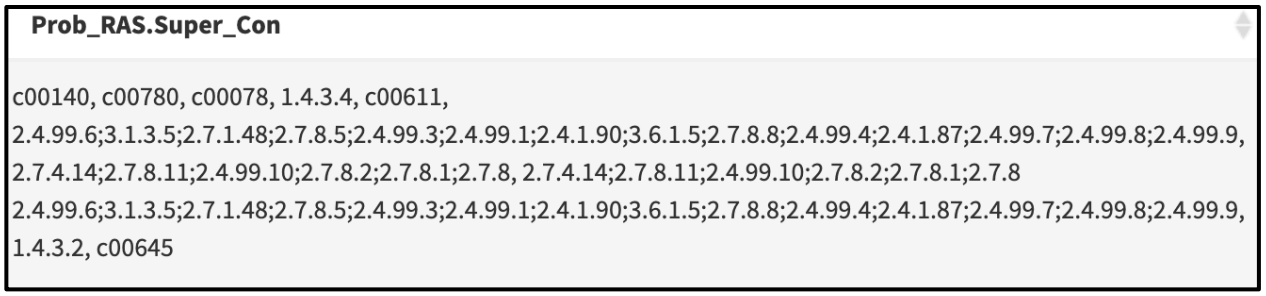

Figure 4.6 A representative term cluster illustrates how STM content covariate estimation can estimate how specific AD metabolic topics (e.g., Topic 4) are described by biochemical terms in specific, clinical phenotypic comparisons. Terms are KEGG chemical and BRENDA enzyme IDs. Distinct (but similar to) overall term loadings onto Topic 4 across all pairwise contrasts, the consolidated comparison of R/OCAS supernormal and preclinical LOAD peripheral metabolomes implicates the metabolism of sialylated complex aminosugars and enzymes involved in their remodeling. It also includes components of lipid, nucleic acid phosphate, and biogenic amine metabolism consistent with Topic 4 loadings across all pairwise comparisons overall.

DISCUSSION

Previous chapters' analyses of the peripheral plasma metabolome across AD cognitive staging comparisons and etiologies suggested that only a modest core of biochemical change characterizes several clinical comparisons (e.g., the MCI/AD versus preclinical contrast across LOAD and ADAD). Because of this substantial residual diversity, alternative statistical approaches were necessary to integratively characterize how this complex distribution of peripherally evident dysmetabolism mapped to a similarly complex distribution of clinical phenotypic comparisons. Because this problem greatly exceeded unaided human consideration, these relationships were instead estimated using text mining approaches (as a natural language processing problem) spanning a biochemical term/token space nominated by upstream Mummichog activity network modeling. Specifically, structural topic models (STMs) estimated five latent metabolic topics which effectively summarized Mummichog activity networks derived from 36 pairwise clinical comparisons across the span of AD clinical staging and etiologies. In explicitly accounting for both prevalence and content structural covariates, these models permitted the highly expressive, yet precise, mapping of implicated biomolecules and clinical comparisons through the inferred biochemical and metabolic topic architectures discovered.

Very interestingly, metabolic Topic 4 specifically implicated the molecule *N*-Acetyl-D-glucosamine (GlcNAc, *Kegg:* **C00140**), which has been previously found to moderate amyloid and tau pathology in AD [472-475]. Specifically, this could be mediated by intracellular post-translational modifications of proteins (including amyloid and tau) by *O*-GlcNAcylation functioning as an activity-dependent regulator and biological signaling component. GLcNAc biosynthesis and its dynamic conjugation to and removal from cellular proteins is dependent

upon nutrient availability, principally that of glucose dysmetabolically impacted in AD [473, 475]. Small-molecule co-factors such as uridine diphosphate (UDP) employed in glycerophospholipid synthesis and the catabolism of some dietary hexose sugars other than glucose (i.e., galactose) also participate in hexosamine metabolism and could prove pathobiologically limiting through their disease-associated dyshomeostasis. Due to the multiply crucial metabolic roles served by these metabolites in AD (and the abnormally aging, dysmetabolic brain specifically), these complex biochemical interdependencies could comprise one discrete CNS-peripheral metabolic axis (likely among others) disproportionately subject to metabolic "failures of compensation" proceeding from "compensations for failure" in abnormal aging. This vicious, feedforward cycle accompanying the development of clinical and cognitive decline could therefore describe a "final common process" in AD etiopathogenesis complexly distributed across metabolic biological systems.

This could explain why the strict "dysmetabolic core" across AD clinical populations including LOAD and ADAD was modest as observed in previous chapters, even if (and perhaps exactly because) most vulnerable biochemistry in AD becomes complexly distributed with its evolution across diverse, impacted biological systems conditional on predisposing risk etiologies. This complexity and its dyshomeostatic compromise in AD (as recapitulated across likely many analogous biological networks similarly constrained by disease processes) could suggest many examples of why and how specific, perturbed biological systems in abnormal aging can only support cognitive stability only indefinitely (i.e., as in the clinical transition from preclinical disease to MCI/AD). To remediate these deficiencies, dysmetabolic limitations rendering brain structure and function incompatible

with cognitive resilience could be therapeutically corrected through dietary, lifestyle, and/or pharmaceutical interventions [33, 207, 476].

This hypothesis strongly agrees with findings that *O*-GlcNAcylation is overall depleted in in the AD brain (similar to AD cortical glucose hypometabolism) and can attenuate neuroinflammation in addition to amyloid/ tau proteopathies [472, 477, 478]. It is also supported by the fact that GLcNAc and broadly hexosamine biosynthesis competes for central carbon pathway metabolites with both the pentose phosphate pathway and glycolysis also homeostatically important in AD [473]. Indeed, UDP-GLcNAc is also depleted in AD, where this could serve as an important nutrient deficiency signal involved in stress response transduction [477, 479, 480]. The only recent attention to brain hexosamine biology in AD belies the importance of this metabolism to it and other metabolically intensive organs such as the pancreas [473]. Like *N*-acetyl-L-aspartate (NAA) metabolism, hexosamines may be particularly important for neurons [329, 332, 475, 481], particularly elements of the extracellular matrix (ECM) including the perineuronal net and sulfated extracellular proteoglycans [482-489]. Metabolic processes involving GLcNAc are enriched in the hippocampus and increased *0*-GlcNAcylation protects against amyloid and tau toxicity [473, 480, 485]. Specifically relating hexosamine to lipid dysmetabolism, high-fat diet has been found to reduce *0*-GlcNAcylation concurrent with mitochondrial deficits in several non-human model systems [474, 477]. This agrees with observations derived from metabolic STM Topic 4, which suggested (in addition to hexosamines) a semantic enrichment for fatty acid metabolic processes compared to all other fitted topics.

Across differing cells and tissues (including neurons and glia in the CNS), these alternative metabolic pathways also serve vital homeostatic functions in successful cognitive

aging which, when sufficiently compromised by AD, could precipitate the failure of GLcNAc-involving processes similarly protective against dementia [336, 338]. Through mechanisms which remain unclear and deserve further mechanistic consideration, this could occur in a manner incompatible with successful cognitive aging once compromised. Increased *O*-GlcNAcylation as measured in cerebrospinal fluid (CSF) or blood might thus represent a specific molecular target remediable by small-molecule therapeutics or functioning as a readout of AD treatment response. Interestingly, comparisons involving cognitively supernormal participants were enriched for contributions from metabolic Topic 4. This very successfully aging population could therefore be uniquely vital for better understanding how the sustained integrity of hexosamine biology mechanistically relates to this exceptional degree of cognitive resilience.

In this supernormal population specifically, content-covariate modeling pursued using STM suggested a semantic cluster of biomolecules again including hexosamine metabolites (e.g., GLcNAc, *N*-acetyllactosamine) and nucleic acid phosphates. Ethanolamine phospholipid processing was, however, additionally implicated through the enzyme ethanolamine phosphotransferase (*EPT1/ SELENOI*) which was also inferred for DS-AD and ADAD comparisons in CHAPTER 2 and CHAPTER 3 using PIUmet modeling [341]. Very interestingly, this content-covariate-level phenotypic comparison further nominated multiple terms involved in the transfer of sialic acid to glycosaminoglycan (GAG) ECM polysaccharides previously implicated in the deposition of amyloid and tau [484, 485]. Also known as siglecs (i.e., sialic-acid-binding immunoglobulin-type lectins), these molecules including the microglial-expressed LOAD GWAS hit **CD33** have been increasingly studied as regulators of chronic neuroimmune dyshomeostases observed from very early in emerging

AD [490-494]. Sulfotransferase deficiencies involving sialic-acid-modified glycans have been associated with AD microglial reactivity AD patient and rodent model brains [495]. Consistent with much prior literature in AD [496, 497], these GAG molecules play roles in innate immune system complement regulation, where resulting deficits in sugar recognition signaling due to disease might precipitate mechanistically catastrophic outcomes and thus cognitive decline [498-503]. Broader glycobiology, like complex lipid biology in AD, might suggest many aspects of complex carbohydrate metabolism related to bioenergetics, but also substantially involving anabolic, signaling roles in the pathways of disease-relevant cells, tissues, and organs. It is again intriguing to consider that dyshomeostases emerging from these additional, potentially competing processes in AD could become pathobiologically limiting in a manner relating to (if not driving) cognitive decline, as suggested here by NLP approaches implementing metabolic STMs.

Importantly, clinical comparisons besides those involving supernormal participants also demonstrated non-zero *theta* coefficient loadings on Topic 4, although these were generally weaker relationships compared to the former. Specifically, some evidence emerged that the ADAD *mutation carrier- non-carrier controls* comparison also loaded modestly on Topic 4, however, several of the implicated enzymes and their genes (*ACAA1-2, HADHB*) are primarily expressed in mature astrocytes, rather than microglia [373]. This suggests the intriguing possibility (consistent with the existence of multiple, complex CNS-peripheral metabolic disease axes) that these dyshomeostatic molecular programs could be complexly and dynamically distributed even across CNS cell types in AD as a function of etiology and cognitive staging. Here too it is possible to appreciate how complex processes of homeostatic compensation and accommodation could fail catastrophically beyond some

critical point in AD with strongly limiting implications for trajectories of successful, resilient cognitive aging.

It is fascinating to consider that these and similar relationships could be alluded to presently by a single metabolic topic identified using STM, even exempting the A) other four fitted topics or B) future extensions incorporating additional, clinical neurological disease comparisons of the peripheral plasma metabolome. Much additional work should be pursued to further unpack the presently fitted biochemical STM and better understand the capabilities of these powerfully expressive, semantic models to accelerate translational, neurodegenerative research. The application of NLP approaches to semantically understand AD pathobiology indexed in peripheral plasma invites exciting, new conceptual-level possibilities for hypothesis generation and downstream, confirmatory research design. In doing so, this generative modeling might suggest highly contextualized, targeted molecular pathways and processes in AD with minimal invasiveness in alive, aging adults themselves.

These possibilities remain to be considered by focused, mechanistic investigations in appropriate AD model systems including patient-derived cell culture [504]; however, semantic-level knowledge generation approaches like these could substantially bridge the gap between A) data-driven and B) hypothesis-testing branches of AD systems biological inquiry in coming years. Benefitting and enriching both research paradigms, this synthesis could prove important for successfully developing biomarker and therapeutic targets associated with abnormal, age-associated cognitive decline, where limited semantic-level, pathobiological understanding of AD currently limits both efforts in precision healthcare. Specifically, applied NLP methods involving scientific text mining could further demarcate diverse and physiologically extended CNS-peripheral dysmetabolic axes in evolving AD. Such

efforts could then systematically suggest important biochemical inferences (for focused follow-up in aging adults) explicitly mapped in terms of clinical and cognitive diversity informing upon these complex biological disease processes.

Where successfully mapped as a function of diverse cognitive status and etiologies, targets resulting from generative NLP modeling of the AD plasma metabolome could precisely direct focused follow-up research, in addition to therapeutic and biomarker targeting. As with all AD modeling efforts, downstream validation will be essential to proactively identify and address the potential limitations of semantic topic extraction NLP methods including STM [340]. As suggested here and by proceeding chapters, however, metabolic perturbations may proceed highly heterogeneously in AD as a function of differing etiologic risk factors and cognitive status. Nonetheless, this dysmetabolic diversity of molecular programs similarly impacted by recurrent "failures of compensation" driven by "compensations for failure" might represent a systems biological, "final common process" across varied AD etiopathogenesis and development. While biochemical pathways, classes, and processes implicated in this way could greatly inform and advance basic AD disease biology, they might also be similarly enriched with diverse translational research targets as a consequence. Befitting their complexity across the dynamic process of Alzheimer's disease, translational systems biology (and metabolomics specifically) will hopefully identify manifold therapeutic targets and precision biomarkers from amongst them to better remediate and monitor this devastating, refractory metabolic disease of abnormal cognitive aging.

CHAPTER 5: Summary, Conclusions, and Future Aims

The coming years will be incredibly important and transformative for the rapid expansion of quantitative, systems biology, not just to advance new paradigms in translational biomedical research, but also to close the sometimes-labyrinthine circle from "bench to bedside." Perhaps no single set of professional skills stands to contribute more intensively and uniformly to all such efforts than does applied systems biology and bioinformatics. If anything, these technologies (and the diseases such as AD increasingly investigated by them) have begun to challenge historically normative expectations regarding the complexity of anticipated pathobiological architectures and mechanisms underlying abnormal aging. At least part of this trend emerges from the field's better understanding of how intricate biological disease processes in AD might, in fact, actually be (as increasingly appreciated through large-data *omics* methods including metabolomics).

Because AD has been historically informed by strong prior expectations concentrated on amyloid-processing, protein-coding genes (i.e., the amyloid cascade hypothesis), this expanded scale of inquiry beyond a finite number of disease subnetworks could be a highly disruptive and translationally fruitful in coming years. In prioritizing the development of methods, platforms, and data integration infrastructure, applied computational optimists within the field may look to these prior efforts in understanding and remediating abnormal, age-associated cognitive decline as the opportunity costs of technology (i.e., machine learning, computation, data) not always evolving in stride with ideal timetables for practical advances in personalized health (i.e., cheap, effective point-of-care tests for emerging cognitive risk). This narrative exalting the power of computation alone to drive innovation in translational science is reductive, but it also remains an underappreciated lens by which

to understand the limited success of many AD therapeutic discovery efforts for decades. We will never know what could have been had a time-travelling bioinformatician (with the benefit of modern compute and *omics* instrumentation) sat with Hardy, Selkoe, and colleagues as they designed and interpreted their seminal experiments. If recent proposals and achievements by the NIH such as Operation Warp Speed and the proposed Advanced Research Projects Agency for Health (ARPA-H) serve as any indication [24], these considerations are, however, very much at the emerging core of tomorrow's transitional medicine for diseases to come and those that remain under-addressed.

The value of systems-scale and platform-driven outlooks on disease is exemplified by few innovations better than metabolomics and the renewed metabolic focus on human disease over the past decade [241]. Not only has this permitted the understanding of pathobiology very proximal to the AD clinical phenotype [25], but it has also forced molecular researchers to confront biases inherited from the protein-coding-gene centric focus of translational molecular research from the late-20th century onwards. In contrast, metabolism as surveyed through metabolomics of peripheral blood might provide an alternative picture of diseases of aging such as AD. Particularly, these experiments might instead suggest interdictable points of homeostatic compromise in the emerging disease process which become pathologically limiting through "failures of compensation" precipitated by "compensations for failure." This specific pattern of failure in time distributed across human structure and function could represent a "final common process" sculpting through its disease-specific particulars many of the well-appreciated, neurodegenerative pathologies of abnormal aging and cognition clinically appreciated in AD.

In this way, the process of specifically mapping this emerging distribution of dyshomeostases (at the pathobiological interface of biochemicals as both functional molecules and metabolites) could turn our attention towards understudied biomarker and therapeutic targets potentially addressing great, unmet clinical needs in diseases of abnormal aging including AD. Particularly because metabolomics contributes such a sensitive and disease-proximal biological readout, these methods will also be of increasing importance for understanding important, but understudied, correlates of clinical variability which must be better contextualized for the effective delivery of personalized monitoring and therapies. Key among these in AD are the effects of distinct predisposing risk etiologies (e.g., LOAD, DS-AD, ADAD) and antemortem cognitive staging (i.e., preclinical disease, MCI, AD), which have been incompletely considered by previous mechanistic models of AD (particularly as they might relate to implicated biology other than amyloid and tau proteopathies).

In 2014, Mapstone and colleagues reported in LOAD that a 10-lipid panel (comprised predominantly of phospholipids and acyl-carnitines) demonstrated depletion in the peripheral plasma of individuals cognitively stable at baseline, but who would go on to objectively decline within a five-year observational period [28]. It remained unclear, however, if these preclinical LOAD participants included in the R/OCAS aging cohort demonstrated broader networks of peripheral metabolic deficits beyond the 10-lipid panel which also anticipated prodromal and clinical impairments. It further remained unclear if these findings were generalizable, even under conditions of clinical and demographic parity, to the peripheral metabolomes of independent participants also meeting criteria for preclinical LOAD.

Consistent with the findings of Mapstone and colleagues, canonical biochemical pathway analyses indeed implicated lipid, and specifically glycerophospholipid, dysmetabolism within R/OCAS preclinical LOAD plasma. They also, however, suggested the importance of potentially related and previously described metabolic pathways including glycolysis and glutaminolysis. In addition to their intrinsic roles in bioenergetics, increasing research at single-cell resolution has suggested that these same molecules may serve vital functions in the vulnerably aging brain which, when compromised, might prohibit cognitive resilience through ultimately abortive "compensations for failure" and "failures for compensation." Across a diverse cross section of metabolites deleteriously affected under similar constraints in abnormal aging, this could constitute a metabolic "final common process" in AD etiopathogenesis and evolution.

Supporting this possibility, the comparison of preclinical LOAD metabolomic "fingerprints" in plasma across the R/OCAS and independent UCI ADRC preclinical participants demonstrated a highly statistically significant, but modest, overlap in implicated canonical pathways. Because this exceeded expectations due to chance alone, it suggests not only that this specific dysmetabolic signature of preclinical LOAD is reproducible at the pathway level, but also that network-scale plasma metabolic perturbations in this population are robustly observed overall. This was further supported by *de novo* modeling driven by these clinical phenotypic comparisons, which identified additional dysregulated metabolic networks in both R/OCAS and UCI ADRC associated with preclinical LOAD status.

Although LOAD is the most prevalent AD etiology, it remained unclear if other vulnerably aging populations (e.g., those with Down syndrome) similarly demonstrated cognitive-decline-associated, network-scale changes to the peripheral metabolome. Even if

this were the case, it remained unclear if any such changes accompanying decline in DS-AD resembled those observed in early LOAD. Because of inherent limitations comparing the cognitive staging of DS-AD and LOAD (due to premorbid intellectual deficits in DS), peripheral plasma metabolic change in DS was examined as the comparison of those trisomic aging individuals who remained cognitively stable versus those who demonstrated clinical AD. As in LOAD, this comparison nominated many network-scale alterations to canonical metabolic pathways in the peripheral blood metabolome, suggesting that these peripheral dyshomeostases do not occur solely as a function of LOAD in the development of AD overall. More specifically, both canonical and *de novo* pathway analyses in DS-AD strongly implicated semantically interpretable patterns of metabolic compromise involving lipids (including phospholipids and acyl-carnitines) and the central carbon metabolism of hexose sugars. This was further supported through the modeling of inferred metabolic enzyme activity from DS-AD peripheral metabolomics, which again implicated proteins with diverse molecular substrates (e.g., lipids, branched-chain amino acids) involved in similarly diverse biochemical programs. Furthermore, targeted metabolomics investigations established multiple, bioenergetically important organic acids (e.g., lactic acid) as dysregulated in the blood of DS individuals with clinical AD.

Although comparisons between LOAD and DS-AD imply necessary limitations within current frameworks as described, these analyses chiefly demonstrated that both etiologies involve peripherally evident changes to the plasma metabolome in relation to cognitive status in AD. Furthermore, these changes were similarly heterogeneous across pathways, even if they did not implicate identical molecules through a strictly delineated “final common pathway”, but perhaps instead a shared “final common process” of emerging dysmetabolic

constraints. Where differentially realized in vulnerably aging biology according to differing cognitive status and risk-conferring etiologies in AD, these dyshomeostases could nonetheless contribute to adverse, shared outcomes involving neuropathological accumulation and associated failures of cognitive resilience.

To further explore this possibility, the ADAD peripheral plasma metabolome was also investigated. This permitted several comparative advantages relative to DS-AD because, unlike this latter population, those with ADAD do not experience premorbid cognitive developmental differences compared to those with LOAD on average. Furthermore, because age to dementia diagnosis can be estimated for given mutations segregating within families, metabolomic changes co-varying with these estimates can be evaluated for mutation carriers, even if these individuals have not yet demented. To explicitly compare the metabolomes of preclinical ADAD to preclinical LOAD participants (versus respective cognitively stable controls), metabolic fingerprint analyses of respectively perturbed canonical pathways again demonstrated a modest, but statistically significant, proportion of overlap between these to a degree exceeding chance alone.

To evaluate the specificity of this overlap for the *preclinical-control* comparison, the plasma metabolomes of preclinical LOAD and ADAD participants were compared to their respective peers demonstrating objective cognitive deficits (i.e., MCI/AD). Not entirely as anticipated, these comparisons across etiologies (and with the benefit of highly harmonized cognitive comparisons) also demonstrated some significant overlap in peripherally indexed, canonical metabolic pathways exceeding chance alone. Much as in LOAD and DS-AD, *de novo* biochemical modeling in ADAD plasma indicated the extensive involvement of diverse networks including lipids of potentially disproportionate importance of hepatic metabolic

processes homeostatically supportive of cognitive resilience. It remained unclear, though, if the content of the blood metabolome from ADAD mutation carriers demonstrated similar, diverse biochemical changes in relation to estimated years until clinical dementia diagnosis. Consistent in other analyses across ADAD, DS-AD, and LOAD, metabolomic network simulations of these observed correlations again implicated diverse metabolites principally including lipids (e.g., cholesterol, fatty acid metabolites), but also sugars of importance to central carbon pathway flux. These findings were recapitulated by analyses of these untargeted data which inferred the activity of inferred enzyme-coding genes (e.g., *SELENOI/EPT1*) again metabolizing lipids, many of which were interestingly expressed in glia.

In sum, the peripheral, systems metabolomic analysis of blood plasma across cognitive staging and etiologies in AD suggested a two-fold distribution of dysmetabolism. On one hand, multiple comparisons of canonical pathway fingerprints evaluated across these populations frequently suggested overlap significantly beyond chance levels, but which was overall modest in proportion. This could suggest that a strict "final common pathway" in AD etiopathology exists ubiquitously across these populations but is overall limited. It could also, however, suggest that a much larger proportion of peripheral metabolomic change in AD remains non-shared and complexly distributed across biochemical pathways as a function of cognitive staging and etiologies. If this were true, such a highly semantically dense and residually complex mapping of metabolic processes to specific clinical phenotypic comparisons presents a non-trivial challenge for understanding these relationships to advance translational aims (i.e., biomarkers and therapeutic targets).

To deconvolve these relationships, this mapping was cast as a natural language processing (NLP) problem in biochemical network space. Specifically, a small number of latent metabolic topics were estimated from counts of metabolic terms extracted from *de novo* Mummichog activity networks for 36 AD clinical phenotypic contrasts. Implemented through structural topic models (STMs), this algorithm also permitted the contextualization of these fitted topics in terms of specific, phenotypic content variable comparisons. In addition, it allowed for the estimation of and correction for nuisance metabolomic variables (e.g., ESI-MS mode, SVA algorithm) which might otherwise confound peripheral metabolic topic mappings.

Consistent with the previously appreciated semantic diversity of the AD peripheral metabolome, none of the five fitted metabolic topics alone significantly associated with specific, clinical phenotypic comparisons (p's > .05). Very interestingly, however, metabolic Topic 4 demonstrated a semantic enrichment for biochemistry involving hexosamine/ aminoglycan metabolism, which has been previously implicated in AD through A) glial dyshomeostases and B) alterations to extracellular structures including the extracellular matrix (ECM) and perhaps the perineuronal net specifically. Moreover, comparisons involving the plasma of aging individuals with exceptional cognitive resilience (i.e., supernormal participants) were disproportionately enriched for these processes, although more limited evidence also suggested relationships to the ADAD *preclinical-control* contrast also evaluated in CHAPTER 3. In the case of the former supernormal comparisons, content-covariate-level modeling using STM even more specifically indicated the importance of glycan sialylation, as described by AD research investigating siglecs (i.e., sialic-acid-binding immunoglobulin-type lectins). Because these molecules include the glycoprotein encoded by

the LOAD GWAS-implicated gene *CD33*, it is intriguing to consider that this observation in supernormal plasma could instead index these same processes as correlates (if not mechanistic mediators) of successful cognitive resiliency resulting from their sustained homeostatic integrity in aging. These possibilities, particularly with respect to cognitive outcomes, remain to be more precisely articulated through refined NLP approaches and mechanistically examined for translational, therapeutic purposes.

In conclusion, these combined findings (across the LOAD, DS-AD, and ADAD peripheral metabolomes) suggest an overwhelming diversity of complex metabolic change contingent upon specific disease etiologies and cognitive status across the development of AD. In this way, a complex distribution of physiologically extended, CNS-peripheral metabolic axes might dynamically participate in and index the emerging cognitive dysfunction nonetheless characterizing all adults who develop AD regardless of specific etiologies. Exactly this finding of cryptic, otherwise masked metabolic diversity in AD (despite overall shared clinical trajectories across etiologies) underscores the importance of *omics* methods (including metabolomics) for unbiasedly and precisely stratifying these individual differences in A) translational research and for B) personalized healthcare in aging.

As a corollary, this pattern of findings also suggests, in highly concrete terms, why etiologically agnostic, linear hypotheses explaining AD pathogenesis and development could substantially and artificially limit the study of what is, in fact, a much more biologically complex and dynamic phenomenon. While this promises substantial opportunities for impactful, quantitative systems-scale research in coming years, it is also a sobering reminder that these strong prior hypotheses provide distinct frameworks for understanding basic AD

pathobiology and pursuing clinical translation which are not without bias. This should be proactively anticipated and actively considered in the pursuit of effective AD biomarkers and therapeutic targets, but also basic disease research as well.

Even if only a modest core of strict metabolic pathways span AD etiologies, this does not exclude the possibility that a dyshomeostatic, "final metabolic process" exerts common constraints across diverse biology in abnormal aging which might otherwise be capable of supporting healthy, resilient cognitive aging. In this capacity, the complexity of homeostatic demands upon metabolism in those aging abnormally (and particularly involving the precariously aging brain) might impose catastrophic limitations across diverse molecular programs in aging nonetheless similarly impacted by recurrent "failures of compensation" driven by "compensations for failure." These themselves may be complexly distributed throughout abnormally aging biological systems conditional on etiologies and cognitive status in a manner which, all the same, similarly converges towards configurations precipitating clinical AD and prohibiting healthy trajectories which might otherwise afford avenues of further cognitive and functional compensation.

If these imperiled CNS-peripheral metabolic axes can be discretely identified and contextualized across differing at-risk clinical populations as pursued in this dissertation, these vulnerabilities might be remediated through highly targeted lifestyle, diet, and/or pharmaceutical interventions consistent with the goals of personalized healthcare. Besides this and contextualized through minimally biased natural language processing approaches, these dynamically dyshomeostatic axes in evolving dementia might also suggest very rationally premised, semantically informed disease biomarkers best indicated to address specific clinical questions in individual aging adults. This does not even begin to address how

similar insights applied to basic AD research might close the loop between A) data-intensive hypothesis generation and B) prioritized, mechanistic investigation of highly non-random and contextually rich target biology thereby implicated in disease. Of great importance for emerging programs like ARPA-H to consider, there will be no such virtuous cycles until open access publishing streamlines the broad dissemination of these taxpayer-funded, public-good insights. This will be especially vital in further scientific text mining efforts in neurodegenerative diseases such as AD, and perhaps should be specifically elevated as a critical concern in the current IT infrastructural design implemented by scientific publishing. Improving this status quo might greatly and more seamlessly aid the exposure of such reported literature to concept/topic-level semantic modeling ahead of human-expert summary review. The possibilities for overburdened PIs with no time to read are tremendous.

Looking to the future of translational AD systems biology, metabolism, and metabolomics, much work remains to be done. The field has, however, made immense strides in the semantic-level understanding of biochemical findings easily and cheaply obtained from blood plasma, but harder to interpret in the pathobiological context of AD as a CNS disorder. Because the extent of these dyshomeostases in aging remain unclear and marked by their striking degree of complexity as described in this dissertation, this conceptual sharpening has been and will continue to be vital. Integrated into an accelerating culture of large-data analysis and platforms in translational AD research, I do not see these trends abating. If anything, I have sensed the early growth of new scientific culture and collaboration befitting an increasingly identifiable approach to translational research.

Unfortunately, these aims are still often talked about more abstractly than they are successfully implemented to the point of concrete insights and deliverables.

At least part of this implies the growing need to nurture this culture and bring more informed perspectives to the conference table or Zoom window. This will likely involve the skillful deployment of existing bioinformatics and computation talent where it exists, but this alone will not be enough. Bioinformatics has no footprint in pre-baccalaureate education and a limited, but growing, footprint at the undergraduate level. Qualified undergraduates might sensibly aim to employ skills well suited to bioinformatics in the handsomely renumerated private tech sector, away from access to many priceless clinical datasets and stipend-level compensation. Similarly, much bioinformatics talent, once terminally trained in academia, churn through a revolving door to industry, which limits the development of bioinformatic culture and institutional knowledge in academia. Although the work to be done and its significance if successful are nearly beyond debate, who will do this work remains far less clear, in addition to the training and educations they will have, the academic culture they will participate in, and the funding mechanisms through which they will best competitively pursue these aims. The most sober, purposeful approach to pursuing translational innovation in AD will proactively address these concerns and create the circumstances for necessary work to flourish and propagate, as it must to succeed.

Along the lines of highly applied and data-centric investigation in disease, it is reassuring to see the NIH embrace innovation in agile, platform/process centric research capabilities and funding through proposed initiatives such as the proposed Advanced Research Projects Agency for Health (ARPA-H) [24]. In aiming to spur collaboration across academic and industry stakeholders consistent with the “Heilmeier Catechism” paradigm of

research prioritization, this will almost definitionally stipulate a culture in which large biomedical data and accompanying data analysis become a common currency of intellectual exchange and appropriate scrutiny. The further normalization and proliferation of these skillsets within academic biomedicine promises exciting opportunities for collaboration and diverse engagement, particularly as this could be driven by existing talent in industry highly acculturated in the construction of durable analysis infrastructure through software. By more optimally distributing computational work amongst invested parties, many time-consuming duties independent of conceptual-level research formulation could be passed to those who stand to gain most from these opportunities (e.g., motivated undergraduates who code and see biology as exactly this, *eager high schoolers through education? As a team sport even?*).

Particularly if this infrastructure can be organized into durable pipelines and databases, the transparency of these processes across analysts could be transformative in allowing translational scientists the bandwidth to follow new lines of inquiry as driven by data and resulting inference, rather than plunging them ever deeper into a quagmire of patchworked software and data. Where this infrastructure is deliberately engineered particularly well, its extensibility might even facilitate the pursuit of new research using novel methodologies and instrumentation which might otherwise only add prohibitive complexity. The prioritization of these capabilities, however, is not guaranteed, as much as doing so would be highly useful. It will be incumbent upon the field of translational AD systems biology and emerging entities such as ARPA-H to aggressively support these front-heavy investments in research infrastructure, which will yield substantial, delayed returns in the study of abnormal human aging consequently. The reward will be an investigative

culture which prioritizes time and resources invested in high-level knowledge generation, diverse communication with stakeholder peers, and biomedical intelligence workup, rather than solitary hours consumed in software code without a thought to the pathobiology of AD.

This is even more important because peripheral investigations of AD alone (such as plasma metabolomics) will be insufficient to mechanistically understand AD and establish causative dysmetabolic relationships within and across different, affected clinical populations. While it remains increasingly clear that concomitant changes to peripheral metabolism accompany cognitive decline in AD, it remains much less clear how these dyshomeostatic CNS-peripheral axes ultimately mediate insults to the brain in abnormal aging which proximally result in failures of cognitive resilience. To probe these questions further in alive, aging participants at molecular resolution, metabolic imaging employing specialized MRI paradigms and/or PET will be necessary to better quantify an intermediate, metabolic "endophenotype" between broad clinical/cognitive status and the blood metabolome. This does not even begin to consider future work to be done further evaluating and clarifying these processes in model systems of AD beyond aging adults themselves (including participant-derived tissues such as iPSCs). Very excitingly, these future studies might themselves implicate other metabolically intensive organ systems in AD cognitive change, which also could prove amenable to imaging or characterization in further model systems. Overall, these expanded workflows will involve substantial considerations and bioinformatics pipelines orthogonal to metabolomics, but which are equally (if not more) data intensive. This is even more reason to invest in large-scale quantitative analysis infrastructure, personnel, and platforms which at least anticipate the scale of these challenges and the software tools to best address them. In doing so, translational researchers

will keep their heads above the matted weeds of methods implementation and remain responsively agile to new avenues of inquiry through rapidly evolving, quantitative research capabilities and platforms.

In all, the translational systems metabolic investigation of AD promises a hard, but hopefully actionable, road to useful therapies and biomarkers in coming years. There remains much to be understood about the disease to the clinical benefit of multiple populations, but the pathobiological architecture underlying AD as suggested by this dissertation much more resembles a sphinxian enigma compared to a cascade. This will be immensely challenging to address for manifold reasons spanning from the conceptual to the methodological and practical. At the same time, it gives promise that the aging biology most promising as AD therapeutics, biomarkers, and mechanistic targets in basic research has not yet been fully appreciated. It is, has been, and will be my privilege as a translational biomedical scientist to continue this vital work advancing precision health aims. In doing so, it is my sincerest hope that we can better monitor and ultimately cure this viciously unrelenting disease of abnormal cognitive aging, and perhaps a few more like it.

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APPENDIX

Appendix 1.1 Biochemical Fingerprints of Canonical Metabolic Pathways Enriched for Dysregulated Metabolite Features in R/OCAS and UCI ADRC Preclinical AD Blood Plasma

Pathway	**R/OCAS**	**UCI ADRC**
Alanine and Aspartate Metabolism	1	0
Androgen and estrogen biosynthesis and metabolism	1	0
Arginine and Proline Metabolism	1	0
Aspartate and asparagine metabolism	1	0
Beta-Alanine metabolism	1	0
Bile acid biosynthesis	0	1
Biopterin metabolism	0	1
C21-steroid hormone biosynthesis and metabolism	0	1
Carbon fixation	1	0
D4&E4-neuroprostanes formation	0	1
De novo fatty acid biosynthesis	0	1
Drug metabolism - other enzymes	1	1
Fatty acid activation	0	1
Fatty Acid Metabolism	0	1
Galactose metabolism	1	0
Glutamate metabolism	1	0
Glutathione Metabolism	1	0
Glycerophospholipid metabolism	1	0
Glycine, serine, alanine and threonine metabolism	1	0
Histidine metabolism	1	0
Limonene and pinene degradation	0	1
Linoleate metabolism	0	1
Methionine and cysteine metabolism	1	0
Nitrogen metabolism	1	0
Prostaglandin formation from arachidonate	1	1
Purine metabolism	1	0
Putative anti-Inflammatory metabolites formation from EPA	1	0
Pyrimidine metabolism	1	0
Saturated fatty acids beta-oxidation	1	0
Sialic acid metabolism	1	0
Tryptophan metabolism	1	0
Tyrosine metabolism	1	0
Urea cycle/amino group metabolism	1	0
Vitamin B3 (nicotinate and nicotinamide) metabolism	1	0
Vitamin B9 (folate) metabolism	1	0

Appendix 1.2 Biochemical Fingerprints of Canonical Metabolic Pathways Enriched for Dysregulated Metabolite Features in Preclinical LOAD and ADAD

Pathway	**Preclinical LOAD**	**Preclinical ADAD**
Alanine and Aspartate Metabolism	1	0
Androgen and estrogen biosynthesis and metabolism	1	0
Arginine and Proline Metabolism	1	0
Aspartate and asparagine metabolism	1	0
Beta-Alanine metabolism	1	0
Bile acid biosynthesis	1	1
Biopterin metabolism	1	0
C21-steroid hormone biosynthesis and metabolism	1	1
Carbon fixation	1	0
D4&E4-neuroprostanes formation	1	0
De novo fatty acid biosynthesis	1	0
Drug metabolism - other enzymes	1	0
Fatty acid activation	1	0
Fatty Acid Metabolism	1	0
Galactose metabolism	1	0
Glutamate metabolism	1	0
Glutathione Metabolism	1	0
Glycerophospholipid metabolism	1	0
Glycine, serine, alanine and threonine metabolism	1	1
Histidine metabolism	1	0
Limonene and pinene degradation	1	0
Linoleate metabolism	1	0
Methionine and cysteine metabolism	1	1
Nitrogen metabolism	1	0
Prostaglandin formation from arachidonate	1	1
Purine metabolism	1	0
Putative anti-Inflammatory metabolites formation from EPA	1	0
Pyrimidine metabolism	1	0
Saturated fatty acids beta-oxidation	1	0
Sialic acid metabolism	1	0
Tryptophan metabolism	1	0
Tyrosine metabolism	1	0
Urea cycle/amino group metabolism	1	1
Vitamin B3 (nicotinate and nicotinamide) metabolism	1	0
Vitamin B9 (folate) metabolism	1	0
Leukotriene metabolism	0	1
Di-unsaturated fatty acid beta-oxidation	0	1
Vitamin H (biotin) metabolism	0	1
Arachidonic acid metabolism	0	1
Porphyrin metabolism	0	1

Appendix 1.3 Biochemical Fingerprints of Canonical Metabolic Pathways Enriched for Dysregulated Metabolite Features in LOAD and ADAD Preclinical versus Objectively Impaired (MCI/AD) Participant Plasma

Pathway	**LOAD Preclinical versus MCI/AD**	**ADAD Preclinical versus MCI/AD**
Alanine and Aspartate Metabolism	1	0
Aminosugars metabolism	0	1
Androgen and estrogen biosynthesis and metabolism	1	0
Arginine and Proline Metabolism	1	1
Aspartate and asparagine metabolism	1	1
Beta-Alanine metabolism	1	0
Carbon fixation	1	0
Drug metabolism - other enzymes	1	0
Fatty Acid Metabolism	0	1
Fructose and mannose metabolism	1	0
Galactose metabolism	1	1
Glutamate metabolism	1	0
Glutathione Metabolism	1	0
Glycerophospholipid metabolism	1	1
Glycine, serine, alanine and threonine metabolism	1	1
Glycolysis and Gluconeogenesis	1	0
Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	0	1
Histidine metabolism	1	0
Methionine and cysteine metabolism	1	0
Nitrogen metabolism	1	0
Porphyrin metabolism	0	1
Purine metabolism	1	0
Putative anti-Inflammatory metabolites formation from EPA	1	0
Pyrimidine metabolism	1	0
Sialic acid metabolism	1	0
Squalene and cholesterol biosynthesis	0	1
Tryptophan metabolism	1	0
Tyrosine metabolism	1	0
Urea cycle/amino group metabolism	1	0
Vitamin A (retinol) metabolism	0	1
Vitamin B3 (nicotinate and nicotinamide) metabolism	1	0
Vitamin B9 (folate) metabolism	1	0
Vitamin D3 (cholecalciferol) metabolism	0	1