The Alzheimer’s Spectrum: Diagnostic Challenges and Nosology in a Clinically Heterogeneous Disease

by

Benjamin Lam

A thesis submitted in conformity with the requirements for the degree of Master of Science

Institute of Medical Science

University of Toronto

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Abstract

Hypothesis: Alzheimer’s disease (AD) is not a unitary condition but a phenotype spectrum.

Rationale: Mounting evidence suggests AD is more complex than previously thought. A new AD conceptualization accounting for this biologic and clinical complexity is needed.

Aim: To review existing evidence for, provide a specific example of, and investigate the nosological impact of AD heterogeneity.

Methods: Three studies were done using (1) literature review, (2) imaging analysis case series, and (3) systematic chart review.

Results: AD is heterogeneous and sub-syndromes exist. Extreme heterogeneity results in syndrome mimicry with blending of imaging markers. Wide variation in diagnostic classification occurs even with standardized application of consensus criteria.

Conclusions: AD is not a single disease but a spectrum of sub-syndromes (core phenotype and atypical sub-syndromes). The AD conceptual framework must evolve to acknowledge, define, and anticipate this complexity, harnessing it to improve diagnostic precision and facilitate treatment discovery.
Acknowledgements

I am greatly indebted to the many individuals and organizations that have supported me throughout this endeavour and made this collaborative work possible.

First are my friends and colleagues at the L.C. Campbell Cognitive Neurology Research Unit, which has been my second family for the past eight years. They and our predecessors gathered the data and conducted much of the preliminary analysis that generated the data for the Sunnybrook Dementia Study upon which this thesis is based. In particular I would like to thank my co-fellows, including Drs. Alexandra Kim, Kie Honjo, Yannick Nadeau, and Laura Middleton, who were ever ready sounding boards for ideas, no matter how eccentric. As a clinician, I would be lost without the guidance of the L.C. Campbell imaging team, including Dr. Joel Ramirez, Christopher Scott, Gregory Szilagyi, Melissa Holmes, and Alicia McNeeley, who helped me better understand the finer details of image acquisition and processing, and gain a better appreciation and respect for the imaging data that makes up much of this dissertation.

Second are my clinical colleagues in the Division of Neurology, who have been role-models, shaping my growth both as an aspiring researcher and physician. They have provided constant encouragement, and made possible that often seemingly impossible task of balancing the tasks of a doctor and being a graduate student. They taught me that each enables and strengthens, rather than occurs at the expense of the other, and that dedicating, as always, is the key ingredient. I am in particular indebted to Dr. Mario Masellis, who has been an unfailing mentor and friend, who provided immeasurable guidance throughout my thesis preparation.
Third are the patients, study participants, and their care partners who made all this research possible. They are possessed of great, and often seemingly supernal resilience and humanity, and they have reminded me how important it was that we never stop seeking better answers, better understanding, and better treatments for this scourge of our day. Their strength in the face of Alzheimer’s also put in perspective the travails of academic toil. Compared to what they did as a matter of course, what were a few sleepless nights spent studying and researching?

Fourth are the donors and funding agencies, without whose generosity none of this would be possible. I would like to sincerely thank the Slaight Family Foundation, the L.C. Campbell Foundation, the Canadian Partnership for Stroke Recovery, the Brill Chair in Neurology, the CIHR Training Program in Neurodegenerative Lipidomics, Roy and Joan Hinsta, and Nancy and Sigmund Levy.

Fifth are the journal editors and reviewers of the papers comprising this thesis. Not only did they take the time to hold a harsh but fair light to these manuscripts, but had the faith to trust and support our findings.

Sixth are my parents Jane and Kin Lam, who never stopped believing in what often seemed like an interminable and insurmountable challenge. Their love has been unfailing, and their support has been unwavering. Although never researchers themselves, they imparted in me that love of learning – that filo sophia – which is so fundamental to the scientific endeavour. I am, always, in their debt for all things.
Last and most certainly not least, is my supervisor and mentor, Dr. Sandra E. Black, and the members of my Program Advisory Committee, Drs. Morris Freedman and Donald T. Stuss, whose ceaseless mentorship and truly inexhaustible patience are beyond what words can describe. If I am now an acceptable researcher, it is because of their herculean efforts and friendship. The trust and confidence they have shown in me, have at times been nothing less than a leap of faith. If I can follow in their footsteps, and join them in the great working of knowledge that is clinical research, I can think of no higher honour.

I dedicate this thesis to my parents, Dr. Sandra E. Black, Dr. Morris Freedman, and Dr. Donald T. Stuss.
Contributions

Please refer to the contributions section within each chapter for a detailing of specific contributions by co-authors. In all cases, the funding agencies had no involvement in the design, analysis, or conduct of the research.
The Alzheimer’s spectrum: Diagnostic challenges and nosology in a clinically heterogeneous disease

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(k and p-values)
List of Abbreviations

**Special Note:** Diagnostic labelling is a key element of this dissertation. The terms Alzheimer’s disease, Alzheimer’s dementia, and Alzheimer’s disease spectrum are related and frequently used interchangeably in broad clinical use. However, there are fine distinctions between them, with incomplete overlap between these labels, particularly in areas of pre-symptomatic disease. It is this complex and at-times unclear nosology, reflecting the complexity of the disease that this thesis speaks to. **Alzheimer’s disease (AD)** can, in one sense, refer to the symptomatic onset of dementia, paired with the most currently accepted construct of Alzheimer’s pathology, namely plaques (beta-amyloid) and neurofibrillary tangles (tau). This conceptualization therefore excludes pre-clinical or at-risk individuals (such as those with positive amyloid-PET imaging), mild cognitive impairment, and dementia. However, AD is sometimes applied more broadly, to include such at-risk, asymptomatic individuals. In keeping with the evolving state of the literature, this thesis will generally use the term AD to apply to specifically to symptomatic disease. However, as our understanding of AD and its conceptualization changes, AD will also be used at times to refer to at-risk, pre-symptomatic cases. When focus is on the whole range of AD, where the variation and distinction between sub-syndromes is being emphasized, the term **AD spectrum** will be used.

<table>
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<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>Aβ</td>
<td>Amyloid beta</td>
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<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>APOE</td>
<td>Apolipoprotein E</td>
</tr>
<tr>
<td>APOE ε4</td>
<td>Apolipoprotein E – epsilon 4 allele</td>
</tr>
<tr>
<td>BPSD</td>
<td>Behavioural and Psychiatric Symptoms of Dementia</td>
</tr>
<tr>
<td>bvFTD</td>
<td>Behavioural Varian Frontotemporal Dementia</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>CBS</td>
<td>Corticobasal syndrome</td>
</tr>
<tr>
<td>CERAD</td>
<td>Consortium to Establish a Registry for Alzheimer's Disease</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
</tr>
<tr>
<td>CSF Aβ42</td>
<td>Cerebrospinal Fluid Amyloid-β 1-42</td>
</tr>
<tr>
<td>DLB</td>
<td>Dementia with Lewy Bodies</td>
</tr>
<tr>
<td>EOAD</td>
<td>Early-Onset Alzheimer’s Disease</td>
</tr>
<tr>
<td>FBI</td>
<td>Frontal Behavioural Inventory</td>
</tr>
<tr>
<td>FTD</td>
<td>Frontotemporal Dementia</td>
</tr>
<tr>
<td>IWG</td>
<td>International Working Group</td>
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<tr>
<td>LOAD</td>
<td>Late-Onset Alzheimer’s Disease</td>
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<tr>
<td>LPA</td>
<td>Logopenic Progressive Aphasia</td>
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<tr>
<td>MMSE</td>
<td>Mini-Mental State Examination</td>
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<tr>
<td>NFT</td>
<td>Neurofibrillary Tangles</td>
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<tr>
<td>NIA-AA</td>
<td>National Institute on Aging – Alzheimer’s Association</td>
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<td>NINCDS-ADRA</td>
<td>National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association</td>
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<td>PCA</td>
<td>Posterior Cortical Atrophy</td>
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<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
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<tr>
<td>PiB</td>
<td>Pittsburgh Compound B</td>
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<tr>
<td>PSEN</td>
<td>Presenilin</td>
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<tr>
<td>SP</td>
<td>Senile Plaques</td>
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<tr>
<td>TDP-43</td>
<td>TAR DNA-binding protein 43</td>
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Foreword

Alzheimer’s disease (AD) is not a single condition. Rather, it is a disease spectrum demonstrating variation along each of its defining elements: age of onset, course, cognitive pattern, behavioural symptoms, functional decline, imaging, co-morbidities, and pathology/biology. When variation is sufficient and recurring, it defines a distinct AD subtype. When variation is extreme, it can render AD clinically indistinguishable from entirely different conditions and imperil attempts at accurate diagnosis. Even when variation is mild, the resulting heterogeneity complicates diagnostic classification, creating controversies in nosology that if not recognized can serve to undermine both patient care and research. In this dissertation, we investigate this variability of AD.

We begin in the Introduction with a discussion of the core features of prototypic AD – the foundation of our modern nosology, which has informed for both good and ill our intuitive conception of this illness. We will end by looking at how these limitations, increasingly recognized, have driven an evolution of the AD paradigm, which continues.

In Chapter 1, we discuss how variation in AD results in distinct sub-syndromes, each with definable and distinct clinical and imaging patterns that may serve to enrich the current nosology of AD. It is the contention of this chapter that recognizing and defining these sub-syndromes aids in future investigations into AD mechanisms, with the ultimate goals being better diagnostic precision and cure discovery.

In Chapter 2, we discuss how extreme variation in AD can result in individuals presenting with the clinical picture of an entirely different illness – Corticobasal Syndrome. This
“syndromic mimicry” serves not only as a caution as to the potential problems arising from AD variation, but illustrates how by defining AD’s sub-syndromes we can simultaneously mitigate these dangers and provide a lens through which to investigate AD’s mechanism.

In Chapter 3, we discuss how phenotypic variation renders AD diagnostic classification an especially difficult challenge. We compare case classification based on five consensus AD criteria, one the former research standard, the other four from the “new generation” of criteria including some that incorporate imaging and CSF biomarkers. We consider how even in the definition of AD there is divergence in thinking resulting in non-equivalency between what by all accounts are diagnostic criteria for the same condition.

We conclude in the General Discussion and Future Directions by considering the immediate and longer term implications of these findings for current research, especially as the field pushes forward with developments in anti-amyloid antibodies and β-site amyloid precursor protein cleavage enzyme (a.k.a. beta secretase or BACE) inhibitors, among other potential therapies. We consider the unanswered question of operational variation and its effect in this context. Specifically, how the definition of specific protocol elements, such as the degree of white matter change necessary to qualify as “significant cerebrovascular disease”, or the definition of “functional decline”. We end on the hopeful note that, if such variation is sufficiently defined, it may aid rather than hinder the disentanglement of the underlying mechanisms of AD, and thus provide signposts on our path to developing a cure.
1. Introduction & Literature Review

Clinical consensus holds that there exists a prototypic Alzheimer’s phenotype. This “typical AD” is the form physicians most often encounter, the course afflicted individuals must most commonly endure, and, justifiably so, the syndrome of primary preoccupation for researchers seeking a cure. This prototype also informs our commonly held conceptualization of AD. It is the paradigm of AD:

- **Age of Onset:** Late
- **Course:** Gradual
- **Cognitive Pattern:** Memory plus others
- **Behavioural Symptoms:** Mild/None
- **Functional Decline:** Present
- **Imaging:** Mesiotemporal and parietal temporal atrophy
- **Co-Morbidities:** Disallowed
- **Pathology and Biology:** Plaques & Tangles

Text-Figure 1.1: Clinical features of typical AD – “the AD paradigm”.

The AD paradigm serves as the foundation for the consensus criteria upon which modern diagnosis, classification, and research into AD are based. Yet it was not an idea that came into existence overnight. It was shaped into being by researchers over 80 years, and crystallized into being with the publication of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for AD in 1984¹.

Today, however, as the boundaries of our understanding of the underlying pathophysiology of AD have expanded, and as ever more cases of atypical AD are catalogued and studied, the AD paradigm has begun to evolve again to help understand this variability
within the disease. As the authors of the International Working Group call it, our increased knowledge demands a “new lexicon” for the conversation on AD to continue\textsuperscript{2}.

1.1. Origins of the AD Paradigm and the Classical View of Alzheimer’s and Dementia

Though dominant today, the AD paradigm emerged very slowly. Due to differences between Alois Alzheimer’s index case (see below) of AD and the most common pattern observed in the elderly, linkage between Alzheimer’s pathology and late onset dementia was not made until pathology studies demonstrated a consistent association between senile plaque and neurofibrillary tangle pathology and the majority of cases of senile dementia\textsuperscript{3,4}. It was not until advances in neuropsychology fashioned tools for investigators to both define and assess cognition to ever finer degrees that it became increasingly clear that this Alzheimer’s pathology-associated group of individuals with dementia shared a common pattern of cognitive deterioration as well.

Terry and Davies summarized these findings in their review of AD, or as it was known at the time – Senile Dementia of the Alzheimer’s Type (SDAT) – providing one of the early classical descriptions for typical AD\textsuperscript{5}. Typical AD presented most commonly in the elderly. Individuals would experience a gradually declining course, with death resulting on average 3-10 years after onset of symptoms (Text-Figure 1.1).

Cognitively, these individuals experienced loss of short term episodic memory. This was often accompanied by difficulties in language, including poor word-retrieval. Parietal dysfunction, including apraxia and agnosia, was often prominent. Over time, judgement and
reasoning were lost. In other words, AD was primarily an amnestic disorder, on top of which other cognitive symptoms evolved. Behaviourally, symptoms were relatively benign though common, frequently taking the form of apathy or “inanition”. Functional decline was present, progressing from deterioration in instrumental activities until eventually the loss of self-care led to the need for long term care. This in turn would contribute to death, often from infection. AD was not itself fatal, but would render affected individuals vulnerable to other ailments which were.

This early work led eventually to two crucial developments. The first was the Diagnostic and Statistical Manual 3rd and 4th editions (DSM III, DSM IV) diagnostic criteria for dementia\textsuperscript{6,7}. The second was the NINCDS-ADRDA consensus criteria for dementia related to Alzheimer’s pathology – i.e. AD\textsuperscript{1}. This pair of criteria collected prior decades’ work into a coherent nosology of AD, thus permitting consistent diagnosis. Although originally intended solely for research, the NINCDS-ADRDA was so successful that its use spread into clinical practice, becoming the clinical and research standard for over 20 years. Around the same time, the DSM-3 and later the DSM-4 played a complementary role by providing the formal requirement for functional decline\textsuperscript{6,7}. Although functional decline was contributory in the NINCDS-ADRDA, it was not required. This formal requirement thus paved the way for the later demarcation between dementia, and the “prodromal” state of Mild Cognitive Impairment (MCI)\textsuperscript{8,9}, with those with cognitive and functional decline defined as having dementia, and those with cognitive but not functional decline defined as MCI.
1.2. Core Features of Prototypic AD

AD and all dementias in general can be conceptualized along a core set of features: (1) age of onset, (2) course, (3) cognitive pattern, (4) behavioural symptoms, (5) functional decline, (6) imaging, (7) co-morbidities, and (8) pathology/biology (Text-Figure 1.2.).

<table>
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<tr>
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<th>Course</th>
<th>Cognitive Pattern</th>
<th>Behavioural Symptoms</th>
<th>Functional Decline</th>
<th>Imaging</th>
<th>Co-Morbidities</th>
<th>Pathology and Biology</th>
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Text-Figure 1.1.: Core Clinical Features for all Dementias

We consider these for typical (“prototype”) AD below.

1.2.1. Age of Onset

Typical AD is a disease of the elderly. Prevalence among the youngest-old (age 65) is relatively low (0.5-0.8%) but increases rapidly with age, doubling with every 5 years of age and reaching 28.5% by age 90\textsuperscript{10–12}. Typical AD is so intertwined with aging that manuscripts frequently discuss the aging population by way of highlighting the threat posed by AD\textsuperscript{13–15}.

1.2.2. Course

Typical AD has an insidious onset and gradual decline\textsuperscript{1,16,17}. Abrupt onset and stepwise deterioration suggest alternative causes of dementia, usually vascular\textsuperscript{18,19}. Annualized progression for typical AD results in a loss of -3 ± 2.4 points per year on the MMSE\textsuperscript{20}. Typical “untreated” AD runs its course over 10 years from time of symptom onset, although factors
including age of onset, level of education, gender, and presence of behavioural-psychiatric symptoms can influence predicted life expectancy\textsuperscript{21}. Of these, age of symptom onset appears particularly important, where those diagnosed earlier in life (60’s and 70’s) might expect 7-10 years of survival, compared to the oldest old (90’s) where median survival is 3 years\textsuperscript{21}. This observation is likely related to common co-morbidities of older age, which are often the eventual cause of death, including pneumonia (45%) and cardiovascular disease (37%)\textsuperscript{21}. However, it is noteworthy that this association gives the appearance that late-onset AD has a more aggressive course, whereas the opposite is demonstrated to be true by studies specifically examining rates of decline\textsuperscript{22,23}. This apparent contradiction lies at the heart of the debate surrounding heterogeneity being the product of stage versus subtype\textsuperscript{24}. Death is often the result of other disease arising as complication of AD, rather than from AD itself. These include complications of immobility, poor nutrition, and pneumonia\textsuperscript{25}.

1.2.3. Cognition

Memory loss is the first and most profound cognitive impairment in typical AD\textsuperscript{1,26–28}, so much so that, similar to old age, memory loss is often synonymous with AD within the public and general medical consciousness. This conception is supported by comparative studies showing correlation between amnestic symptoms with AD while non-AD dementias correlate with decline in other cognitive domains\textsuperscript{29}.

The pattern of memory impairment in typical AD is specific, usually affecting short-term, episodic (i.e. experience and context related) recall. This is in contrast to other dementias, such as semantic dementia, wherein semantic (i.e. the fund of knowledge or personal ‘database’ of
information) recall is more likely affected, versus subcortical dementias (including Huntington’s and Parkinson’s disease dementia) where working memory and memory retrieval is impaired. It should be noted that the specificity of such patterns are stage dependent, as dementias tend to acquire the characteristics of their peers with progression. Patients with AD, for example, developed impaired semantic recall although not usually with the same prominence as in semantic dementia, at least early on. Studies have also found high correlation between episodic memory loss and hippocampal atrophy (which is also a signature of typical AD; see below), leading to the syndrome designation of Amnestic Syndrome of the Hippocampal Type, linked in turn to AD. Such studies have led to the recommendation that episodic memory testing be used to establish the cognitive phenotype when diagnosing AD.

Due to its pre-eminence, the presence of short-term episodic memory loss is a requirement in the diagnosis of AD for all major consensus criteria in current use, with the sole exception of the NIA-AA. Its primacy has likewise informed cognitive screening tools recommended for the early detection of AD, including the Folstein Mini-Mental State Examination.

1.2.4. Behaviour

Behavioural or neuropsychiatric symptoms are frequent in AD, affecting 60-80% of individuals. Apathy, depression, and irritability/agitation are especially common, while psychosis may develop later in the disease course. Euphoria by contrast is relatively infrequent in AD, compared to other non-AD dementias. However, these symptoms are mild early in the disease course, and early prominent behavioural symptoms raise doubts of an AD diagnosis.
Early neuropsychiatric symptoms are more in keeping with other aetiologies, chief among them the behavioural variant of frontotemporal dementia (bvFTD)\textsuperscript{40}.

1.2.5. Function

Functional decline is a hallmark of typical AD, beginning with higher-level activities of daily living and progressing to impairment of daily activities of self-care\textsuperscript{41}. Effects can present first with alterations in an individual’s routine, rather than an out-right loss of abilities\textsuperscript{16,17}. Occupational distress resulting in ‘early retirement’ can occur. So critical is it to the phenotype of AD that function serves as a mandatory co-primary outcome measure in AD treatment trials in the United States\textsuperscript{42}. Further, functional decline serves as the demarcation between prodromal disease and frank dementia. In the current conceptualization of dementia, the absence of functional decline despite presence of other diagnostic elements, especially memory loss, serves as the basis for the diagnosis of Mild Cognitive Impairment (MCI)\textsuperscript{9,9,43} instead.

1.2.6. Imaging

The original use of imaging was not as a tool for diagnosis, but one of diagnostic exclusion\textsuperscript{3}. The chief aim of performing computed tomography (CT) was to rule out other co-morbidities that might be the “true” cause of a patient’s dementia (see next section). However, as more studies were conducted into the imaging signatures of AD and other dementias, a pattern emerged wherein AD pathology at death mirrored atrophy in life, in particular ventricular enlargement, sulcal widening, and gyral narrowing\textsuperscript{5}. Later, PET studies demonstrated a pattern of bilateral hypometabolism in AD brain, focused predominantly within the parietal regions and extending...
into the posterior temporal and anterior occipital areas. Frontal lobes by contrast were relatively spared\textsuperscript{44,45}. Regional function was thus linked to regional atrophy. Eventually, these reproducible patterns of atrophy and hypometabolism led to their incorporation as supportive imaging biomarkers within current AD diagnostic criteria\textsuperscript{16,17,32}.

1.2.7. Co-morbidities

Leading up to the NINCDS-ADRDA codification in 1984, typical AD was viewed as a disease with AD pathology existing in isolation, in line with the older concept of AD being a diagnosis of exclusion\textsuperscript{1}. This emerged from early pathological studies which described the then recognized forms of “organic psychosis”: dementia that occurred in the presence of no pathology (normal aging), atherosclerotic changes (vascular dementia), or amyloid plaques and neurofibrillary tangles (senile dementia, later further specified as being of the Alzheimer’s type)\textsuperscript{4}. AD was diagnosed only after these other causes were eliminated, and their presence was taken to mean that AD was not the driving factor of disease. As noted in previous sections, diagnostic investigations were once relegated to ruling out non-AD diagnoses. It was not until neuroimaging signatures were described that these investigations switched from looking for exclusions to identifying inclusionary features of AD\textsuperscript{41}. At the same time, imaging and pathology studies increasingly indicated that some of these “exclusionary” conditions potentially played a contributory role in the clinical expression of AD\textsuperscript{46}.

Currently, testing for Vitamin B12 levels and thyroid function are the standard of care. Neurologic conditions including cerebrovascular disease (including major stroke, multiple smaller strokes, or severe white matter disease), Parkinson’s disease, dementia with Lewy
Bodies, frontotemporal dementia, Huntington’s disease, significant or repetitive traumatic brain injury, normal pressure hydrocephalus, epilepsy, and psychiatric conditions such as major depression and schizophrenia must be excluded\textsuperscript{1,16,17,32,47}. Only when all such conditions, along with any other general medical illness that might reasonably be suspected as being present are excluded can the diagnosis of AD considered probable\textsuperscript{1,16,17}. Some consensus criteria do allow for the presence of these co-morbidities so long as they are not felt to be the sole or primary cause for cognitive deterioration, but even then the diagnostic classification in the presence of such conditions is for “possible AD”\textsuperscript{16,17}.

**1.2.8. Pathology and Biology**

What unifies AD as a clinical entity is pathology.

Early neuroscientists suspected that dementia emerged from an assortment of causes, with the best known at the time being traumatic injury, storage diseases, and toxic-metabolic conditions\textsuperscript{5}. One common theory was that AD was caused by a combination of patient vulnerabilities, perhaps genetic and age related, along with some external factor such as a slow infection, perhaps an unidentified virus\textsuperscript{48,49}. However, the nature of the underlying pathology remained unclear. Eventually, however, studies began demonstrating a strong association between late-onset dementia, and the presence of amyloid plaques and tau neurofibrillary tangles on histology\textsuperscript{50}, leading to a watershed moment with the publication of the amyloid hypothesis by Hardy and Higgins\textsuperscript{51}. The amyloid hypothesis was based on the observed association between mutations in the amyloid precursor protein (APP) gene and dementia, and the accelerated onset of dementia in those with Trisomy 21 (as APP is located on chromosome
Hardy and Higgins postulated that amyloid must therefore play some role, likely early, and almost certainly central, in a cascade of events including tau pathology that resulted in the development of AD.

Braak and Braak around the same time showed a tight correlation between clinical disease severity and presence of NFT tau pathology in key brain regions, suggesting that amyloid and tau were direct mediators of disease. These regions included the transentorhinal cortex (stage I-II), hippocampus and entorhinal cortex (stage III-IV), and parietotemporal and finally frontal association areas (stage V-VI). In contrast, the primary motor, sensory, and visual areas were spared. Due to the consistency of this clinical-pathological association, the paradigm finally took hold that AD was a common disease of the elderly, defined by neuropathology. This clinicopathologic definition of AD remains dominant today.

1.2.9. Summary: Prototypic AD

In summary, typical AD is a condition of the elderly, presenting with insidious onset and gradual decline first and chiefly in memory, followed by likewise gradual deterioration in other cognitive domains including language, visuospatial, and finally executive function. Behaviour is generally intact until late in the disease course. Cognitive decline results in loss of function, with symptoms first interfering with instrumental activities before progressing to a loss of independence. Imaging demonstrates hippocampal atrophy, spreading to parietal regions with the frontal lobes being spared until very late in the disease course, and is mirrored topographically by functional imaging. Co-morbidities may occur, although their presence reduces diagnostic certainty, and in some cases precludes an AD diagnosis altogether. Definitive
diagnosis is established at autopsy with the histopathological confirmation of the presence of amyloid plaques and neurofibrillary tangles composed of tau.

Perhaps aptly and ironically, Alois Alzheimer’s index case for the disease that now bears his name didn’t fit this mold very well.

1.3. A Cautionary Case: The Story of Auguste Deter

In 1901, Alois Alzheimer admitted a patient to the Frankfurt hospital where he was chief of staff. He described her as having “a peculiar disease of the cerebral cortex”\textsuperscript{56}. The patient was Auguste Deter, the index case of AD. A review of her case against the background of what was considered typical AD is informative.

Deter was younger at onset, and her course was more rapid. She was a 51 year old woman who, prior to admission was living at her Frankfurt apartment with her husband and her daughter. Her family reported that she had declined rapidly, progressing from the first signs of disease to her present state of distress over only a year and a half. Progression from onset of symptoms to death was only 4.5 years.

Cognitively, there were some similarities to typical AD, but more differences. Mrs. Deter’s memory was severely impaired. She would forget conversational details and misplaced items, with both symptoms becoming increasingly frequent over time. She also had profound apraxia, having lost the ability to use eating utensils. Somewhat unusual for current concepts of AD, Deter developed language symptoms very early on, primarily with poor word-retrieval. She would exhibit circumlocution, phonemic paraphasias, and frequent use of neologisms\textsuperscript{57}. Alzheimer’s clinical notes described automatic speech and empty speech\textsuperscript{57}, wherein she would
reply with her first name “Auguste” regardless of Alzheimer’s questions. This phenomenon, while common in late stage typical AD, is more commonly associated with the semantic dementia subtype of frontotemporal dementia when occurring early in the disease course\textsuperscript{58,59}.

Also somewhat atypically, Deter presented early in her course with severe, erratic \textbf{behavioural symptoms}. Alzheimer’s described marked psychosis and paranoia, particularly directed at her husband. She accused him of being unfaithful, and “of wishing her ill”. Alzheimer further elaborated that Deter was convinced her husband was planning to assault her, though there was no material evidence there was any such danger.

\textbf{Functionally}, Deter became unable to look after her home and then herself. She would drag her bedding around their apartment without apparent goal. Following admission her functional decline accelerated until she was often found lying in the fetal position in bed. Due to this and despite nursing care, she developed pressure sores. Deter also became incontinent over her admission. Typical of AD, Deter’s motor function was relatively spared, and she continued to walk without difficulty, and had intact limb dexterity, but progressing to being bed-ridden toward the end of her life.

\textbf{Imaging} did not exist at the time, and Alzheimer could pursue no further investigations. He left Frankfurt in 1903, but continued to follow Deter’s case. When she passed away in 1906 he undertook a thorough pathological assessment of her brain.

\textbf{New pathology} stains had recently become available at the time, and Alzheimer’s availed himself of these developments. He employed new techniques developed by his friend and colleague, Franz Nissl, who had been the one to recruit Alzheimer to Frankfurt originally in
1899. These he used alongside the new staining methods developed by Bielschowsky.

Alzheimer described neurofibrils throughout the neocortex, appearing in some but not all cells. In the most severe instances, he observed that the nucleus and cell body had disintegrated, leaving “only a tangle of fibrils indicat[ing] the place where a neuron was previously located.”

Alzheimer noted that the neurofibrils in diseased and dead cells stained, while those within apparently healthy cells did not. He hypothesized that “some chemical change must have occurred” to allow for this difference in staining reaction, presaging the revelation of tau hyperphosphorylation as a critical event in neurofibrillary tangle formation.

Although there was no clinical mention of co-morbidities, it is of great interest that Alzheimer noted that “a growth appears on the endothelia” (presumably atheroma). As we will discuss later, the idea of vascular pathophysiology in AD, long neglected, has come once more in vogue.

Finally, Deter’s genetic status was recently examined, with one group reporting a mutation in the PSEN1 gene, although this could not later be replicated. These authors did, however, confirm the presence of an APOE ε3 genotype, which some have postulated is in keeping with Deter’s atypical, early-onset phenotype.

Auguste Deter’s case is a cautionary tale. Although some of her features – notably early memory loss and classical AD pathology – are archetypical for AD, her presentation included many atypical features not often associated with the more common sporadic form of AD. She had early language symptoms including some reminiscent of primary progressive aphasia. She had prominent early behavioural symptoms more consistent with behavioural variant
frontotemporal dementia. She was too young. Her course was too rapid, though this pace would be more typical for familial AD. That these inconsistencies with the AD paradigm should exist in the index case of AD makes clear the reality that AD is a protean disease.

It would be up to the pioneering efforts of later investigators to reveal the rich kaleidoscope of AD variability, and allow the paradigm of AD to evolve.

1.4. The AD Syndrome Paradigm Evolves

Evolution of the AD Syndrome paradigm can be seen in every aspect of typical AD, and generally relates to one of three shifts:

- **A broadening of the acceptable range and definition of individual syndrome elements:**
  Examples include the easing of age, cognitive pattern, and functional decline requirements.

- **Increasing use of biomarkers:** Perhaps the most significant single shift in the conceptualization of AD, movement in recent years has been away from post mortem pathology, and towards biology through the use of in vivo biomarkers, enabling recognition in earlier stages of disease.

- **Recognition of the association and potential interaction between individual syndrome elements:** Examples include the association between early age of onset, atypicality, and the relative sparing of hippocampal neuropathology.

These shifts feed into each other. Loosening of clinical definitions relies on biomarkers to retain diagnostic precision, while the expanded range of allowable phenotypes permits a more sophisticated view on AD’s syndromic complexity. This complexity in turn gives rise to the idea
of individual AD sub-syndromes. Sub-syndromes in turn provide anchorage to the otherwise dizzying breadth of phenotypic variation.

Using the same framework of dementia elements as typical AD, we consider these shifts in turn.

1.4.1. Evolution of Age of Onset

Current consensus holds that AD can present much earlier in life and that age should no longer serve as a diagnostic criterion\textsuperscript{16}. Recent evidence further suggests that the age-driven model for dementia incidence in general may not adequately account for complex interactions between age and other risk factors, particularly when dementia incidence is assessed globally\textsuperscript{62}. Additional factors, such as national economic development enabling high standard of living, with associated higher levels of education and lower rates of potential general medical risk factors such as cardiovascular disease, may have the effect of reducing age-specific incidence.

Reflecting this, Early Onset AD (EOAD) is now a recognized nosological entity, distinguishing it from Late Onset AD (LOAD). In general, EOAD presents with relative sparing of the hippocampus and conversely greater cortical involvement evident in structural and metabolic imaging\textsuperscript{63–65}. EOAD has a more aggressive clinical course\textsuperscript{66,67}, a distinct pattern of memory loss involving less loss of semantic memory than LOAD\textsuperscript{68}, and more frequent association with co-morbidities that may play a causative role in the expression of what might otherwise have been subclinical pathology\textsuperscript{69}. Clinical divergence between EOAD and LOAD has been hypothesized to arise from the presence or absence of \textit{APOE} ε4 allele\textsuperscript{22}. EOAD cases lacking the ε4 allele more often present with non-amnestic, atypical disease while the same
absence in LOAD is associated with a more typical picture. This is discussed further in Chapter 1.

1.4.2. Evolution of Course

AD’s course is definable by variable rates of progression\(^70\). Among predictors of rate of decline are age of onset, presence of behavioural and psychiatric symptoms, and presence of other medical co-morbidities\(^21\). For example, EOAD is associated with a more aggressive course\(^71\). Importantly for drug development, these variable rates result in significant differences in rates of conversion from MCI to dementia, time to conversion, and survival\(^70,72\). Given these often serve as endpoints in drug discovery, accounting for this variability becomes crucial.

1.4.3. Evolution of Cognition

The primacy of memory symptoms has also come under revision. Although some consensus criteria still require the presence of memory decline (DSM-5, ICD-10)\(^17,47\), and consider it both necessary and sufficient (IWG)\(^32,2,73\), others allow for non-amnestic presentations of AD (NIA-AA)\(^16\). In general, the shift has been to recognize non-amnestic AD cognitive subtypes, given converging lines of evidence as to their existence\(^74\). This is discussed in greater detail in Chapter 1 where major cognitive sub-syndromes of atypical AD are reviewed, and in Chapter 3 where the required cognitive phenotype for an AD diagnosis among different criteria is shown to influence diagnosis.

1.4.4. Evolution of Behaviour

In typical AD, the emergence of behavioural and psychiatric symptoms of dementia (BPSD) occur, but typically not early in the disease course. This, however, is precisely the pattern
observed in some AD subtypes, in particular EOAD and autosomal dominantly inherited AD. In these, BPSD especially anxiety and depression may be notable even in the earliest, mildest stages of disease \(^{75,76}\). In extreme cases, behaviour can even present as the primary symptom in AD, defining the behavioural/dysexecutive variant of AD\(^{77}\).

As with cognition, it is now recognized that BPSD can be classified into distinct sub-syndromes. Forrester and colleagues, in examining the National Alzheimer’s Coordinating Centre data identified three distinct cohorts – a **severe cluster** with agitation, apathy, and disinhibition, an **affective cluster** with depression and irritability, and an **asymptomatic cluster**\(^{78}\). At even finer degrees of gradation, individual BPSDs can themselves be subdivided and demonstrate discrete linkage to biology. For example in the case of delusional symptoms, persecutory thoughts often present early and have genetic associations, while visual misidentification and other perceptual derangements occur later and are associated with disease severity\(^{79}\). Specific BPSDs also demonstrate tight topographical linkage, as evinced by tractographic, SPECT, and PET associations between the anterior cingulate gyrus and apathy\(^{80–82}\). BPSDs can clearly not only be sub-syndrome defining, but may hint at underlying disease mechanisms in the same way that distinct cognitive sub-syndromes may. Indeed, very recently the concept of Mild Behavioural Impairment (MBI), a behavioural-psychiatric analogue of MCI, has been put forward\(^{83}\). Recognition of this syndrome may help predict future progression to MCI/AD, as well as have prognostic value in predicting greater caregiver burden, institutionalization, and poorer quality of life.
1.4.5. Evolution of Function

Although the assessment of functional decline generally remains an important element for both diagnosis and monitoring of AD progression, some have begun to question this foundational pillar of the AD paradigm. They argue that function is arbitrarily and inconsistently defined. Further, they point out that by the time clinically evident functional decline is present, pathology has often progressed to the point where the opportunity for treatment has already been lost. Another argument for eliminating functional decline from the AD framework is that it holds little or no prognostic value. Doody and colleagues examined the predictive value of baseline functional symptoms on subsequent progression as measured by annualized decline in MMSE, and on conversion from MCI to dementia. They found no difference between individuals showing baseline functional decline and those free of such symptoms at the outset.

Proponents of retaining function within the AD framework note its crucial role in distinguishing dementia from MCI. Function serves as a useful and relatively easily assessed marker of disease in the clinical setting. However, MCI as a diagnostic entity is itself problematic, suffering from low sensitivity and specificity, and demonstrating poor internal consistency when minor alterations are placed upon MCI criteria. The International Working Group cites this lack of reliability in the MCI construct as a reason for the elimination of function in the diagnostic framework – bypassing what they call the inconsistencies and associated pitfalls of the MCI label in the process. Along these lines and more extremely, they advocate moving away from the term dementia itself, and instead linking nosology to the
presence of the disease biology – i.e. a **neurobiological paradigm** that shifts away from the traditional neuropathological definition of AD\(^{32,2,73}\).

Function is unlikely to disappear entirely, however, given its regulatory requirement by the FDA for the approval of any prospective AD therapy\(^{42}\), and its face validity in the clinical setting, though this may change as in vivo disease biomarkers become more widely available.

### 1.4.6. Evolution of Imaging

Rapid developments in the field of neuroimaging in dementia preclude an exhaustive discussion of its evolving complexity. However, one key area that bears special mention is the elucidation of focal atrophy syndromes. These have corresponding patterns of hypometabolism that demonstrate consistent topographic patterns on structural and metabolic imaging\(^{29,44,45,89–94}\). Common patterns include (1) left versus right asymmetry, (2) limbic versus extra-limbic involvement, and (3) focal regional involvement. These variations correlate with specific, often atypical, non-amnestic patterns of clinical MCI or dementia, and are discussed in detail in Chapter 1. One key implication of these variations includes differences in observed progression among types\(^{95}\). Some focal patterns, such as the parietal-predominant, periorolanic-involving pattern of corticobasal syndrome (see Chapter 2), even show distinct linkage to pathology.

### 1.4.7. Evolution of Co-morbidities

Among the greatest shifts in the AD paradigm has been a growing awareness and allowance for co-morbidities. Although the presence of such conditions still increase the uncertainty of an AD diagnosis, several newer criteria now give well-defined schemes under which co-morbidities can be recognized within the context of AD. These employ the term possible AD, such as
“clinically possible, due to etiologically mixed AD” in the case of the NIA-AA. Among allowed co-morbidities are: (1) “substantial” cerebrovascular disease (i.e. temporally related stroke or presence of multiple/extensive infarcts), (2) core features of DLB, (3) other active neurologic disease, (4) other active non-neurologic disease, and (5) cognitive-affecting medication use. Others remained prohibited, including: (1) prominent features of bvFTD, and (2) prominent features of PPA (either semantic dementia or progressive non-fluent aphasia).

Strong associations between co-morbidities and AD have driven much of this shift. Considering only dementia-associated pathologies, the frequency of non-classical AD findings (including neocortical Lewy bodies, hippocampal sclerosis, microinfarcts) ranged in one study from 9.7-43%. The same study found that such co-morbidities independently correlated with cognitive decline, suggesting that quantity of total pathology, rather than specific pathologies, determined disease severity. This was despite most such cases being labeled as being solely AD. Among medical comorbidities, obesity and diabetes are strongly associated with both LOAD and EOAD, although the prevalence is greater among LOAD (86.5% vs 58.2%). Even when considering conditions more commonly associated with late-life, including endocrine, nutritional, and metabolic disease, one third of EOAD cases were shown to harbour these co-conditions.

Why is there this association, and do they point at mechanism? Along these lines, alternative and companion pathophysiological processes to the amyloid cascade hypothesis have enjoyed a renewed support (or at least consideration), wherein some “co-morbidities” are increasingly viewed as facilitators of Alzheimer’s pathology in the expression of clinical AD, with
some authors calling them AD aetiologies in their own right. This is especially true for cerebrovascular pathology including small vessel disease such as is manifest in white matter hyperintensities (WMH).

1.4.7.1. Evolution of Co-morbidities: The special case of Small Vessel Disease

Small vessel disease (SVD), including silent lacunar strokes and white matter hyperintensities (WMH), are common in both AD and aging\(^9^8\).

Its underlying pathology is heterogeneous\(^9^8,9^9\). In the setting of cognitive decline, SVD suggests vascular cognitive impairment or vascular dementia\(^1^0^0\), previously serving to exclude probable AD\(^1\). Although some criteria continue this tradition of exclusivity\(^1^7,4^7,7^3\), others have begun permitting it to degrees\(^1^6\). Differences in philosophy on whether to allow for co-morbid SVD is also reflected in observational studies that tended to filter out SVD (e.g. Alzheimer’s Disease Neuroimaging Initiative; ADNI\(^1^0^1\), versus those permitting it (e.g. Sunnybrook Dementia Study)\(^1^0^2\), resulting in widely divergent observed frequencies of co-morbidity\(^1^0^2\). The central controversy hinges on the nature of SVD’s role — whether it is merely additive (implying SVD is a bystander) or mechanistically integral).

Increasingly, evidence suggests that SVD should be considered integral to the pathogenesis of Alzheimer’s disease\(^1^0^3,1^0^4\). The strongest support for this position comes from evidence of the intimate relationship between WMH and AD, including WMH’s role in: (1) cholinergic pathway disruption\(^1^0^5,1^0^6\), (2) distributed network disruption\(^1^0^7\), (3) failure of brain amyloid clearance via the glymphatic system, and (4) astrocytic dysfunction and decoupling of
the neurovascular unit. Cholinergic pathway and network disruption are discussed further in 

**Chapter 1.** We consider glymphatic failure and astrocytic dysfunction below.

1.4.7.1.1. SVD Role: Failure of amyloid clearance and the brain’s glymphatic system

A relatively recent discovery regarding the link between SVD and AD has been the identification of perivascular lymphatics or “glymphatics” as a key clearance system for cellular by-products otherwise toxic to the brain\(^{108,109}\). Tied to an individual’s sleep cycle, compromise of this system was first observed to potentiate the formation of A\(\beta\) plaques in rodent models\(^{110}\). Theoretical models postulate vessel wall stiffness and heart rate as factors determining pathogenicity in glymphatic failure, in support of the “heart-brain axis” hypothesis\(^{111}\). The importance of clearance pathways is only now beginning to be better understood, and additional pathways are being discovered that likely work in tandem to ensure homeostatic control of by-products, including the recent discovery of the meningeal lymphatic system\(^{112}\).

1.4.7.1.2. SVD Role: Astrocytes, Clasmatodendrosis, and Decoupling of the Neurovascular Unit

WMH are not exclusively tied to neuronal pathology alone. Astrocytic dysfunction, expressed as clasmatodendrosis\(^{113}\), may be an underappreciated mechanism of WMH and subcortical injury, and a distinct hallmark of neurovascular dysfunction\(^{114,115}\). Normally, neurovascular coupling serves to accommodate the brain’s extreme metabolic demands. Perfusion, and thereby metabolite trafficking, is tightly regulated through cell-to-cell signalling involving neurons and endothelial smooth muscle of the microvasculature, with astrocytes playing a key role\(^{116,117}\). The result is an increase in blood flow in response to neuronal function, also referred to as functional hyperemia. With age, this process can become impaired, in part due to alterations in
production of neuronal-derived nitric oxide\textsuperscript{118} resulting from differential expression patterns of inducible nitric oxide synthase\textsuperscript{119}. In addition to neurovascular decoupling, this may further exacerbate pathology through pro-inflammatory effects. Conversely, adaptive restoration of neurovascular coupling as the result of aerobic exercise may mitigate the deleterious effects of dementia\textsuperscript{120}.

1.4.7.1.3. SVD Role: Amyloid Angiopathy

Cerebral amyloid angiopathy (CAA) is a general term describing both the specific pathology of intra-mural amyloid deposition and the resulting, observable complications due to this damage (most often lobar cerebral haemorrhage)\textsuperscript{121}. Previously, CAA was a post-mortem diagnosis. However, validated imaging-based diagnostic criteria can now identify individuals with CAA in life\textsuperscript{122}. This ante mortem diagnosis is crucial, as CAA is a self-potentiating (feed-forward) process that can simultaneously result in further vascular injury and greater \(\text{A}^\beta\) deposition, and thus represents another SVD link to AD\textsuperscript{121}. Observable sequelae of CAA in addition to lobar hemorrhage include (1) microbleeds, (2) cortical superficial siderosis, (3) microinfarcts, and (4) WMH.

As a bridging pathophysiological process between SVD and AD, the distribution of CAA shares genetic risk factors with AD, including APOE\textsubscript{ε}4, \textit{PSEN1}, and \textit{APP}\textsuperscript{123,124}. In the case of APOE\textsubscript{ε}4, carriers show greater occipital CAA burden in association with greater occipital myelin loss\textsuperscript{123}. In \textit{PSEN1} carriers, occipital CAA is associated with T-cell activation\textsuperscript{124}. Such inflammation and myelin injury may explain CAA’s correlation with impaired structural connectivity, as demonstrated by a dose-dependent reduction in network efficiency proportionate to degree of
amyloid burden on PiB-PET\textsuperscript{125}. Clinically, CAA is associated with a dysexecutive syndrome and reduced processing speed\textsuperscript{126}. CAA is a clear example where SVD doesn’t simply enhance Alzheimer’s pathology – it is Alzheimer’s pathology.

\textbf{1.4.7.2. SVD and Implications for the Role of Co-Morbidities}

In summary, SVD plays a central role in the clinical expression of AD and cannot be excluded from the taxonomy. The presence and impact of amyloid/tau pathology versus SVD in individual cases distribute along a continuum. While examples of “pure” diseases exist at extremes of the spectrum, most cases represent a multifaceted interaction of these “co-morbidities”; understanding AD thus requires a greater understanding of the role of SVD.

Caution, however, must be exercised so as not to eliminate entirely the nosological categorizations that do in actuality hold great value, particularly on the clinical front lines. The key is recognizing that such categorizations need not be ones of exclusion, but descriptions of continuums.

\textbf{1.4.8. Evolution of Pathology and Biology}

Although the amyloid and tau hypotheses remain cornerstones of modern models of the AD pathophysiology, there is increasing consensus that neither is able to fully explain the full spectrum of AD pathology and phenotype. Ball and Murdoch, for instance, cautioned against drawing a linkage of equivalency between Alzheimer’s pathology and the diagnosis of AD, citing inconsistencies between Braak staging and clinical phenotype\textsuperscript{127}. Authors of the revised NIA-AA criteria for AD take a similar stance\textsuperscript{55}. The incompleteness of this linkage likely arises in part from the complex interactions between these classical processes of AD and AD’s co-morbidities,
as just discussed. However, even only considering the classical AD pathological processes, there is increasing evidence of variation in topography which, as with variations in patterns of cognition, function, and behaviour, can define subtypes of AD\textsuperscript{128}. Further, AD histology now also appears to be variable, expanding the coterie of pathologies which one might consider “Alzheimer’s”\textsuperscript{129}. At the molecular level, distinct amyloid sub-species, in particular for Aβ\textsubscript{1-40}, have been demonstrated, with the degree of fibril structure variation in being associated with typical AD and PCA, versus rapidly progressing AD\textsuperscript{130}. The pathological conception of AD therefore bears reconsideration in light of this variation. This variation also simultaneously illustrates the importance of biomarkers (which can improve diagnostic certainty by identifying classical Alzheimer’s biology), and cautions against over-reliance on them (as they miss non-classical Alzheimer’s biology).

1.4.8.1. Pathologic Variation by Place – Topographic Heterogeneity

Not all cases of AD follow the pathologic progression of Alzheimer’s pathology described by Braak and Braak\textsuperscript{53}, Thal\textsuperscript{131}, and CERAD\textsuperscript{132}. The topographic distribution of these changes varies between cases, and in the extremes define distinct subtypes of disease\textsuperscript{128}. More recent work suggests topographic subtypes may be variably associated with other neuropathology. Josephs and colleagues demonstrated frequent co-occurrence of TDP43 in typical and limbic predominant subtypes, but TDP43 deposition was relatively rare in hippocampal sparing cases\textsuperscript{133}. Interestingly, this same study found an association between memory scores on cognitive testing and TDP43 burden, despite TDP43 not traditionally being considered an Alzheimer’s pathology. This view may require revising, as TDP43 may be a more common presence in AD than previously thought, being demonstrable in upwards of 57% of cases\textsuperscript{134,135}.  

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1.4.8.2. Pathologic Variation by Type – Histologic Heterogeneity

Beyond distribution, the histology of Alzheimer’s pathology also varies, and such heterogeneity is observed even in the presence of causative AD mutations\textsuperscript{129}. Cotton Wool Plaques (CWP), for example are observed in association with \textit{PSEN1} mutations and spastic paraparesis\textsuperscript{136,137}. We present another example of pathologic variation in the setting of \textit{PSEN1} in Chapter 2. Variation also occurs in non-neuronal Alzheimer’s pathology, such as with microglia\textsuperscript{138}. Finally, if one allows for the inclusion of non-classical Alzheimer’s pathology within the spectrum of “contributor pathologies”, yet further heterogeneity arises due to inter-case differences in the degree of contributions from such pathologies. This situation, as applied to cerebrovascular disease, is summarized by Kalaria\textsuperscript{139}. As with co-morbidities, caution must be exercised so as not to be too permissive in the inclusion of non-classical Alzheimer’s pathology, as distinctions between diseases do exist and pathological spectrums do not always conjoin\textsuperscript{140}. 
1.5. Summary

Typical AD is characterized by late onset and gradual worsening in cognition, primarily memory, with associated functional decline. Behaviour is spared until late in the disease course, although apathy, anxiety, and low mood may be present. Imaging demonstrates mesiotemporal atrophy, with extension into the limbic circuits and temporal-parietal regions with pathological progression of disease. Co-morbidities raise caution when attributing an unspecified dementia presentation to AD, resulting in a downgrading of diagnostic certainty to possible disease or eliminating AD as the aetiology altogether. However, the original index case of AD did not conform to the profile of typical, sporadic AD. Auguste Deter was a young woman whose course was rapid. She presented with language symptoms as severe as her memory loss, and had prominent behavioural symptoms early in her course. Were it not for her pathology, and more recently her genetic status indicating familial AD, a different aetiology might have been considered such as FTD. Her case illustrates the clinically heterogeneous nature of AD.

More recently, recognition of AD heterogeneity has increased, reflecting an evolution in the AD paradigm previously dominated by typical AD. However, typical AD remains a useful starting point in defining this marked variation. Just as typical AD is characterized by a set of core features, atypical AD can be characterized by variation within any of these features, with each thus acting considered an axis around which a specific subset of clinically variant cases can be arrayed. This model is useful conceptually for two reasons. First, it renders the task of disentangling AD more feasible, as investigating AD heterogeneity in its totality can quickly lead to an overwhelming diversity of cases. This has the immediate consequence of requiring any such studies to have enormous, impractical cohort sizes, so as to capture the full spectrum of
AD along with its rare variants. By focusing on each axis of heterogeneity in turn, studies enhance their statistical power, and thus bring sample sizes back down to a reasonable level. Second, division along core disease features sets up a situation more favourable to the discovery of underlying disease mechanisms, by taking advantage of the endophenotype concept\textsuperscript{141,142}. AD is not unlike many diseases of psychiatry in that they are complex traits, resulting from the interplay of host, in particular genetic, factors\textsuperscript{143,144}. As such the use of endophenotypes whereby clinical features are considered emergent traits of more fundamental consequences of underlying processes is well-suited for uncovering these basic mechanisms of disease.

As can be seen in the evolving conception of the features that comprise AD, variation along distinct axes often correlate. This can be seen in the association between young-onset (age of onset), non-memory predominant decline (cognitive pattern), and lateralization (imaging). There is insufficient information to draw causal relationships between these elements; however, what can be inferred in at least some cases is the clustering of atypical cases into definable atypical sub-syndromes of AD. Alzheimer himself commented:

Considering everything, it seems we are dealing here with a special illness. An increasing number of similar cases have been observed during the last years. This fact should persuade us not to be satisfied with classifying clinically undetermined cases by forcing them into the categories of recognized illnesses. There are certainly more psychiatric illnesses than are listed in our textbooks. A histological examination will enable us to determine the characteristics of some of these cases. This process will gradually lead to
a clinical distinction of specific illnesses from the more general categories of our
textbooks and it will enable us to define them clinically in greater detail. – Alois
Alzheimer\textsuperscript{56}, translated in\textsuperscript{57}.

In other words, with everything that we have learned as a field, it is time to define and
investigate these “specific illnesses”, these atypical AD sub-syndromes, and use that
classification in order to elucidate in greater detail, not just the clinical presentation of these
variants, but the mechanisms which permit them, and by extension typical AD, to occur. We
discuss the axes of heterogeneity, and the variant sub-syndromes they define in \textbf{Chapter 1}. 


2. Aims and Hypotheses

2.1. Aims

This thesis aims to describe the spectrum of AD heterogeneity, and to touch upon its potential impacts on diagnosis with implications for research. First, it reviews the established evidence supporting a re-conceptualization of AD not as a unitary disease entity, but as a syndrome spectrum comprising a core phenotype (the typical, “prototypic” syndrome) and discrete sub-syndromes. Although sub-syndromes can be defined using any dementia element, the focus will be on cognitive variants of AD. Second, this thesis will provide concrete support of the proposed AD spectrum framework by illustrating an example of extreme phenotypic variation resulting in syndrome mimicry. Gene sequencing confirms pathological mutations in the \textit{PSEN1} gene, while quantitative imaging comparisons elucidate the imaging signature of this new entity – corticobasal syndrome secondary to familial AD. Finally, from the specific to the general, this thesis examines discordance between major consensus diagnostic systems currently in use, underscoring the hurdles in reaching a cogent nosology for even typical AD, and explores the further complication that may arise when phenotypic variation is accounted for.

2.2. Hypotheses

2.2.3 Study One: AD Heterogeneity Exists

AD is not a unitary syndrome, but a spectrum of phenotypes comprising discrete sub-syndromes. These sub-syndromes are distinguishable on the basis of cognitive pattern. These distinctions are not merely artificial, but are reflected in differences in surrogate markers (e.g. imaging), and underlying biology (e.g. pathology and genetics), as well as co-pathologies.
2.4 Hypothesis of Study Two: Extreme AD Heterogeneity Can Mimic Other Conditions while Retaining Disease Signatures that are Coherent within the Spectrum of AD Phenotypes

Extreme cases within the AD spectrum can result in syndrome mimicry. This can occur even in the presence of confirmed, causative genetic mutations. Despite the unexpected existence of such sub-syndromes, they nonetheless exhibit disease signatures (in this case, imaging) that fit well within the context of other syndromes within the AD syndrome framework, and other non-AD diseases.

2.5 Hypothesis of Study Three: Even Considering Mainly Prototypic AD Diagnosis is Challenging, and Potentially Complicated by Heterogeneity

At least four major consensus diagnostic systems exist for AD. Although similar, they are not equivalent in conceptualization or operationalization. When applied systematically to an AD clinical cohort, these differences give rise to differences in diagnostic classification. In some cases, the divergence is expected to be substantial. Given the significant divergence that some AD sub-syndromes can exhibit when compared to the core phenotype upon which most of these classification systems are based, AD heterogeneity may contribute to some of the observed discrepancies between these consensus frameworks.
3. Chapter One: The Syndrome Spectrum of Alzheimer’s disease

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3.1. Abstract

With increasing knowledge of clinical in vivo biomarkers and the pathological intricacies of Alzheimer’s disease (AD), nosology is evolving. Harmonized consensus criteria that emphasize prototypic illness continue to develop to achieve diagnostic clarity for treatment decisions and clinical trials. However, it is clear that AD is clinically heterogeneous in presentation and progression, demonstrating variable topographic distributions of atrophy and hypometabolism/hypoperfusion. AD furthermore often keeps company with other conditions which may further nuance clinical expression, such as synucleinopathy exacerbating executive and visuospatial dysfunction and vascular pathologies (particularly small vessel disease that is increasingly ubiquitous with human aging) accentuating frontal-dysexecutive symptomatology. That some of these atypical clinical patterns recur may imply the existence of distinct AD variants. For example, focal temporal lobe dysfunction is associated with a pure amnestic syndrome, very slow decline, with atrophy and neurofibrillary tangles limited largely to the hippocampus. Left parietal atrophy and/or hypometabolism/hypoperfusion are associated with language symptoms, younger age of onset, and faster rate of decline – a potential “language variant” of AD. Conversely, the same pattern but predominantly affecting the right parietal lobe
is associated with a similar syndrome but with visuospatial symptoms replacing impaired executive function. Finally, the extremely rare frontal variant is associated with executive dysfunction, relatively preserved memory, and may have prominent behavioural symptoms. Genotypic differences may underlie some of these subtypes; for example, absence of ApoE ε4 is often associated with atypicality in younger onset AD. Understanding the mechanisms behind this variability merits further investigation, informed by recent advances in imaging techniques, biomarker assays, and quantitative pathological methods, in conjunction with standardized clinical, functional, neuropsychological and neurobehavioral evaluations. Such an understanding is needed to facilitate “personalized AD medicine”, and eventually allow for clinical trials targeting specific AD subtypes. For although the focus legitimately remains on prototypic illness, continuing efforts to develop disease-modifying therapies should not exclude the rarer AD subtypes and common comorbid presentations, as is currently often the case. Only by treating them as well, can we address the full burden of this devastating dementia syndrome.

3.2. Introduction

Alzheimer’s disease (AD) most commonly presents in later life as an amnestic syndrome, with impairment in other domains including language and executive function emerging as disease progresses\(^1\). Symptoms occur in association with a breakdown in the brain’s acetylcholine network\(^{145,146}\) and pathological degeneration, the hallmarks of which are beta-amyloid senile plaques (SP) and neurofibrillary tangles (NFT) containing hyperphosphorylated tau\(^{53}\). In addition to this disease prototype, there are AD subtypes. Variations in cognitive profile, age of onset, and rate of decline, exemplify the heterogeneity of AD\(^{16}\).
Similar patterns of variability within neuropsychiatric features may define behavioural AD subsyndromes with consistent symptom-lesion correlates and association with specific neurotransmitter dysfunction, such as dopamine. Familial AD syndromes may also exist, resulting from highly penetrant but variably expressed mutations in key genes, such as presenilin-1, presenilin-2, and amyloid precursor protein. As with sporadic AD syndromes, there is significant heterogeneity among the different mutations. These behavioural and familial-genetic subsyndromes represent a potentially rich area of inquiry, which is beyond the scope of the current discussion. Instead this review will focus on sporadic cognitive subsyndromes.

The growing recognition in recent years of AD variability reflects increasing understanding of its complexity, building upon convergent streams of evidence from clinical, imaging, and pathological studies. While it is legitimate to focus on prototypic illness, as this represents the majority of cases and thus bulk of disease burden, understanding the rarer AD subtypes and the influence of common comorbidities may open new targets for therapy and better allow the tailoring of existing ones – personalized medicine. Concurrently, knowledge of AD’s syndromic inhomogeneity may disentangle its confounding effects in clinical trials, as efforts to exclude atypical subtypes are not always successful.

3.2.1. Origins of the AD Heterogeneity Concept: A Historical Perspective

The observation that AD demonstrates phenotypic heterogeneity is not new. In 1969, McDonald identified two distinct subgroups among dementia patients in a chronic geriatric hospital setting. Using simple tests of memory, parietal function, and aphasia, he noted that...
some had difficulties predominantly with praxis, visual construction, and cortical sensation. They exhibited more severe progression on follow-up. These he termed the “parietal group”. Other patients had predominantly memory dysfunction, later age of onset, and slower disease progression. These he termed “benign memory dysfunction of aging”\textsuperscript{149}. Unfortunately, this early demonstration of AD heterogeneity failed to generate widespread recognition for subtype variability. In large part this was due to the prevailing theory of the time, which held that clinical variation arose from observing the disease at different stages of progression (phase hypothesis), rather than truly distinct disease phenotypes (subtype hypothesis)\textsuperscript{24}.

Additional support for the subtype hypothesis would later be provided by early positron emission tomography (PET) studies. These demonstrated that, just as there were clinically distinct profiles of AD, there were distinct topographic patterns of brain hypometabolism. Asymmetry in PET imaging among AD subjects\textsuperscript{89,150} was consistently associated with greater language impairment if the left hemisphere was more affected. Conversely, visuospatial impairment predominated in those with mainly right parietal hypometabolism\textsuperscript{151,152}. This variability exhibited good anatomic correlation. Left angular hypometabolism, for example, was associated with Gerstmann’s syndrome in AD\textsuperscript{152}. Importantly, longitudinal follow-up demonstrated that these different syndromes remained distinct over time\textsuperscript{153}, and with clinical worsening of disease\textsuperscript{154}.

The one remaining observation that still supported the phase hypothesis was that these variant cases were associated with an earlier age of onset\textsuperscript{155,156}. This was later addressed in studies taking advantage of standardized neuropsychological assessments. Using the CERAD
database, Fisher and colleagues, demonstrated neuropsychologically defined subtypes of AD\textsuperscript{157}. Anomic, apraxic, and mixed patterns mirrored the left, right, and general subgroups of the earlier PET studies. Importantly, Fisher’s subtypes had the same age of onset, strongly suggesting that these observed differences were not merely alternate stages, but true variants of AD. As with PET studies, these subtypes remained distinct over longitudinal follow-up, again supporting the idea of true distinct subtypes of disease\textsuperscript{158}.

### 3.2.2. AD Syndromes

#### 3.2.2.1. Typical AD

Prototypic AD is a late-onset AD (LOAD) syndrome with amnestic impairment predominating in association with hippocampal and temporal-parietal atrophy and/or decreased perfusion/metabolism\textsuperscript{32}. It is the most commonly observed AD phenotype in the clinical setting and serves as a good starting point against which the rare AD subtypes can be compared. Clinically, memory decline is accompanied by similar worsening in other cognitive domains, setting typical AD apart from temporal variant AD where memory decline occurs in isolation, and the other variants wherein non-amnestic presentations predominate. Relatively symmetric and generalized atrophy and hypometabolism/hypoperfusion distinguish typical AD from the more focal topography of temporal variant (hippocampal), frontal variant (frontal), language variant (left parietal), and visuoperceptive variant (right parietal) AD. Typical AD progresses more quickly than temporal variant AD, and slower than language, visuoperceptive, and frontal variant AD.
3.2.2.2. Temporal (Pure Amnestic) Variant AD

Focal temporal lobe dysfunction, pure amnestic AD, and temporal variant AD all refer to the LOAD syndrome of isolated episodic memory impairment with notably slow decline (Figure 3.1)\textsuperscript{159}. SPECT imaging in temporal variant AD demonstrates hypoperfusion limited to the mesiotemporal lobes, while the temporal-parietal changes seen in typical AD are absent\textsuperscript{160}. Longitudinal studies of temporal variant AD individuals demonstrate slow or no change in MMSE scores, and even when memory is significantly impaired, visuospatial and executive function remain borderline to normal\textsuperscript{159,161}. Pathologically, studies have demonstrated a subgroup of patients with plaques and neurofibrillary tangles limited to the limbic regions with little or no spread to the neocortical areas\textsuperscript{162}. Clinically these individuals have a later age of onset and slower rates of cognitive decline. Although the genetic factors contributing to temporal variant AD remains unknown, there is some evidence to suggest that the APOE ε4 allele is absent\textsuperscript{159}. Unique among atypical AD variants, temporal variant AD is a LOAD syndrome, and may present even later than typical AD.

3.2.2.3. Left (Language) Variant and Logopenic Progressive Aphasia

Language variant AD is often an early-onset AD (EOAD) syndrome of gradually worsening non-fluent speech typified by significant agrammatism, phonemic paraphasias, relative preservation of memory, and often atrophy of the left perisylvian region on imaging (Figure 3.2)\textsuperscript{162–164}. These individuals have pathologically confirmed AD with a topographically atypical distribution of neurofibrillary tangles predominantly within the left neocortex, sparing in some cases the hippocampus. The early non-fluent language impairment of this subtype distinguishes it from
the aphasic syndrome of typical AD, which is generally semantic in nature, with surface dyslexia occurring as a feature of later stage disease\textsuperscript{165,166}.

This non-fluency also distinguishes language variant AD from the second AD language syndrome, Logopenic Progressive Aphasia (LPA). In LPA, speech rate is slowed but grammar and articulation are preserved. Rather, impaired repetition typifies LPA\textsuperscript{167}. LPA is commonly associated with AD pathology\textsuperscript{59}, demonstrates left posterior temporal and inferior parietal hypoperfusion, and strong association with $\beta$-amyloid deposition on PiB-PET\textsuperscript{168}.

Despite their shared features, both being language predominant syndromes, the basis for these two forms of different aphasic manifestations is unclear.

**3.2.2.4. Right (Visuoperceptive) Variant (Visuoperceptive AD)**

Visuospatial dysfunction does not commonly occur as the initial or predominant symptom in AD. When it does, it may portend the onset of PCA (Figure 3.3)\textsuperscript{169}. In other cases, visuospatial dysfunction suggests a subtler, non-memory variant, associated with greater right vs. left hemisphere pathology and atrophy (Figure 3.4); a distinct pattern that is maintained over time and with disease progression\textsuperscript{151,152,154,158}.

As with language variant AD and LPA, the relationship between visuoperceptive AD and PCA remains relatively unknown. Also unknown is whether individuals with visuoperceptive impairment represent a prodromal stage of DLB, rather than a true AD subtype, as sometimes happens anecdotally. Pathologic and biomarker imaging studies may help resolve some of these questions.
### 3.2.2.5. Frontal (executive) variant (frontal variant AD)

Frontal variant AD is an extremely rare EOAD subtype, associated with significant frontal cognitive and behavioural symptoms (Figure 3.5), first described by Johnson and colleagues who found 3 cases among 63 individuals with pathologically confirmed AD\textsuperscript{170}. Alladi and colleagues likewise identified only 2 instances among their 100 case series, of whom only one had a true dysexecutive syndrome, the other having only behavioural features\textsuperscript{168}. Pathologically there is a predominance of NFT in the frontal regions (10-fold increase over typical AD), with comparable loading in the entorhinal cortex and other regions\textsuperscript{170}. Amyloid plaques and the lack of the cell loss, microvacuolarization, and gliosis in layers II and III distinguish frontal variant AD from FTD. Biochemically, frontal variant AD shows focally reduced calcium-independent phospholipase A2 activity within the frontal regions as compared to typical AD on post-mortem protein assay (8.8 pmol/mg versus 15.2 pmol/mg), a neuronal-specific isoform of phospholipase and indirect marker of neuronal health\textsuperscript{171}. In one case report, CSF amyloid beta was found to be decreased, although tau levels were only borderline\textsuperscript{172}.

In part because of its rarity, the clinical identification of frontal variant AD has proven troublesome. One study used the Frontal Behavioural Inventory (FBI) to define a “high frontality” AD group based on the presence of frontal symptoms such as apathy, aspontaneity, loss of empathy (negative symptoms) and disinhibition, utilization behaviour, alien limb phenomenon (positive symptoms)\textsuperscript{173}. However, except for three items on the FBI – hyperorality, perseveration, and aspontaneity – there was no distinction between Woodward’s “high frontality” group and FTD and there were no between-group differences on global cognition screening tests. Furthermore, Woodward and colleagues in another study found that
frontal variant AD can be associated with co-occurring FTD pathology\textsuperscript{174}. In light of this overlapping pathology and diagnostic uncertainty, CSF biomarker profiles and amyloid imaging will likely be needed to convincingly demonstrate frontal variant AD in a cohort large enough to perform the necessary neuropsychological, imaging, and genetic studies to define this subtype.

3.2.3. Common Features and Potential Factors Contributing to AD Heterogeneity

3.2.3.1. Age of Onset

EOAD accounts for 32\% of atypical, i.e. non-amnestic, cases of AD, in contrast to only 6\% of typical AD\textsuperscript{23}. In retrospect, this is consistent with earlier studies, wherein atypical subgroups of AD were consistently associated with younger age. As alluded to previously, these early-onset individuals evince a more aggressive disease course, in distinction from the more gradual progression of typical AD, and in contrast to the very slow decline of temporal variant AD. The mechanism and significance of this association remain unclear.

3.2.3.2. Disease Topography

The distribution of AD pathology and associated atrophy varies among individuals\textsuperscript{175} and may affect phenotype. For example, histopathological asymmetry with associated left (language) and right (visuospatial) syndromes, similar to those found in PET studies have been described\textsuperscript{176}. Conversely, focal cortical, non-amnestic presentations can be associated with underlying AD in 34\% of cases, including bilateral posterior cortical atrophy (PCA), aphasia, behavioural executive syndromes, and cortical basal syndrome (CBS), all of which tended to be EOAD\textsuperscript{168}.  

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In one recent study, Armstrong and colleagues used principal components analysis to identify three clusters of NFT and SP distribution: cortical (cingulate gyrus, gyrus rectus, orbital frontal gyrus, occipital lobe), deep grey (thalamus, nucleus basalis of Meynert, striatum), and limbic (ventral tegmentum, raphe, amygdala). Similarly, Murray and colleagues described three pathologically distinct patterns of AD in a large cohort of 889 cases: hippocampal-sparing, limbic-predominant, and “typical”. These subdivisions were based on NFT and SP burden, as defined by silver-stain counts, comparing between mesiotemporal lobe (CA1, subiculum) and cortical (middle frontal, inferior parietal, superior temporal association areas) regions of interest. Hippocampal-sparing AD occurred in younger individuals (mean age 72 years, versus 79 for typical and 86 for limbic-predominant AD), consistent with the association between atypicality and EOAD. Hippocampal-sparing AD revealed the fastest rate of cognitive decline (-4.8 on MMSE per year, versus -2.8 for typical and -1.4 for limbic-predominant AD). In keeping with their neocortically-predominant pathological burden, hippocampal-sparing cases were more likely to have an atypical, non-amnestic clinical onset (30% of cases), versus individuals with typical pathologic distribution where this occurred less frequently (17%).

3.2.3.3. Genetics

ApoE genotype may be one important factor contributing to heterogeneity in sporadic AD, as non-ε4 status among EOAD patients correlates with atypically. What underlies this relationship is unknown; perhaps the absence of ε4, rather than the presence of ε2 or ε3, is important. ApoE ε4 is associated with greater hippocampal atrophy, suggesting that symptoms in non-carriers may instead reflect damage to areas normally eclipsed by hippocampal pathology (e.g. parietal, temporal or frontal regions). This is in keeping with the
“cortically predominant” topographies of disease observed among non-ε4 EOAD carriers. This greater degree of cortical damage could be reasonably expected to result in significant, widespread neurologic dysfunction, potentially explaining the observation that such individuals experience a more rapid clinical decline. Alternately, this same “hippocampal effect” of ApoE ε4 may mask the influence of other genetic and epigenetic factors. If so, such factors may have a greater role in the absence of ε4; their variability in turn explaining the greater heterogeneity of non-ε4 EOAD.

Another consideration is the role of autosomal dominant mutations causing familial AD. Although a complete discussion of genotype-phenotype correlations in familial AD is beyond the scope of this review, mutations in presenilin-1 (PSEN1) and amyloid precursor protein (APP) can both produce non-amnestic, atypical early onset AD. For example, PSEN1 can result in non-fluent aphasia in addition to more typical amnestic AD, while APP can be associated with severe cerebral amyloid angiopathy presenting with hemorrhage and seizures along with memory decline. The same mutation may even result in different syndromes. PSEN1 has been described in spastic paraparesis, frontotemporal dementia, myoclonus with seizures, and predominantly psychiatric presentations. Even within the same family, the APP mutation presented with bradykinesia and hallucinations in one individual, memory and behavioural changes in another, and memory decline followed by intracerebral hemorrhage from angiopathy in a third.

Ultimately, imaging-genetic endophenotype studies may provide a link between genetics and disease topography by elucidating those areas of the brain most associated with
known and potential pathologic genotypes. Should these patterns correlate with the topology of syndromic phenotypes of AD, it may lend support that genotype underlies at least some of these phenotypic variations. Until such links can be established, and given the variability with which genetic mutations/polymorphisms can present, decisions around genetic testing when a suspected case of atypical AD is encountered must be adjudicated on a case by case basis.

3.2.3.4. Co-Occurring Pathology: Lewy body Pathology

Lewy bodies comprised in part of alpha-synuclein aggregates are the hallmark of Lewy body disease, presenting in prototypic cases with visual hallucinations, extrapyramidal symptoms, and marked clinical fluctuations\(^\text{184}\). These same alpha synuclein inclusions can be found in pathologically confirmed AD, with neocortical Lewy bodies occurring in 10-30% of cases at autopsy\(^\text{185,186}\). These co-occurring Lewy bodies also appear associated with AD proven cases of PCA\(^\text{163,187}\). More generally, the presence of Lewy bodies in AD is associated with more perceptual impairment, though milder than what occurs in “pure” DLB\(^\text{188}\).

3.2.3.5. Co-Occurring Pathology: White Matter Hyperintensities

Best seen on T2 and FLAIR imaging by MRI, white matter hyperintensities (WMH) reflect an array of pathophysiological processes, the precise causes of which remain under active investigation\(^\text{99}\). They occur in 95% of healthy adults over the age of 65 (Figure 3.6)\(^\text{189}\), 92% of individuals with mild cognitive impairment age 45 to 87\(^\text{190}\), and is more common in the presence of pathologically confirmed AD (57% vs. 33% in aged matched normal controls)\(^\text{191}\). WMH appear associated mostly with impaired executive function, speed of processing, and mental flexibility\(^\text{192–198}\), functions attributable to frontal lobe networks. More generally, it is
associated with global decline, dementia, depression, and death\textsuperscript{199–202}. WMH frequently occur in the frontal regions\textsuperscript{190,203–205}, although some evidence suggests that WMH is associated with frontal-executive symptoms regardless of location\textsuperscript{206}. The importance of WMH topography remains an area of active investigation, with periventricular WMH possibly related to venous collagenosis\textsuperscript{207,208}.

As the degree of WMH varies widely, investigators have explored whether a dose-effect relationship for WMH exists, and if so what minimum degree of WMH is necessary for cognitive changes to be observed. To this end, Boone and colleagues identified a “threshold” of >10 cm\textsuperscript{3} for cognitive changes to be observed\textsuperscript{209}. DeCarli and colleagues demonstrated similar findings using PET, showing that a threshold of >0.5% of brain parenchymal fraction was significantly associated with poorer performance on executive function and mental flexibility tasks (phonemic fluency, Trails B). They also demonstrated an association with worsened visual memory, suggesting that the influence of WMH is potentially more widespread\textsuperscript{210}. Importantly, even when only accounting for those cases where WMH met or exceeded the minimum “threshold” for associated clinical symptoms, co-pathology was common (6-20%)\textsuperscript{189,209,210}, underscoring its importance in overall AD phenotype.

A major challenge to investigating independent contributions of WMH in AD is disentangling its contributory role to cerebral atrophy. Controlling for atrophy has been can eliminate the observed influence of WMH on cognition\textsuperscript{210,211}, although Swartz and colleagues, using factor analysis in a dementia population, discerned an independent association between WMH burden on executive function and memory even after atrophy was accounted for\textsuperscript{212}. 
Furthermore, vasculopathy, especially lacunar infarcts appears to contribute to the expression of AD dementia in pathology series\textsuperscript{213,214}.

3.2.3.6. Co-Occurring Pathology: Other Pathologies

TDP-43 inclusions and agyrophillic grains are two other pathologies that have been associated with AD pathology and which may nuance the phenotype of AD\textsuperscript{215}. A full review of their association is beyond the scope of this review, although given their association with frontotemporal dementia (FTD), one might expect that they would result in a blending of FTD symptomatology with prototypic AD.

3.3. Future Directions

3.3.1. Framing Heterogeneity and Complexity in the Alzheimer’s Disease Syndrome

AD phenotype is most strongly affected by age of onset, genetic profile, and comorbidities. AD subtypes reflect the interactions between these three axes. When the onset is later, ApoE ε4 is present, and comorbidities absent, the resulting pattern is generally prototypic AD. Changes along any of these three axes shift the observed phenotype towards one of the rarer AD variants (Figure 3.7).

Several important questions remain. Frontal variant AD appears to be the rarest variant and the potential role of WMH in its expression needs to be further explored, in particular the role of venous collagenosis, which may contribute to executive control network dysfunction. The relationship between visuoperceptive AD and PCA, and between language variant AD and LPA remain unclear. ApoE ε4 is hypothesized to be absent in the early onset subtypes: frontal, visuoperceptive, and language variant AD, but its relative absence in temporal variant
AD, the only late onset subtype, is also worthy of further investigation. Finally, the role of rarer co-pathologies such as TDP43, and genetic polymorphisms, other than ApoE, and epigenetic factors on clinical profile remain to be explored.

### 3.3.2. Translational Implications: Diagnosis and Therapy

Phenotypic heterogeneity among AD subtypes and co-pathology may have particular importance for biomarker-based ante mortem diagnosis. Factors such as ApoE status, gender, age, education, and brain size appear associated with differences in CSF A\(\beta_{42}\) levels\(^{216}\), with education inversely correlated with CSF A\(\beta_{42}\) in early disease\(^{217}\). Such findings suggest that at least in the case of CSF A\(\beta_{42}\), biomarkers must be interpreted in the context of phenotype. This interpretation in turn requires a greater understanding of AD phenotypic variation than is presently available. As atypical subtypes are relatively rare, multi-site studies specifically addressing them are urgently needed to delineate the full spectrum of AD and its interaction with biomarkers.

Beyond the clinical need for diagnostic certainty, the ability to recognize and control for phenotypic variation is important to continuing clinical trials design. The very slow rate of decline in temporal variant AD is but one example of how subtype heterogeneity can confound results. More generally, as it appears that all AD subtypes exhibit divergent trajectories of symptom progression from prototypic disease, characterizing the onset and decline across the AD syndrome spectrum\(^{70,72}\), including comorbid cases, is a priority. Similarly, it is unknown how often an initially typical AD case evolves into an atypical subtype over time, or vice versa. As the occasional inclusion of an atypical individual in clinical trials may thus be unavoidable,
understanding these subtypes may allow researchers to disentangle their influence from their findings.

Phenotypic variation may likewise affect response to therapeutic strategies, requiring a personalized medicine approach that will only be possible with a greater understanding of AD subtypes and their causes. For example, the presence of comorbid WMH involving peri-insular cholinergic pathways in a case-controlled cohort study was associated with more favourable response to cholinesterase therapy\textsuperscript{106}, in contrast to more generally distributed WMH, which show no such association. Cholinergic therapies may therefore be particularly appropriate in such co-morbid cases. Another example is the observation that synapse loss, a key correlate of cognition, is greater in early onset AD\textsuperscript{218,219}, implying a more aggressive neuronal degeneration. Clinical trials in these subgroups would therefore have to target symptoms much earlier than would normally be done for prototypic disease, in order to address the more severe cellular damage of EOAD. Along similar lines, it has been observed that early onset AD is associated with more widespread neurotransmitter dysfunction\textsuperscript{220}, affecting noradrenaline, \(\gamma\)-amino-butyric acid, and somatostatin levels in addition to acetylcholine. Hence, combination therapy that restores multiple neurotransmitters may be more symptomatically effective than cholinergic agents alone in EOAD. While the medications for such a clinical trial are already available, the lack of clear diagnostic criteria for EOAD subtypes hampers implementation, further underscoring the urgent need for further research in AD heterogeneity.
3.4. Conclusions

Prototypic and atypical AD subtypes exist along continuums of age, genotype, and co-pathology within the Alzheimer’s Dementia Syndrome, presenting challenges and opportunities for both researchers and clinicians. While the pursuit of treatments and salient criteria for the more common AD prototype understandably remains a priority, these rarer subtypes pose a substantial burden of disease faced by those affected by them and their caregivers. Furthermore, unless atypical variants are understood and recognized, controlling for their potentially confounding effects in clinical trials will be more difficult, hindering treatment development even for prototypic disease.
Figure 3.1. Temporal variant AD. 69 y.o. woman presenting with short-term memory loss and very slow progression with conversion to mild dementia about 5 years later. Her last neuropsychological testing after 11 years of follow up revealed normal language and visuospatial function, with very mild executive dysfunction and significant impairment on memory items. 10 years into her course, imaging including coronal T1 MRI at the level of the hippocampus (bottom left) demonstrated marked hippocampal atrophy (white arrows) while SPECT done at the same time (bottom right) showed mesiotemporal hypoperfusion (red arrows). Axial T1 MRI (top left) and SPECT (top right) at the level of the basal ganglia demonstrated no atrophy in the parietal and frontal association areas and no hypoperfusion in the same areas.
Figure 3.2. Left/Language Variant AD. 50 y.o. man presenting with significant aphasia and mild short-term memory loss, which progressed rapidly over the next few years. Axial T1 MRI at the level of the basal ganglia (left) demonstrated subtly greater left parietal atrophy and expansion of the lateral ventricle (white arrows). SPECT done at the same time (right) demonstrated left parietal hypoperfusion (red arrow). The patient died 8 years after symptom onset, and autopsy confirmed AD (Braak V/VI).
Figure 3.3. Posterior Cortical Atrophy (PCA). 67 y.o. man presenting with mild memory loss and striking visuospatial difficulties, later developing parkinsonism. Imaging 6 years into the disease course, including axial T1 MRI at the level of the midbrain (top left) and basal ganglia (bottom left) demonstrated temporal-occipital atrophy, posterior atrophy and ventricular expansion (white arrows), slightly greater on the right. Axial SPECT imaging done at the same time (right) demonstrated bilateral posterior hypoperfusion (red arrows), greater on the right. The patient died 7 years in the course of disease, and autopsy revealed AD (Braak IV/VI) with co-occurring Lewy bodies (Braak VI/VI).
Figure 3.4. Right/Visuoperceptive Variant AD. 64 y.o. man presenting with short term memory loss and difficulty with visually-guided tasks (reading clocks and music). Imaging done 3 years into the course of illness, including axial T1 MRI at the level of the midbrain (top left) demonstrated subtle right, greater than left, temporal and parietal atrophy with relative expansion of right lateral ventricle and increased right parietal sulcal markings (white arrows), less evident at the level of the basal ganglia (bottom left). At the same time, axial SPECT (right) demonstrated right hypoperfusion, particularly in the parietal region (red arrows). The patient died 5 years into the course of disease, and autopsy confirmed AD (Braak V/VI).
Figure 3.5. Frontal Variant AD. 57 y.o. woman presenting with mild short term memory loss and inability to perform simple daily and occupational tasks. Prominent frontal symptoms were noted 2 years into the course of her illness, with prominent apathy, loss of empathy, and socially inappropriate behaviours. Axial T1 MRI at the level of the basal ganglia (left), done 1 year after symptom onset, demonstrated mild frontal atrophy with increased sulcal markings (white arrows). Axial SPECT done within a few months at the same level (right) demonstrated frontal hypoperfusion, especially on the left (red arrows). The patient died 5 years after the start of her symptoms, and autopsy revealed AD (Braak V/VI).
Figure 3.6. White Matter Hyperintensities (WMH) in AD. 68 y.o. man presenting with short-term memory decline. Imaging done 7 years into the course of illness, including axial T1 MRI at the level of the centrum semiovale (upper left) demonstrated severe generalized atrophy. Axial T2 (top right) and proton density (bottom left) images at the same level showed marked WMH, especially posteriorly (white arrows). One year prior, axial SPECT at the corresponding level (bottom right) demonstrated mild parietal hypoperfusion bilaterally (red arrows). The patient died 9 years later and autopsy confirmed AD (Braak V/VI), with diffuse atherosclerosis throughout the white matter and basal ganglia, lacunar infarcts, remote microhaemorrhages and cortical microinfarcts. There had been no history of visual hallucinations, but parkinsonism developed very late into the disease course, and he was also found to have diffuse Lewy bodies within the brain stem and cingulum.
Figure 3.7. Proposed framework for AD subtypes. Bubbles represent subpopulations of AD by subtype, located along each of the three axes of heterogeneity: age of onset, ApoE status, and co-pathology. Exact size, positioning and degree of overlap for each subtype remain uncertain areas of active research.
4. Chapter Two: A case of extreme syndromic mimicry – using outliers cases as a probe into Alzheimer’s disease heterogeneity

Published as:


4.1. Abstract

Introduction: Corticobasal syndrome (CBS) resulting from genetic Alzheimer’s disease (AD) has been described only once. Whether familial CBS-AD is a distinct clinical entity with its own imaging signature remains unknown.

Methods: Four individuals with CBS from two families underwent detailed assessment. For two individuals, regional atrophy and hypoperfusion was compared to autopsy-confirmed typical late-onset AD and corticobasal degeneration, as well as genetically proven PSEN1 cases with an amnestic presentation.

Results: One family harbored a novel mutation in PSEN1:p.Phe283Leu. MRI demonstrated severe parietal, perirolandic, and temporal atrophy, with relative sparing of frontal and ipsilateral hippocampal regions. Autopsy confirmed pure AD pathology. The other family harbored a known PSEN1 mutation:p.Gly378Val.

Discussion: This report confirms familial CBS-AD as a distinct clinical entity, with a parietal-perirolandic-temporal atrophy signature. It illustrates the clinical heterogeneity that can occur
Despite a shared genetic cause, and underscores the need for biomarkers such as amyloid imaging during life.

4.1. Introduction

Understanding of potential mechanisms of disease requires correlating accurate syndromic diagnosis with pathology, genetics, and imaging. Advancement in each provides increased precision. We demonstrate this improved linkage in Corticobasal Syndrome (CBS), a progressive, neurodegenerative condition characterized by asymmetric motor symptoms in the setting of apraxia, cortical sensory impairment, and in prototypic cases, alien limb phenomenon.\textsuperscript{221,222} Previously thought to be associated with specific pathological changes termed Corticobasal Degeneration (CBD), confirmed CBS due to CBD (CBS-CBD) affects only a subset of individuals.\textsuperscript{223} Increasingly, it has been recognized that CBS occurs in association with other pathologies including: sporadic Alzheimer’s disease (AD), Dementia with Lewy Bodies (DLB), Creutzfeldt-Jakob disease (CJD), and Progressive Supranuclear Palsy (PSP).\textsuperscript{224,225} Genetic causes of clinical CBS also vary, with causative mutations identified in Microtubule-Associated Protein Tau (MAPT), Progranulin (PGRN), and hexanucleotide repeat expansions in C9orf72.\textsuperscript{229} Although mutations in the Presenilin-1 gene (PSEN1) are responsible for the majority of early-onset familial Alzheimer’s disease (AD) cases, and are associated with a heterogeneous array of presentations, they have only recently been associated with CBS, in a single case carrying a Met233Leu mutation.\textsuperscript{230}

We report on two families that segregate the CBS phenotype,\textsuperscript{222,231} due to two different mutations in PSEN1, with one being novel. These families presented a unique opportunity to
examine using quantitative imaging and autopsy whether CBS-AD is a distinct, reproducible clinical entity.

4.2. Methods

4.2.1. Subjects and Clinical Assessment

Family 1 included two brothers seen in cognitive neurology assessment at Sunnybrook Health Sciences Centre (supplemental Figure s-4.1). Family 2 included a brother and a sister seen at the Sam and Ida Ross Memory Clinic at Baycrest (supplemental Figure s-4.2). Work-up for probands was conducted by three experienced neurologists (TC, MM, SEB), and included: clinical interview, physical examination, blood biochemistry, and screening cognitive testing with the Behavioural Neurology Assessment (BNA)\textsuperscript{232}. Brothers from Family 1 underwent quantitative MRI and semi-quantitative 99mTc ethyl cysteinate dimer single-photon emission computed tomography (SPECT). Siblings from Family 2 underwent clinical MRI and SPECT.

For quantitative imaging comparisons with Family 1, data from three comparator groups were drawn from the Sunnybrook Dementia Study, a prospective longitudinal cohort across major dementia subtypes including AD and CBD (ClinicalTrials.gov Identifier: NCT01800214). The first comparator group consisted of six age-approximated, clinically typical AD cases with pathologically confirmed AD, and no white matter hyperintensities (sporadic typical AD group). The second comparator group was a sample of two familial early-onset AD cases, with genetically confirmed \textit{PSEN1} mutations, but typical amnestic presentation (familial EOAD group). The third comparator group was a sample of five CBS cases with pathologically confirmed CBD (CBS-CBD group), previously described by Misch et al\textsuperscript{233}. 
4.2.2. Imaging and Image Processing

Brain MRIs were acquired on a 1.5T General Electric Signa (Milwaukee, WI) system (T1-weighted axial 3D SPGR with an interleaved PD-T2 axial dual-echo spin echo, fluid attenuation inversion recovery, and gradient recalled echo sequences). The Lesion Explorer (LE) and SABRE segmentation and parcellation procedure\textsuperscript{234} was used to provide an initial analysis of lobar and basal ganglia/thalamus volumes. Detailed analysis of volumes in select regions (temporal divisions, perirolandic gyri, precuneus, superior parietal lobule, parahippocampus) was performed using FreeSurfer\textsuperscript{235} (http://surfer.nmr.mgh.harvard.edu/), alongside the automated parcellation system developed by Destrieux et al\textsuperscript{236}, with pre-processing enhancement using LE and SABRE as previously described\textsuperscript{233}. These regions were chosen for their previously described association with CBS in general, CBS-AD, and CBS-CBD\textsuperscript{223,237,238}. Hippocampal volumes were obtained using the fully automated segmentation technique developed by Nestor et al\textsuperscript{239}.

Brain SPECT scans were acquired with a triple-head gamma camera (Prism 3000×P; Phillips Medical Systems Inc., Cleveland, OH, USA) following administration of 20 mCi (740 MBq) 99mTc ethyl cysteinate dimer. Image reconstruction involved a ramp-filtered back projection algorithm followed by a three-dimensional restoration post-filter (Wiener filter, multiplier 1.0) as previously described\textsuperscript{240}. Regional cerebral blood flow maps (rCBM) were generated using a standardized template with each region normalized within-subject to cerebellar perfusion, providing mean normalized perfusion values\textsuperscript{241}.

Comparing topographically analogous regions was done using z-scores. A cut-off of less than -1.5 or greater than +1.5 was considered a relevant difference. Given the asymmetric nature of CBS, left/right regions for probands and the CBS-CBD group were flipped as
appropriate, such that compared ROIs were matched for the side contralateral to the affected hemibody.

### 4.2.3. Pathology

Initial neuropathological assessment of Family 1 was performed by two experienced neuropathologists (JK and JB). Paraffin-embedded sections were stained with Luxol fast blue-haematoxylin and eosin, tau (AT8), beta-amyloid, ubiquitin, alpha-synuclein, p62, TDP-43, and Gallyas stains (supplemental Table s-4.1). Additional assessment was done by JK, with sections taken from: (1) superior and middle frontal gyrus, (2) superior and middle temporal gyrus, and (3) hippocampi, for staging.

### 4.2.4. Genetics

Initial mutation analysis was performed for case III.2 Family 1, with the initial focus on causative mutations for frontotemporal dementia, given previously reported associations between such mutations and familial CBS. Screening was done for repeat expansion of C9orf72, as reported previously. However, before MAPT and PGRN screening were completed, the patient passed away and decision was made to defer testing until definitive pathological diagnosis was made. Subsequent autopsy demonstrated the unexpected finding of AD pathology, and as such genetic screening for frontotemporal dementia was abandoned and causative mutations for familial AD were screened for instead, with sequencing of the entire open reading frame of PSEN1, PSEN2, and APP including exon/intron boundaries as previously described. Commercial testing for subjects III.1 Family1, III.4 Family 2, and III.2 Family 2 (Athena Diagnostics, Worcester, Massachusetts; www.athenadiagnostics.ca) was concurrently
directed at AD causative mutations given the autopsy findings of III.2 Family 1. This assay used automated sequencing with PCR amplification of coding regions and 10 bases of intronic DNA surrounding each exon in \textit{PSEN1}, \textit{PSEN2}, and \textit{APP}. Highly conserved flanking intronic sequences of splice junctions were also sequenced. Sequence variants were confirmed by bi-directional Sanger sequencing.

\subsection*{4.2.5. Standard Protocol Approvals, Registrations, and Patient Consents}

In all cases, spouses provided collateral history and informed consent for genetic testing. The Sunnybrook Dementia Study was approved by the local Research Ethics Board.

\subsection*{4.3. Results}

\subsubsection*{4.3.1. Cases and Families}

Key clinical features for cases and comparator groups are detailed in Table 4.1, and Boeve\textsuperscript{231} and Armstrong\textsuperscript{222} diagnostic criteria findings are summarized in the online material (supplemental Table s-4.2), with additional familial characteristics, pedigrees, and detailed case descriptions (supplemental sections S.4.1.).

Initial clinical diagnosis was made according to the Boeve et al criterion, given that the current Armstrong criterion was non-existent at time of clinical assessment. Pathological analysis of III.1 Family 1 and III.2 Family 1 confirmed the presence of AD pathology and an absence of CBD pathology. As such these cases did not represent CBS-CBD with AD co-pathology, which can occur in at least 14\% of CBS cases, but were pure CBS-AD\textsuperscript{222}. 
4.3.2. Quantitative Imaging Analysis

General volumetric measures expressed as a percentage of total intracranial capacity (TIC) for III.1 Family 1 and III.2 Family 1, as well as the sporadic typical AD and CBS-CBD comparator groups are detailed in supplemental Table s-3. Gross regional volume comparisons are summarized in Table s-4. Relevant differences, defined by Z-scores less than -1.5 or greater than 1.5 are highlighted.

There was asymmetric atrophy in key regions in keeping with observed phenotypes. For III.1 Family 1, who had marked visuospatial impairment, parietal atrophy was worse on the right. By contrast for III.2 Family 1, where semantic difficulties predominated, temporal atrophy was greater on the left. Gross regional comparisons also demonstrated asymmetric sparing of the frontal lobes in familial CBS-AD compared to CBS-CBD. Asymmetry was again demonstrated on detailed parcellation comparisons (Table 4.2), where positive z-scores on the hemisphere ipsilateral to the affected hemibody were seen in temporal and precentral regions when familial CBS-AD cases were compared against AD and CBS-CBD.

Refined volumetric comparisons (Table 4.2) demonstrated severe precuneal and inferior temporal atrophy in our familial CBS-AD cases compared to CBS-CBD, with a similar pattern of relative inferior temporal hypoperfusion (supplemental Table s-4.5) in familial CBS-AD compared to CBS-CBD. SPECT comparisons also showed greater perirolandic hypoperfusion in familial CBS-AD cases compared to AD. Similarly, the familial CBS-AD cases demonstrated more severe perirolandic atrophy, which extended into the parietal regions, compared to typical amnestic familial EOAD (Table 4.2).
Parahippocampal volumes (Table 4.2) were relatively spared in familial CBS-AD cases compared to AD, while being comparable to CBS-CBD and familial EOAD.

Hippocampal volumes (Table 4.3) on the side ipsilateral to the affected limb were likewise spared in familial CBS-AD compared to typical AD. Conversely, III.1 Family 1 demonstrated more severe hippocampal atrophy on the side opposite to the affected limb, when compared to familial EOAD. Hippocampal volumes also demonstrated asymmetry, being larger in familial CBS-AD cases on the side ipsilateral to the affected hemibody.

4.3.3. Neuropathology

4.3.3.1. Case III.1 Family 1

The brain weighed 1280 grams and there was diffuse moderate gyral atrophy without a fronto-temporal or pre-central gyral predilection. Coronal sectioning confirmed the diffuse cortical atrophy, especially pronounced in the left inferior parietal lobule and affecting the mesial temporal structures, and there was a reduction in the volume of white matter with thinning of the corpus callosum (Figure 4.1A). The deep grey structures were normal. On H&E/LFB stained histologic sections the cerebral cortex diffusely showed atrophy with neuronal loss, gliosis and superficial spongiosis, which was symmetrical between the frontal cortices (Figures 4.1B, 4.1C) and temporal cortices bilaterally. No ballooned neurons were seen. Beta-amyloid immunohistochemistry showed amyloid angiopathy and frequent neuritic plaques in the frontal, temporal and parietal cortices bilaterally (Figure 4.1D) and amyloid was present in the brainstem. There were (+) tau AT8 immunopositive neurofibrillary tangles in the temporal and frontal cortices (Figure 4.1E), the left inferior parietal cortex had a heavy burden of tangles.
(Figure 4.1F) and there were (+++) tangles in CA1 of the hippocampi. The putamen was gliotic and contained frequent neuritic amyloid plaques and (i) neurofibrillary tangles and the remainder of the deep grey nuclei appeared normal. These findings were consistent with a diagnosis of Alzheimer’s disease (Aβ3B3C3)55. There were no tau immunopositive glial inclusions suggestive of a FTLD-tau and no TDP43 or alpha-synuclein immunopositive inclusions present.

4.3.3.2. Case III.2 Family 1

The brain weighed 1380 grams, there was mild symmetrical gyral atrophy in a frontotemporal distribution and the substantia nigra was pale. Microscopy confirmed mild cortical atrophy characterized by thinning, superficial spongiosis and neuronal loss symmetrically affecting the frontal (Figures 4.2A, 4.2B), temporal and parietal lobes, both hippocampi were small (Figure 4.2C), and no ballooned neurons were seen. Beta-amyloid immunohistochemistry showed frequent neuritic plaques in the frontal, temporal and parietal cortices bilaterally (Figure 4.2D) and amyloid was present in the brainstem. There was (+++) tau AT8 immunopositive cortical neurofibrillary tangles, and some tangles were present in the primary motor cortex (Figure 4.2E) and substantia nigra (Figure 4.2F). The putamen was gliotic and contained (i) neurofibrillary tangles, though the gliosis was less pronounced than in the previous case, and the remainder of the deep grey structures were normal. These findings were consistent with a diagnosis of Alzheimer’s disease (Aβ3B3C3)55. There were no tau immunopositive glial inclusions suggestive of a FTLD-tau, no TDP43 pathology was present, and rare alpha-synuclein immunopositive Lewy bodies were present in the amygdala.
4.3.4. Genetics

Sequencing of two affected Family 1 members demonstrated a novel nonsynonymous point mutation, the \textit{PSEN1}:NM\_000021:exon8:c.T849G:p.Phe283Leu, affecting a highly conserved codon (online supplemental material \textbf{Figure s-4.6}). This mutation was not found in databases of the Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org/), including sequencing data from >60,000 unrelated individuals, as well as 1000 Genomes and Exome Variant Server. Sequencing of Family 2 members revealed the known early onset AD-related mutation \textit{PSEN1}:NM\_000021:exon11:c.G1133T:p.Gly378Val, previously described\textsuperscript{129}. Both variations are predicted to have a damaging effect on PSEN1 protein function, according to SIFT, Polyphen and MutationTaster. There were no sequence variations in \textit{PSEN2} or \textit{APP} in either family. \textit{C9orf72} screening on III.2 Family 1 confirmed normal genotype with 2/5 repeats.

4.4. Discussion

This report (1) expands on the already remarkable phenotypic variability associated with \textit{PSEN1} mutations\textsuperscript{244}; (2) illustrates the clinical heterogeneity that can occur even when a common genetic cause is present and within the same family; (3) adds further evidence in support of a CBS-AD imaging signature, and (4) underscores the challenge of early diagnosis, especially in young-onset, atypical cases of dementia\textsuperscript{69}. In such cases biomarkers play a key role. Cerebrospinal fluid amyloid beta-42 and tau levels have been shown for instance to identify individuals otherwise presenting with CBS but who on quantitative imaging and formal neuropsychological assessment are more consistent with AD\textsuperscript{245}. Changes in cerebrospinal fluid markers have similarly been associated with asymptomatic autosomal dominant AD mutation carriers\textsuperscript{69}. 
This report confirms the clinical association between CBS and familial AD previously identified in a single case that did not have detailed quantitative structural and functional neuroimaging assessment\textsuperscript{230}, which likely represents a unique subset of CBS-AD cases more commonly caused by sporadic disease.

4.4.1. Clinical features of familial CBS-AD

CBS due to AD pathology has been previously described in association with sporadic AD\textsuperscript{246–249}. Our cases in contrast describe Boeve criteria-defined clinically probable CBS with marked, asymmetric motor and imaging findings\textsuperscript{231} in familial AD with identified \textit{PSEN1} mutations and verified pathologically. III.1 Family 1, III.2 Family 1, and III.4 Family 2 additionally met the newer Armstrong et al. criteria for probable CBS\textsuperscript{222}, with III.2 Family 2 meeting the Armstrong criteria for possible CBS. Cognitive symptoms are common in CBS\textsuperscript{222} and were reported in all four cases. Prominent features included inattention, disorganization, and difficulty with novel task learning, consistent with previous studies\textsuperscript{250–252}. Memory symptoms were reported in three cases. In III.1 Family 1 where formal neuropsychological testing was available, this was associated with improvement with cueing, more consistent with CBS than AD\textsuperscript{250}. However, the presence of memory complaints may have been an early clue to the underlying pathology being AD. Three of the cases share the same right-hemisphere predominant phenotype of the previously described Spanish case\textsuperscript{230}, with visuospatial and prominent behavioural changes. III.2 Family 1 in contrast had a unique left-hemisphere predominant phenotype with language symptoms initially and relatively preserved behaviour.
4.4.2. Regional imaging comparisons between familial CBS-AD cases against typical AD, familial EOAD, and CBS-CBD comparator groups

Cases III.1 Family 1 and III.2 Family 1 demonstrated greater parietal atrophy compared to CBS-CBD, familial EOAD, and to a lesser degree than typical AD groups, with greater involvement of the hemisphere opposite the affected hemibody. Both patterns were in keeping with previous imaging studies of sporadic CBS-AD\textsuperscript{223,237,238,249}. This severe relative parietal volume loss was also in keeping with prior studies of early-onset AD, which demonstrated precuneal involvement even in minimally symptomatic mutation carriers\textsuperscript{253}. That our familial CBS-AD subjects had such severe relative parietal atrophy may thus represent a double hit – the first associated with CBS, and the second associated with familial AD. However, caution must be taken in this interpretation, given the very small number of subjects. Detailed comparisons of parcellated temporal regions showed markedly worse inferior temporal atrophy as compared to CBS-CBD, consistent again with prior suggestions of CBS-AD being a more parietal-temporal form of CBS\textsuperscript{238,249}. This temporal predilection was also seen on mean normalized perfusion comparisons. This relatively worse temporal atrophy, predominantly distributed superiorly, was also seen in the familial CBS-AD subjects when compared to familial EOAD. Perirolandic involvement, a hallmark of CBS regardless of underlying pathology\textsuperscript{223}, was expectedly greater in our cases compared to typical AD and familial EOAD. Interestingly, perirolandic involvement was also greater than CBS-CBD, with the postcentral gyrus being most affected.

By contrast, there was relative frontal sparing compared to CBS-CBD. There was also mesiotemporal sparing compared to typical AD, but not when compared to familial EOAD. This relative hippocampal sparing in familial CBS-AD compared to typical AD, but not EOAD would
be in line with the observation that early-onset AD is more often associated with atypical, extra-
hippocampal presentations versus conventional, later-onset AD. As with the observed parietal
involvement, hippocampal volumes were markedly asymmetric, being smaller contralateral to
the affected hemi-body.

Overall, the distribution of atrophy and regional hypoperfusion is consistent with the
current understanding of imaging signatures for CBS (due to any pathology), CBS-AD, familial
AD, and early-onset AD (familial and sporadic), with our cases presenting a blend of features
from each. While the key regional patterns of CBS (parietal and perirolandic involvement) and
CBS-AD (temporal involvement with frontal sparing) are present, there is a bias of imaging
changes posteriorly (postcentral worse than precentral gyrus; parietal involvement worse than
typical AD, familial EOAD, or CBS-CBD), in keeping with the posterior predominance of AD.
Hippocampi are relatively spared when compared to typical late-onset AD (consistent with
early-onset AD) with asymmetry in parietal and hippocampal atrophy (consistent with CBS).

4.4.3. Phenotypic divergence despite shared genotype

It was intriguing that the brothers from Family 1 exhibited divergent left- and right-
predominant presentations despite having the same pathological mutation. Possible
contributors to this include contextual genetic factors, and environmental effects.

PSEN1 codes for Presenlin-1 (PS-1), a transmembrane protein that first undergoes
endoproteolysis after which it modulates γ-secretase activity on APP and thereby amyloid beta
production resulting in AD. Many of the causative mutations occur within PSEN1’s
transmembrane regions as with Family 2, while a subset involves the N-terminal side of the
endoproteolysis site (residues 292-299) within exon 8, as with Family 1. Over 200 pathogenic,
usually missense mutations have been identified, though no clear correlation has yet been made between mutation location and phenotype. The lack of genotype-phenotype concordance among our cases is therefore consistent with this previously described clinical heterogeneity\textsuperscript{148,182,255}.

Clinical heterogeneity may also be due to epigenetic factors. In one study, there was a trending association between higher degree of promoter methylation for \textit{APP} and \textit{PSEN1} and longer disease duration, though no correlation was observed between methylation status and cognition\textsuperscript{256}. As epigenetic elements can by definition differ despite identical genotype, such differences could have lead one brother to develop a right-predominant presentation, with a contralateral presentation in the other.

Another possible contextual factor is modifier genes. \textit{PSEN1} p. Ser170Phe mutation was associated with severe cerebellar symptoms in all five affected members of an Austrian family\textsuperscript{257}, but only two of four previously published cases. Further, three of these four cases manifested seizures, absent in the Austrian cohort. On further analysis, all affected members from the Austrian family also carried a variant in the Cathepsin D gene (\textit{CTSD}), a known causal factor for Neuronal Ceroid Lipofuscinosis (NCL), the adult form of which is associated with marked cerebellar, brainstem, and extrapyramidal symptoms. This family’s cerebellar-predominant presentation may therefore have resulted from interaction between their \textit{PSEN1} and \textit{CTSD} mutations.

Finally, environmental factors including occupational experience and cognitive reserve have been associated with disease presentation and imaging findings\textsuperscript{258}. Both brothers were
highly accomplished professionals with significant and divergent educations. It could be hypothesized that their different experiences lead to divergent disease lateralization.

Despite these divergent clinical features, our cases nevertheless presented with a similar age of onset (range 43-51 years), in line with previous observations in \textit{PSEN1} mutation carriers, including those carrying the G378V mutation found in Family 2\textsuperscript{129}.

\textbf{4.5. Conclusion}

These cases expand on the already marked clinical variation of pathogenic \textit{PSEN1} mutations, and characterize the recently identified clinical entity of familial CBS-AD. They also underscore the challenge of establishing clear clinicopathologic correlations even when shared causative mutations are present, and the resulting difficulties in dementia diagnosis especially in early-onset presentations. Quantitative imaging comparisons suggest severe involvement of the parietal lobe extending into the inferior temporal region, frontal and hippocampal sparing, and asymmetric atrophy more prominently affecting the hemisphere opposite the affected hemibody. This may eventually serve as an imaging biomarker specific to familial CBS-AD and require verification as additional familial CBS-AD cases emerge. Significant questions remain with regards to the genetic mechanisms leading to this atypical and variable presentation. These will require \textit{in vivo} specific biomarker studies, such as amyloid and tau imaging, in larger case series examining earlier stages of disease.

Three individuals shared a similar right-hemisphere predominant, visuo-behavioural presentation with a previously described Spanish case, while one presented with a left-hemispheric, language syndrome. In two cases from the same family, autopsy confirmed AD pathology. Quantitative imaging comparisons against pathologically confirmed sporadic typical
AD and CBS-CBD comparator groups demonstrated an imaging signature involving severe parietal atrophy, less severe inferior temporal volume loss, relative frontal sparing, and asymmetric atrophy more prominently affecting the hemisphere contralateral to the affected hemibody.

These cases confirm that CBS due to familial AD pathology is not a singular occurrence, but reflects a small and potentially important subset of CBS-AD, with a specific pattern of clinical presentation, imaging findings, and genetics, which we term familial CBS-AD. This extreme example of phenotypic variation, despite the presence of a causative mutation, underscores the pitfalls of clinical diagnosis and the need for confirmatory biomarkers.
Table 4.1: Clinical, neuropsychological, and neuroimaging features of probands and comparators

<table>
<thead>
<tr>
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<th>Family 1 III.1</th>
<th>Family 1 III.2</th>
<th>Family 2 III.4</th>
<th>Family 2 III.2</th>
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<th>CBS-CBD group (n=5, means shown)</th>
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<td>Male</td>
<td>Female</td>
<td>50%</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Years of Education</td>
<td>20</td>
<td>18</td>
<td>17</td>
<td>17</td>
<td>12</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Side of Affected Limb</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Left</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Initial Symptoms</td>
<td>Inattention, dysexecutive syndrome, memory, personality change, low mood</td>
<td>Inattention, dysexecutive syndrome, low mood</td>
<td>Inattention, memory, personality change</td>
<td>Inattention, memory, personality change, low mood</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Presenting MMSE</td>
<td>4/30</td>
<td>16/30</td>
<td>8/30</td>
<td>6/30</td>
<td>19.5/30</td>
<td>22/30</td>
<td>n/a</td>
</tr>
<tr>
<td>Presenting BNA MRI</td>
<td>24/114</td>
<td>34/114</td>
<td>26/114</td>
<td>27/114</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>MRI</td>
<td>Moderate right-generalized atrophy; moderate right-sided WMH</td>
<td>Moderate left temporal and parietal atrophy</td>
<td>Moderate right-greater-than-left atrophy</td>
<td>Generalized atrophy</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Total Brain Volume (%TIC)</td>
<td>80.2</td>
<td>80.7</td>
<td>n/a</td>
<td>n/a</td>
<td>67.2 (SD 3.6)</td>
<td>71.4 (SD 2.3)</td>
<td>80.6 (SD 4.6)</td>
</tr>
<tr>
<td>SPECT</td>
<td>right-sided hypoperfusion</td>
<td>Left temporal and parietal hypoperfusion</td>
<td>Right temporal and parietal hypoperfusion</td>
<td>Right frontal hypoperfusion</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Abbreviations: BNA, Behavioural Neurology Assessment (<84/148 considered below average, and suggestive of clinically significant cognitive decline); WMH, white matter hyperintensities
Figure 4.1: Neuropathology of III.1 Family 1. (A) coronal section through the formalin fixed brain shows moderate gyral atrophy, especially affecting the left inferior parietal cortex, reduction in the calibre of white matter, and normal deep grey structures, H&E/LFB of the left (B) and right (C) frontal cortices showing symmetrical atrophy; (D) beta-amyloid immunohistochemistry showing frequent neuritic plaques in the frontal cortex; (E) (+++) tau AT8 immunopositive neurofibrillary tangles in the frontal cortex; (F) abundant neurofibrillary tangles in the left inferior parietal cortex
Figure 4.2: Neuropathology of III.2 Family 1. H&E/LFB of the left (A) and right (B) frontal cortices showing symmetrical atrophy; (C) atrophy of the left hippocampus; (D) frequent beta-amyloid immunopositive neuritic plaques and amyloid angiopathy in the frontal cortex; (E) AT8 immunopositive tangles in the primary motor cortex; (F) tau AT8 immunopositive neurites and neurofibrillary tangles in the substantia nigra.
Table 4.2: Detailed comparisons of regional volumes in selected ROIs (Destrieux parcellation)

### Versus Sporadic Typical AD comparator group (n=6)

<table>
<thead>
<tr>
<th>Family 1 Case</th>
<th>Temporal</th>
<th>Perirolandic</th>
<th>Parietal</th>
<th>Parahippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Superior</td>
<td>Middle</td>
<td>Inferior</td>
<td>Precentral Gyrus</td>
</tr>
<tr>
<td>Opposite affected limb</td>
<td>III.1 0.3</td>
<td>0.4</td>
<td>-0.4</td>
<td>-0.9</td>
</tr>
<tr>
<td></td>
<td>III.2 0.3</td>
<td>0.5</td>
<td>-1.0</td>
<td>-1.3</td>
</tr>
<tr>
<td>Ipsilateral affected limb</td>
<td>III.1 2.2*</td>
<td>2.4*</td>
<td>0.6</td>
<td>-1.8*</td>
</tr>
<tr>
<td></td>
<td>III.2 1.0</td>
<td>0.6</td>
<td>0.3</td>
<td>0.8</td>
</tr>
</tbody>
</table>

### Versus Familial Early-Onset AD (typical amnestic phenotype) comparator group (n=2)

<table>
<thead>
<tr>
<th>Family 1 Case</th>
<th>Temporal</th>
<th>Perirolandic</th>
<th>Parietal</th>
<th>Parahippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Superior</td>
<td>Middle</td>
<td>Inferior</td>
<td>Precentral Gyrus</td>
</tr>
<tr>
<td>Opposite affected limb</td>
<td>III.1 -14.5*</td>
<td>-7.3*</td>
<td>-1.0</td>
<td>-11.5*</td>
</tr>
<tr>
<td></td>
<td>III.2 -3.0*</td>
<td>-0.5</td>
<td>-1.8*</td>
<td>-4.9*</td>
</tr>
<tr>
<td>Ipsilateral affected limb</td>
<td>III.1 -0.2</td>
<td>1.3</td>
<td>-0.3</td>
<td>-5.6*</td>
</tr>
<tr>
<td></td>
<td>III.2 -7.3*</td>
<td>-4.4*</td>
<td>-0.5</td>
<td>-6.5*</td>
</tr>
</tbody>
</table>

### Versus CBS-CBD comparator group (n=5)

<table>
<thead>
<tr>
<th>Family 1 Case</th>
<th>Temporal</th>
<th>Perirolandic</th>
<th>Parietal</th>
<th>Parahippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Superior</td>
<td>Middle</td>
<td>Inferior</td>
<td>Precentral Gyrus</td>
</tr>
<tr>
<td>Opposite affected limb</td>
<td>III.1 -0.3</td>
<td>-0.1</td>
<td>-4.3*</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>III.2 0.4</td>
<td>-0.6</td>
<td>-4.7*</td>
<td>0.4</td>
</tr>
<tr>
<td>Ipsilateral affected limb</td>
<td>III.1 3.4*</td>
<td>2.5*</td>
<td>0.5</td>
<td>-0.6</td>
</tr>
<tr>
<td></td>
<td>III.2 -0.2</td>
<td>0.4</td>
<td>-0.4</td>
<td>3.0*</td>
</tr>
</tbody>
</table>

*Z-scores with relevant differences, as defined by a cut-off of less than -1.5 or greater than +1.5
### Table 4.3: Hippocampal volumes for Cases 1 and 2, typical AD group, and CBS-CBD group

<table>
<thead>
<tr>
<th></th>
<th>Case III.1 Family 1 (left arm affected)</th>
<th>Case III.2 Family 1 (right arm affected)</th>
<th>Sporadic Typical AD group (n=6) (cm³; means and standard deviations)</th>
<th>Familial Early-Onset AD group (n=2) (cm³; means and standard deviations)</th>
<th>CBS-CBD group (n=5) (cm³; means and standard deviations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volumes</td>
<td>Opposite affected arm 2.38 2.99</td>
<td>Ipsilateral affected arm 2.66 2.85</td>
<td>Right 2.26 (0.5) 2.12 (0.4)</td>
<td>Left 2.7 (0.2) 2.8 (0.2)</td>
<td>Opposite affected arm 2.49 (0.3) 2.60 (0.3)</td>
</tr>
<tr>
<td></td>
<td>Opposite affected arm 2.66 2.85</td>
<td>Ipsilateral affected arm 2.66 2.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z-Score vs. AD group</td>
<td>0.74</td>
<td>1.72*</td>
<td>0.22</td>
<td>2.04*</td>
<td></td>
</tr>
<tr>
<td>Z-Score vs. Familial EOAD group</td>
<td>-2.45*</td>
<td>0.75</td>
<td>0.50</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Z-Score vs. CBS-CBD group</td>
<td>0.61</td>
<td>0.88</td>
<td>-0.37</td>
<td>1.36</td>
<td></td>
</tr>
</tbody>
</table>

*Z-scores with relevant differences, as defined by a cut-off of less than -1.5 or greater than +1.5
S.1. GENERAL FAMILIAL CHARACTERISTICS

Family 1 (Figure s-1) is a Canadian family of British descent. The proband (III.1 Family 1) had one affected brother (III.2 Family 1) and one unaffected brother. His mother presented in her 40’s with dressing apraxia, apathy, and progressive memory loss until her death at age 51. Pick’s disease was suspected, though no autopsy was performed and genetic analysis was not available. His maternal grandmother had a similar presentation in her 40’s, passing away at age 56. No further details were available.

Family 2 (Figure s-2) is a Canadian family of Chinese descent. The proband (III.4 Family 2) had one affected sister (III.2 Family 2), an unaffected brother, and one unaffected sister. His father developed similar symptoms at age 42, passing away at age 52. No genetic or pathological data was available from his case. His father had one unaffected sister and two unaffected brothers. Both paternal grandparents lived into late age and had no dementia symptoms.

S.1.1. Case 1 (III.1 Family 1)

Case 1 was a right-handed business professional with a childhood diagnosis of Attention Deficit Hyperactivity Disorder, treated with methylphenidate. He presented with executive (difficulty organizing work projects, inability to use computer database) and attention (misplacing items, abandoning incomplete tasks) deficits at age 48, and he was forced to retire at age 50. Simultaneously he developed left-hand clumsiness, apathy, and withdrawal. Over time he became repetitive, irritable, and uncharacteristically profane. Neurologic examination at this time showed mild left facial droop, left pronator drift, increased left-sided reflexes, and marked impersistence. Neuropsychological testing demonstrated impaired processing speed, impaired
sustained attention, and visuospatial deficits, but intact language. Verbal and to a lesser degree visual memory was also impaired, although verbal recall improved with cuing. He was diagnosed with an atypical dementia syndrome and donepezil was tried but with no effect. He presented to Sunnybrook at age 56, by which time he had developed difficulties in word finding, speech cadence, increasing disorganization, declining empathy, and gait instability. Examination revealed marked left arm myoclonus, left arm rigidity, left-greater-than-right ideomotor limb apraxia, impaired stereognosis and graphesthesia bilaterally, and orofacial apraxia. MRI showed generalized right-predominant atrophy with moderate right-predominant periventricular white matter hyperintensities. SPECT showed right-predominant hypoperfusion over the frontal, temporal, and parietal regions (Figure s-3). A provisional diagnosis of probable CBS was made. The patient’s deterioration accelerated rapidly and he passed away at age 58.

S.1.2. Case 2 (III.2 Family 1)

Brother 2 was a previously well right-handed health care professional presenting with executive (difficulties using the telephone, operating household appliances, and finances), and attention (misplacing items, leaving tasks unfinished) deficits at age 51, with significant anxiety, withdrawal, and depression. Neuropsychological assessment revealed poor concentration, reduced attention span, and significantly reduced speed of processing. Visuospatial performance was poor, and with low-average verbal comprehension. He was subsequently referred to Sunnybrook at age 55 for suspected atypical neurodegeneration. By this time he had frequent attention lapses, word-finding difficulties, and gait and balance issues. Clinical cognitive testing on the BNA revealed global deficits, worst in attention, and with relative sparing of memory. He exhibited reduced verbal fluency, had difficulty with object naming, and
was unable to reproduce simple figures or draw a clock. His MMSE was 16. Examination revealed markedly asymmetric right-predominant rigidity, right arm myoclonus, right hand tremor, right-sided visual and sensory extinction, difficulty with rightward saccades, errors on right-sided ideomotor praxis testing, and impaired right-sided graphesthesia. MRI showed moderate atrophy, predominantly in the left temporal and parietal regions. SPECT showed global hypoperfusion, more severe in the temporal and parietal regions (supplemental Figure s-4). He was given a diagnosis of CBS. Deterioration occurred rapidly, with MMSE dropping to 3 within one year, accompanied by functional dependence, bladder incontinence, irritability, and emergence of pseudobulbar affect. Right-sided myoclonus became disabling, with suspected complex partial seizures superimposed just prior to his death at age 57.

S.1.1.3. Case 3 (III.4 Family 2)

Case 3 is a previously well right-handed former communications professional. He presented to Baycrest at age 43 with short term memory loss and personality changes. Previously an “affectionate hockey dad,” he became withdrawn, physically aggressive, and apathetic. Self-care declined rapidly, with eating obsessions and bowel incontinence. His MMSE was 8/30. On examination he was distractible, fluctuating between blunted affect and emotional lability. He had perseverative and hypophonic speech, bradyphrenia, and micrographia. He had increased latency in upward vertical gaze, decreased blink frequency, postural instability, and slowed gait. Cogwheel rigidity and multi-segmental myoclonic jerks were noted in the left arm. There was no orofacial or ideomotor apraxia. Cognitively he had poor comprehension, but could name to confrontation. Figure copy demonstrated binding to stimulus, but visuospatial function in general was difficult to assess due to poor comprehension. MRI demonstrated moderate
right-left-sided atrophy (Figure s-5). SPECT showed right temporal and right-left parietal hypoperfusion. After the initial presentation, he developed dysarthria, dysphagia, and drooling. Frequent, progressive left-sided myoclonus emerged, progressing to generalized tonic-clonic seizures that were not controlled despite a combination of valproic acid, gabapentin, and clobazam. He continued to experience seizures on average monthly. Over the course of 8 years, he developed frontal release signs, with speech deteriorating to short sentences, anomia, and marked aprosodia, then mutism.

S.1.1.4. Case 4 (III.2 Family 2)

Case 4 is a previously well right-handed project manager. She presented to Baycrest at age 46 with short term memory loss and progressive confusion, eventually resulting in her losing her job. Family noted increasing distractibility, disorientation, robotic affect, and loss of libido, with increasingly childlike and dysphoric behaviours. She was started on valproic acid and quetiapine for her behavioural symptoms, and zopiclone for deteriorating sleep. On examination her MMSE was 6/30. She had difficulty repeating questions, was non-fluent, with frequent word-finding pauses; there was evident speech apraxia. She was agraphic and alexic. She had left arm apraxia and cogwheel rigidity. Her posture was stooped, with mild postural tremor. MRI showed generalized atrophy. SPECT showed right frontal hypoperfusion. Although not initially aggressive, she started to yell and grab as symptoms worsened over the next 8 years, with bouts of unprovoked laughter and crying. She developed dysphagia, with spasmodic, hoarse, and load vocalizations. Family also reported multi-segmental myoclonus. Self-care rapidly declined.
**Table s-4.1: Immunostaining Details**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Manufacturer</th>
<th>Dilution</th>
<th>Detection System</th>
<th>Instrument</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tau (AT8)</td>
<td>Nitrogen</td>
<td>1/500</td>
<td>Mach 4</td>
<td>Labvision</td>
</tr>
<tr>
<td>Beta-amyloid</td>
<td>Dako</td>
<td>1/100</td>
<td>Mach 4</td>
<td>Labvision</td>
</tr>
<tr>
<td>TDP43</td>
<td>Cederlane</td>
<td>1/500</td>
<td>Mach 4</td>
<td></td>
</tr>
<tr>
<td>p62</td>
<td>BD LAB</td>
<td>1/250</td>
<td>Mach 4</td>
<td>Labvision</td>
</tr>
<tr>
<td>Alpha-synuclein</td>
<td>Vector</td>
<td>1/200</td>
<td>Mach 4</td>
<td>Labvision</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>Dako</td>
<td>1/700</td>
<td>Mach 4</td>
<td>Manual</td>
</tr>
</tbody>
</table>
### Table s-4.2: CBS features required for the Boeve 2003 and Armstrong 2013 Criteria among probands

<table>
<thead>
<tr>
<th></th>
<th>Family 1 Case (brother) 1</th>
<th>Family 1 Case (brother) 2</th>
<th>Family 1 Case (brother) 3</th>
<th>Family 2 Case (sister) 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Boeve 2003 Criteria Features</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insidious onset and progressive course</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>-</td>
</tr>
<tr>
<td><strong>Cortical dysfunction reflected by at least 1 of the following:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal or asymmetrical ideomotor apraxia</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Alien limb</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cortical Sensory Loss</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>-</td>
</tr>
<tr>
<td>Visual or sensory neglect</td>
<td>Present</td>
<td>Present</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Constructional Apraxia</td>
<td>Present</td>
<td>Present</td>
<td>-</td>
<td>Present</td>
</tr>
<tr>
<td>Focal or asymmetric myoclonus</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Apraxia of speech/nonfluent aphasia</td>
<td>-</td>
<td>Present</td>
<td>-</td>
<td>Present</td>
</tr>
<tr>
<td><strong>Extrapyramidal dysfunction as reflected by at least 1 of the following:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal or asymmetric appendicular rigidity lacking prominent and sustained L-dopa response</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Focal or asymmetric appendicular dystonia</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Supportive investigations:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal or asymmetric atrophy</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Focal or asymmetric hypoperfusion</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Focal or lateralized cognitive function</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>-</td>
</tr>
<tr>
<td><strong>Conclusion</strong></td>
<td>Meets criteria</td>
<td>Meets criteria</td>
<td>Meets criteria</td>
<td>Meets criteria except for acute onset confusion</td>
</tr>
</tbody>
</table>

### Armstrong 2013 Criteria Feature

<table>
<thead>
<tr>
<th></th>
<th>Family 1 Case (brother) 1</th>
<th>Family 1 Case (brother) 2</th>
<th>Family 1 Case (brother) 3</th>
<th>Family 2 Case (sister) 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Two of the following limb features:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limb rigidity or akinesia</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Limb dystonia</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Limb myoclonus</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td><strong>Two of the following cortical features:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apraxia (limb or orofacial)</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Cortical Sensory loss</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>-</td>
</tr>
<tr>
<td>Alien limb</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Asymmetric</strong></td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td><strong>Conclusion</strong></td>
<td>Meets criteria for probable CBS</td>
<td>Meets criteria for probable CBS</td>
<td>Meets criteria for probable CBS</td>
<td>Meets criteria for possible CBS</td>
</tr>
</tbody>
</table>

**Figure s-4.1**: Pedigree for Family 1
Figure s-4.2: Pedigree for Family 2
Figure s-4.3: MRI and SPECT of Case 1

Left-hemibody predominant CBS with visuospatial impairment. T1 MRI demonstrates asymmetric atrophy of the right hemisphere (red arrows) with hypoperfusion over the right temporal and parietal areas on SPECT (yellow arrows).

Figure s-4.4: MRI and SPECT of Case 2

Right-hemibody predominant CBS with language impairment. T1 MRI demonstrates asymmetric atrophy of the left temporal lobe (red arrow) with subtle hypoperfusion over the left temporal area and more prominently over the parietal region on SPECT (yellow arrows).
Left-hemibody predominant CBS with behavioural-personality deterioration. Generalized atrophy with marked asymmetry in the frontal, temporal, and parietal lobes. Note the more pronounced expansion of the right temporal horn and lateral ventricle (red arrows).
Table s-4.3: Volumetric data (%TIC) for Cases 1 and 2, typical AD group, and CBS-CBD group

<table>
<thead>
<tr>
<th></th>
<th>Case (Brother) 1 (%TIC)</th>
<th>Case (Brother) 2 (%TIC)</th>
<th>Typical AD group (n=6) (%TIC; means and standard deviations)</th>
<th>CBS-CBD group (n=5) (%TIC; means and standard deviations)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Affected Arm</strong></td>
<td>Left</td>
<td>Right</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>TIC (cm³)</strong></td>
<td>1351</td>
<td>1364</td>
<td>1227</td>
<td>1131</td>
</tr>
<tr>
<td><strong>Total Brain Volumes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Parenchyma</td>
<td>80.2</td>
<td>80.7</td>
<td>67.2 (3.6)</td>
<td>71.4 (2.3)</td>
</tr>
<tr>
<td>Grey Matter</td>
<td>40.7</td>
<td>38.1</td>
<td>37.9 (2.3)</td>
<td>41.2 (2.7)</td>
</tr>
<tr>
<td>White Matter</td>
<td>48.4</td>
<td>30.6</td>
<td>29.3 (2.4)</td>
<td>30.4 (4.9)</td>
</tr>
<tr>
<td><strong>Lesion Explorer (SABRE) Regions (Total Parenchyma)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
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</tr>
<tr>
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<td>7.7</td>
<td>8.0</td>
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<tr>
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<td>4.1</td>
</tr>
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<td>1.5</td>
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<tr>
<td><strong>Lesion Explorer (SABRE) Regions (Grey Matter)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Opposite affected arm</td>
</tr>
<tr>
<td>Lateral frontal</td>
<td>4.4</td>
<td>4.8</td>
<td>4.9</td>
<td>4.3</td>
</tr>
<tr>
<td>Medial frontal</td>
<td>2.8</td>
<td>2.9</td>
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<td>2.6</td>
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<tr>
<td>Temporal</td>
<td>5.6</td>
<td>6.0</td>
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</tr>
<tr>
<td>Parietal</td>
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<td>4.3</td>
<td>3.5</td>
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<tr>
<td>Occipital</td>
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<td>2.1</td>
</tr>
<tr>
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<td>0.7</td>
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<tr>
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<td></td>
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<td>1.8</td>
<td>1.9</td>
<td>1.4</td>
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<td>3.3</td>
<td>3.8</td>
<td>3.6</td>
</tr>
<tr>
<td>Parietal</td>
<td>2.9</td>
<td>3.6</td>
<td>3.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Occipital</td>
<td>1.6</td>
<td>1.7</td>
<td>1.8</td>
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<td><strong>Lesion Explorer (Destrieux) Regions</strong></td>
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<tr>
<td>Parahippocampal gyrus (volume in cc)</td>
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<td>Parahippocampal gyrus (%TIC)</td>
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**Table s-4.4:** Gross regional comparisons of brain volume (MRI)

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<th>Temporal</th>
<th>Parietal</th>
<th>Occipital</th>
<th>Basal Ganglia/Thalamus</th>
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<tbody>
<tr>
<td><strong>Hemisphere contralateral affected arm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brother 1</td>
<td>0.6</td>
<td>1.0</td>
<td>-1.5</td>
<td>1.1</td>
<td>-0.6</td>
</tr>
<tr>
<td>Brother 2</td>
<td>0.4</td>
<td>2.4</td>
<td>-2.6</td>
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<td>-0.3</td>
</tr>
<tr>
<td><strong>Hemisphere ipsilateral affected arm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brother 1</td>
<td>0.4</td>
<td>3.6</td>
<td>-1.7</td>
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<td>0.6</td>
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<th>Parietal</th>
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<th>Basal Ganglia/Thalamus</th>
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<td></td>
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</tr>
<tr>
<td>Brother 1</td>
<td>1.6</td>
<td>0.0</td>
<td>-1.7</td>
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<td>0.0</td>
</tr>
<tr>
<td>Brother 2</td>
<td>2.5</td>
<td>-0.5</td>
<td>-1.4</td>
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<td>-0.5</td>
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<tr>
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</tr>
<tr>
<td>Brother 1</td>
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<td>4.2</td>
<td>0.4</td>
<td>-3.0</td>
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Table s-4.5: Gross regional comparisons of mean normalized perfusion (ECD-SPECT)

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<th>Middle</th>
<th>Inferior</th>
<th>Perirolandic Precentral</th>
<th>Perirolandic Postcentral</th>
<th>Caudate/Putamen</th>
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<tr>
<td><strong>Hemisphere contralateral affected arm</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Brother 1</td>
<td>-0.2</td>
<td>0.8</td>
<td><strong>1.8</strong></td>
<td>-2.4</td>
<td>-2.2</td>
<td><strong>3.5</strong></td>
</tr>
<tr>
<td>Brother 2</td>
<td>0.4</td>
<td>-1.0</td>
<td>-0.3</td>
<td>-1.6</td>
<td>-0.1</td>
<td>1.3</td>
</tr>
<tr>
<td><strong>Hemisphere ipsilateral affected arm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brother 1</td>
<td><strong>3.9</strong></td>
<td><strong>2.8</strong></td>
<td><strong>1.6</strong></td>
<td>-0.5</td>
<td>0.9</td>
<td><strong>3.1</strong></td>
</tr>
<tr>
<td>Brother 2</td>
<td>1.0</td>
<td>1.0</td>
<td>1.1</td>
<td>-1.4</td>
<td>0.3</td>
<td><strong>1.9</strong></td>
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<table>
<thead>
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<th>Inferior</th>
<th>Perirolandic Precentral</th>
<th>Perirolandic Postcentral</th>
<th>Caudate/Putamen</th>
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<td></td>
<td></td>
</tr>
<tr>
<td>Brother 1</td>
<td>-0.9</td>
<td>-1.8</td>
<td>-1.4</td>
<td>-0.9</td>
<td>-0.9</td>
<td><strong>5.7</strong></td>
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<tr>
<td>Brother 2</td>
<td>-0.5</td>
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</tr>
<tr>
<td>Brother 1</td>
<td>0.3</td>
<td>-0.9</td>
<td>-1.0</td>
<td>-0.5</td>
<td>0.3</td>
<td><strong>1.8</strong></td>
</tr>
<tr>
<td>Brother 2</td>
<td><strong>-1.6</strong></td>
<td><strong>-1.5</strong></td>
<td><strong>-2.0</strong></td>
<td><strong>-1.5</strong></td>
<td><strong>-0.8</strong></td>
<td><strong>1.7</strong></td>
</tr>
</tbody>
</table>
Figure s-4.6: Analysis of PSEN1 mutation in Family 1
5. Chapter Three: Comparing consensus diagnostic Alzheimer’s disease criteria

Submitted as:


5.1. Abstract

**Background:** Alzheimer’s disease (AD) diagnosis can be based on different consensus criteria, which vary in core phenotype and biomarker technologies (cerebrospinal fluid and imaging) used. Equivalence among these criteria and the former benchmark NINCDS-ADRDA is unknown.

**Methods:** Clinical and imaging data was retrospectively reviewed for 155 individuals meeting NINCDS-ADRDA criteria for possible (n= 55) or probable (n=100) AD. Four diagnostic classification systems were re-applied systematically to these cases, and diagnoses were compared against this benchmark and each other using percentage agreement and Cohen’s kappa.

**Findings:** Percentage agreement with the NINCDS-ADRDA is excellent for the NIA-AA (94%) and DSM-5 (96%), but poor for the ICD-10 (55%) and IWG-1 (54%). Pairwise, concordance was very high between the NIA-AA and DSM-5 ($\kappa=0.88$, $p<0.005$), and substantial between the NIA-AA and IWG-1($\kappa=0.62$, $p<0.005$). All other pairs demonstrated only moderate agreement. Only 53 subjects (34%) met all five criteria.

**Interpretation:** Current AD criteria are not equivalent, despite areas of overlap. Comparing studies that employ different criteria requires caution.
**Funding:** The study was supported by the Canadian Institutes of Health Research (MOP 13129), as detailed in ClinicalTrials.gov (NCT01800214).

### 5.2. Introduction

Diagnostic accuracy and precise classification of disease are pivotal to the success of patient care and clinical trials. However, accurate diagnosis of Alzheimer’s disease (AD) faces particular challenges, especially during early stages of disease when clinical features are sparse. These include AD’s highly variable clinical presentation\(^\text{259}\), non-AD pathologies that can mimic the AD syndrome\(^\text{224}\), and the high prevalence of co-morbidities such as cerebrovascular disease that can complicate, even obscure, the underlying biology.

How these factors are weighted and incorporated into a given consensus diagnostic framework can potentially have dramatic impact on the end result. This in turn can lead to the identification of markedly different cohorts depending on the system used. Considering the analogous case of Vascular Cognitive Impairment (VCI), different classification systems yield widely divergent estimates of prevalence even when applied to the same cohort\(^\text{260–262}\). Such differences affect interrater reliability\(^\text{263}\), and are further exacerbated by operational choices\(^\text{264}\). For AD, even the gold standard of pathology demonstrates differences among staging systems\(^\text{265}\). With the recent advent of multiple consensus AD criteria, the question arises as to whether, and to what degree, such non-equivalency exists between these different diagnostic systems.
The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria (NINCDS-ADRDA) has been the standard for research for 27 years, and has influenced the general conceptualization of AD in clinical practice\(^1\). However, in recent years AD diagnosis has undergone significant revision in response to advances in biomarker research, and an expanded understanding of AD’s syndromic complexity. This has led to the new National Institute on Aging – Alzheimer's Association (NIA-AA) criteria (2011)\(^{16}\), the International Working Group (IWG) criteria (2007 and 2014)\(^{32,2,73}\), the International Classification of Disease (ICD-10; 2010)\(^{47}\), and the Diagnostic and Statistical Manual of Mental Disorders 5\(^{th}\) Edition (DSM-5; 2013)\(^{17}\). The equivalency of these criteria has yet to be examined in detail.

The objectives of this study are: (1) to assess the comparability of the four newer criteria against the previous NINCDS-ADRDA standard, (2) to determine the degree of discordance between the four newer criteria, and (3) to determine if any classifications are significant outliers that may impact study interpretation or clinical use.

5.3. Methods

5.3.1. Participants

A convenience sample of 155 participants from the Sunnybrook Dementia Study (ClinicalTrials.gov NCT01800214) who had previously been diagnosed with probable or possible Alzheimer’s disease (AD) by the NINCDS-ADRDA criteria was reviewed retrospectively by three experienced neurologists (BL, AK and KH). Data including clinical history, function (Alzheimer’s Disease Functional Assessment of Change Scale)\(^{266}\), cognitive screening (MMSE and Behavioural
Neurology Assessment (BNA\textsuperscript{232}), cognitive testing (Dementia Rating Scale (DRS)\textsuperscript{267}), MRI, and 99mTc ethyl cysteinate dimer single photo emission computed tomography (SPECT), were used in accordance with each consensus criteria.

5.3.2. Standard Protocol Approvals, Registrations, and Patient Consents

Participants or primary caregivers with power of attorney provided informed consent for clinical assessment, neuropsychological testing, imaging, blood work, and data collection. The Sunnybrook Dementia Study was approved by the local Research Ethics Board.

5.3.3. Neuropsychological Testing

Use of cognitive measures for the purpose of diagnostic classification was restricted to the MMSE, BNA, and DRS. The BNA was designed for clinician use to cover all major cognitive domains including attention, memory, visuospatial, language, executive function, and praxis, providing a more comprehensive level of assessment than tests such as the MMSE, but not as extensive as formal neuropsychological testing\textsuperscript{232}. As such it was considered ideal for providing objective verification of cognitive decline in specific domains as required by the different consensus criteria. Longitudinal, objective cognitive assessment was provided by the DRS, as its subscores (attention, initiation and perseveration, construction, conceptualization, and memory) similarly covered major cognitive domains and it has been shown to have good validity\textsuperscript{267}. The DRS thus provided further objective verification of cognitive impairment as well as surveillance for cognitive impairments as they emerged over time, and in turn informed the final diagnostic classification.
5.3.4. Imaging Acquisition and Interpretation

Brain imaging protocols were as follows: For MRI, acquisition was on a 1.5T General Electric Signa (Milwaukee, WI) system (T1-weighted axial 3D SPGR with an interleaved PD-T2 axial dual-echo spin echo, fluid attenuation inversion recovery, and gradient recalled echo sequences). For SPECT, acquisition was on a triple-head gamma camera (Prism 3000×P; Phillips Medical Systems Inc., Cleveland, OH, USA) following administration of 20 mCi (740 MBq) 99mTc ethyl cysteinate dimer. Image reconstruction involved a ramp-filtered back projection algorithm followed by a three-dimensional restoration post-filter (Wiener filter, multiplier 1.0) as previously described.

MRI was reviewed for cerebrovascular disease and other possible confounding diagnoses, with the extent of white matter hyperintensities scored using the modified Fazekas Scale. MRI was qualitatively assessed for regional atrophy, with hippocampal shrinkage estimated using the Schelten’s Scale. SPECT was used in place of FDG-PET when applying the NIA-AA and IWG-1 criteria.

5.3.5. Clinical Review Procedure

Given its prominent role in research and in shaping the conceptualization of AD, the NINCDS-ADRDA criteria were selected as the “silver standard” for the diagnosis of AD. Four other frequently used criteria were compared to this: the National Institute on Aging – Alzheimer’s Association criteria (NIA-AA), the International Working Group criteria (IWG-1), the International Classification of Diseases (ICD-10), and the Diagnostic and Statistical Manual (DSM-5). As cerebrospinal fluid analysis and amyloid-PET imaging were not available, the
revised IWG-2\textsuperscript{73} criteria could not be applied. Instead, the IWG-1 criteria were used, as they allow for atrophy and hypo-metabolism imaging biomarkers in rendering a diagnosis of AD.

Information for each case was reviewed in chronological order to better approximate the availability of data available to the clinical team at each follow-up visit.

5.3.6. Diagnostic Criteria

Comparative features among consensus criteria are detailed in Table 5.1.

The NINCDS-ADRDA criteria require the presence of three elements: cognitive decline, functional impairment, and mood and behaviour changes. This triad of symptoms serves as the core phenotype, and is reflected to varying degrees in all subsequent criteria. The NINCDS-ADRDA criteria are sparse in defining cognitive decline, although memory loss is required. Investigations serve as much to exclude alternate diagnoses as to rule-in AD, with no investigation being either necessary or sufficient. Predating the concept of Mild Cognitive Impairment (MCI), functional decline is supportive but not necessary in establishing a diagnosis.

As the direct successor to the NINCDS-ADRD criteria, the NIA-AA criteria formalize the requirement for functional decline (distinguishing AD from MCI), and integrate biomarkers for research diagnosis. They represent a compromise along the continuum of biomarker-driven versus clinically-driven diagnostic systems. They also expand the allowable AD phenotypes to include non-memory predominant subtypes of disease including language, frontal-executive, attentional, and visuospatial variants.

By contrast, the IWG-1 criteria focus on memory exclusively, remove the requirement for functional decline, and do not require the presence of mood and behavioural changes. Co-
pathologies are strictly excluded. Biomarkers are required, and in the revised IWG-2 criteria\textsuperscript{73}, the definition of an appropriate biomarker is further narrowed, in that the biomarkers must demonstrate AD biology. Regional atrophy and hypometabolism are downgraded to reflecting downstream damage only, and cannot serve to establish underlying AD etiology. The intention is to identify a cohort of prototypic AD, distilled to its purest form, so both iterations of the IWG criteria could thus be considered the biomarker-driven extreme of diagnostic criteria.

At the other extreme is the ICD-10 criteria, where the only investigation required is some structural brain imaging confirming brain atrophy. Emphasis is instead placed on clinical features, namely the presence of cognitive, functional, and behavioural decline. Unique to the ICD-10 criteria, cognition and function are linked in the operational definition of cognitive decline and must occur together. Also unique is the absolute requirement for behavioural changes. By being relatively free from often costly investigations, the ICD-10 is well-positioned for deployment in the wide range of global clinical realities.

The DSM-5 resembles the NIA-AA in its core clinical phenotype, but eliminates investigational requirements entirely. It is also lexically distinct, using the de-stigmatizing term neurocognitive disorder (NCD) in place of dementia. It has the most extensive operational support, with copious examples for impairment in each cognitive domain.

\textbf{5.3.7. Comparisons and Statistical Analysis}

Agreement between criteria was calculated as percentage of agreement and also used the kappa statistic ($\kappa$). The kappa was chosen for its ability to compare agreement observed versus what would be expected from chance alone, and as such represents a more objective indication
of agreement between diagnostic classifications. This was based on a similar approach used to compare diagnostic agreement in vascular cognitive impairment\textsuperscript{263}. Similar qualitative cut-offs for interpreting the magnitude of calculated kappas were used: \(\kappa = 0.00 – 0.20\) (slight agreement), \(\kappa = 0.21 – 0.40\) (fair agreement), \(\kappa = 0.41 – 0.60\) (moderate agreement), \(\kappa = 0.61 – 0.80\) (substantial agreement), and \(\kappa = 0.81 – 1.0\) (almost perfect agreement). Kappa calculations were done using the IBM SPSS Statistics 23.0 analysis software package.

5.3.8. Role of funding source

This work was supported by the Canadian Institutes of Health Research (grant number MT13129). The sponsor had no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

5.4. Results

5.4.1. Comparing New Criteria with NINCDS-ADRDA Benchmark

Percentages of agreement between new consensus criteria and the NINCDS-ADRDA are show in Table 5.2. Agreement was highest with the NIA-AA and DSM-5, and significantly lower with both the IWG-1 and ICD-10. When diagnostic certainty (probable vs. possible AD) was considered (Table 5.3), percentage agreement between the NINCDS-ADRDA with the NIA-AA, and the NINCDS-ADRDA with the DSM-5 remained very high. However, degree of diagnostic certainty affected agreement between the NINCDS-ADRDA with the IWG-1, and the NINCDS-ADRDA with DSM-5. Percentage of agreement improved among those with probable disease, and dropped significantly among those with possible disease. Percentage agreement was
especially poor between the NINCDS-ADRDA and the IWG-1 among individuals classified with possible AD.

5.4.2. Comparing Among New Criteria

Kappa statistics for pair-wise comparisons of the four new criteria are show in Figure 5.1. Using the recommended $\kappa$ cut-offs there was almost perfect agreement between the NIA-AA and DSM-5, and substantial agreement between the IWG-1 and the NIA-AA. All other comparisons showed only moderate agreement, with the weakest agreement between the ICD-10 and its peers.

5.5. Discussion

Our data confirm significant areas of divergence between current consensus AD criteria, and a lack of backwards compatibility between some classification systems and the prior NINCDS-ADRDA benchmark. Although some classification systems overlap better than others, none are entirely equivalent. In the extreme, the percentage agreement was so low as to exclude one half of individuals when cases were re-classified using a second system.

5.5.1. Discordance of the ICD-10

The greatest non-equivalence was between the ICD-10 and its counterparts. Pairwise concordance between it and other newer criteria was only moderate, and against the NINCDS-ADRDA percentage agreement was poor. This was especially true for cases of lower diagnostic certainty (i.e. possible AD). Even among higher certainty cases (i.e. probable AD), 29% of those diagnosed with AD by the NINCDS-ADRDA would have been missed by the ICD-10. The ICD-10 generated the most classification outliers among the newer criteria, and missed 17% (26/155)
of cases classified as AD by its counterparts. This suggests the ICD-10 is capturing a different cohort than its peers. Despite its ease of application, due to a relative lack of requirements for investigations and biomarkers, these findings may raise questions about the applicability of the ICD-10 when investigational tools are available.

5.5.2. Discordance of the IWG-1

The IWG-1 demonstrated poor overlap with the NINCDS-ADRDA benchmark, both in general and following fractionation of cases into probable vs. possible disease. The percentage disagreement is similar to that found by Oksengard et al. in a smaller cohort of AD individuals, where only 12 of 23 subjects diagnosed with AD by the NINCDS-ADRDA met the IWG-1 definition for AD. Pairwise comparisons were marginally better between the IWG-1 and other newer criteria than was the case with the ICD-10, with the most substantial concordance between the IWG-1 and the NIA-AA. Although the IWG-1 had the most missed cases among the newer criteria, it had the same number of classification outliers as the DSM-5, and half as many as the ICD-10. This degree of non-equivalence is similar to the ICD-10. However, unlike the ICD-10, which was designed for wide clinical use, the IWG-1 was targeted towards purer forms of AD demonstrating a hippocampal (amnestic) phenotype, with hippocampal pathology, and in the context of Alzheimer’s biology. This clinicobiological shift from the traditional clinicopathological paradigm represents a novel departure from the older, more established model. The resulting classification of individuals would expectedly also diverge. As such, the IWG-1’s relative lack of agreement with its counterparts in fact confirms this classification system’s ability to ascertaining the distinct cohort of prototypic “pure” AD.
5.5.3. Concordance of the NIA-AA and the DSM-5

There was near perfect concordance between the NIA-AA and DSM-5, and both criteria had excellent overlap with the NINCDS-ADRDA regardless the level of diagnostic certainty. This likely reflects these criteria’s nearly overlapping clinical definitions for AD. The main difference between their core phenotypes is the allowance for non-amnestic variants of AD by the NIA-AA where memory symptoms are absent. There is, however, significant discrepancy between the NIA-AA and the DSM-5 in their use of investigations, primarily imaging and biomarkers. The NIA-AA employs them to improve diagnostic certainty, while the DSM-5 does not require them at all. This raises the question of what biomarkers and imaging are contributing in the strictly clinical setting given their apparent equivalence. Could a purely clinical criterion be sufficient to capture all affected AD individuals, when research confirmation of underlying biology is not sought?

It must be noted that the above findings were based on diagnoses made late in the disease course. Not only were these individuals suspected of having AD at the outset, but they had been assessed at a tertiary referral clinic, where they had been followed for a minimum of one year, and had three follow-ups. More of the clinical syndrome therefore had emerged, making a diagnosis on purely clinical grounds much easier. Whether this near equivalency between the NIA-AA and the DSM-5 would apply to individuals at earlier stages of illness is unclear, and it may be here that diagnostic investigations would demonstrate meaningful benefit.
5.5.4. Implications of Non-Equivalency and Future Directions

AD is already highly heterogeneous, even when considering only its cognitive profile. When variation in disease course, imaging signatures, and variability in genetic contributions are added, the potential for inhomogeneity is extreme. The introduction of further heterogeneity into study samples through the use of non-equivalent classification systems only serves to further complicate matters, rendering meaningful inference difficult if not impossible.

When associations or causations can be inferred, the interpretation of these findings in light of this non-equivalence becomes problematic. Research findings should not automatically be compared between studies employing different criteria without first accounting for likely differences to arise due to differences in these criteria. A study employing the IWG-1, for instance, might be expected universally to have memory complaints, but may include individuals without functional decline who would be considered MCI by a study employing the NIA-AA. Criteria selection becomes a key element in study design. Our data suggest that use of either the NIA-AA or DSM-5 will result in twice as many recruited participants as the IWG-1, potentially translating to halving the time window necessary to meet recruitment targets. However, use of the NIA-AA or DSM-5 would likely include more individuals with co-morbidities, and some who may have no or only subtle memory symptoms.

One strategy for reconciling differences among criteria may be to use them in parallel or complementary pairs. The first criteria would be more permissive, such as the NIA-AA or DSM-5, and would be used exclusively for clinical diagnosis and patient care. Such criteria would also be well-suited for observational studies, as their allowance for atypical and variant cases would
ensure better detection of unexpected and emergent disease phenomenon. The second criteria, perhaps applied afterwards, would then isolate the subgroup of individuals most prototypic of AD phenotype and biology. Such a criteria, for example the IWG-1, would serve to select a homogenized research cohort best suited for detecting interventions effects, a strategy increasingly used in clinical trials of anti-amyloid therapies.

5.5.5. Biomarker Reliance and Social and Ethical Considerations for Selection among Non- Equivalent Diagnostic Constructs

Even as this study was being conducted sea change has been underway, with some advocating re-conceptualization of AD to address continuing failures in therapeutics development. The goal is to shift from current “reductionist” to “dynamic, systems based” models that decouple AD from clinical presentation, linking it instead to biomarkers that allow pre-clinical diagnosis. Such approaches also tackle the profoundly complex biology of AD and related dementias, complexity often leading to clinical heterogeneity that confounds clinically-reliant diagnostic models. For example, recent molecular biology investigations reveal morphologic variation among amyloid-β 40 fibrils distinguishing rapidly progressive AD from typical AD and posterior cortical atrophy. Furthermore, specific molecular conformational “strains” of tau can reproduce clinically distinctive histologic phenotypes when seeded into mice. However, despite such insights, the cautionary note for such strictly biomarker-driven approaches, exemplified by the IWG criteria, is that complexity and costs of the diagnostic work-up and management of AD may become unfeasible for many. The impact of incorporating advanced investigations such as CSF structural MR, and PET imaging, can increase diagnostic costs fivefold, overwhelming already many under-resourced health care systems.
may be the proposed A/T/N scheme that dichotomizes presence or absence of Amyloid, Tau, and Neurodegenerative biomarkers to describe the pathologies underway, and then use clinical tools to stage the clinical continuum from presymptomatic or prodromal or symptomatic with functional decline\textsuperscript{276}. The eventual choice of diagnostic system and conceptualization must balance the necessities of research with the resources and realities of the clinical front lines.

5.6. Conclusion

The major consensus criteria for AD currently in use for research and clinical diagnosis are not equivalent. Even when significant overlap occurs, cases excluded by one criterion may be included by another. Cohorts in studies that employ different criteria cannot therefore be considered entirely analogous, and study results should only be compared or combined after careful consideration for the criteria used.

This non-equivalence poses interesting questions as to what constructs should be used in the diagnosis of AD, and underscores the impact decisions relating to this construct can have on the final appearance of the disease population. How the core phenotype should be defined, what and whether investigations and biomarkers are required, and which and whether co-morbid conditions should be allowed, are not fine distinctions but can radically alter who is considered to have AD.
Table 5.1: Comparative elements among consensus diagnostic criteria for AD

<table>
<thead>
<tr>
<th>Cognitive Syndrome</th>
<th>NINCDS-ADRSA</th>
<th>NIA-AA</th>
<th>IWG-1</th>
<th>ICD-10</th>
<th>DSM-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memory</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Executive Function</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Visuospatial</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Language</td>
<td>+</td>
<td>{Any 1}</td>
<td>Any 2</td>
<td>+</td>
<td>{Any 1}</td>
</tr>
<tr>
<td>Attention</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Social Cognition</td>
<td>(+)</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

| Functional Decline | (+)          |        |       | +      | +     |

| Behavioural Syndrome | (+)          |        |       | +      |       |

<table>
<thead>
<tr>
<th>Higher Cortical Features</th>
<th>(+)</th>
<th></th>
<th></th>
<th>(+)</th>
<th>(+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apraxia</td>
<td>(+)</td>
<td></td>
<td></td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Agnosia</td>
<td>(+)</td>
<td></td>
<td></td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Aphasia</td>
<td>(+)</td>
<td></td>
<td></td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Imaging and Biomarkers</th>
<th>(+)</th>
<th>Temporal/Parietal</th>
<th>Medial temporal/Parietal</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrophy</td>
<td>(+)</td>
<td>Any</td>
<td>Any</td>
<td>+</td>
</tr>
<tr>
<td>Hypometabolism</td>
<td></td>
<td>Temporal/Parietal</td>
<td>Temporal/Parietal</td>
<td></td>
</tr>
<tr>
<td>Amyloid PET</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>↑CSF tau or phospho-tau</td>
<td>+</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>↓CSF Aβ</td>
<td>+</td>
<td>+</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

| Other Features           | (+) | +                 | +                        |   |
| Insidious onset with     |     |                   |                          |   |
| gradual decline          |     |                   |                          |   |
| Causative gene present   |     |                   |                          |   |

<table>
<thead>
<tr>
<th>Co-morbid Conditions</th>
<th>Reduces certainty to “possible”</th>
<th>Reduces certainty to “possible”</th>
<th>Exclusionary</th>
<th>Exclusionary</th>
<th>Reduces certainty to “possible”</th>
</tr>
</thead>
</table>

+ = mandatory element; (+) = optional
Table 5.2: Breakdown of re-classified diagnoses among new criteria for NINCDS-ADRDA defined AD (n=155)

<table>
<thead>
<tr>
<th></th>
<th>NIA-AA</th>
<th></th>
<th>NIA-AA</th>
<th></th>
<th>NIA-AA</th>
<th></th>
<th>NIA-AA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%*</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Dementia due to AD</td>
<td>146</td>
<td>94%</td>
<td>83</td>
<td>54%</td>
<td>86</td>
<td>55%</td>
<td>149</td>
<td>96%</td>
</tr>
<tr>
<td>Not Dementia (incl. MCI and mild NCD)</td>
<td>9</td>
<td>72</td>
<td>69</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Dementia due to AD includes: (1) for NINCDS-ADRDA criteria - probable and possible AD; (2) for NIA-AA criteria - probable and possible AD; (3) for IWG-1 criteria – probable AD; (4) for ICD-10 criteria – dementia due to AD; (5) DSM-5 criteria – major neurocognitive disorder due to probable or possible AD.

* “%” refers to percentage of agreement with NINCDS-ADRDA criteria.
Table 5.3: Breakdown of re-classified diagnostic subcategories among new criteria for NINCDS-ADRDA defined AD subgroups; probable (n=100) and possible (n=55)

<table>
<thead>
<tr>
<th></th>
<th>NINCDS-ADRDA Probable AD (n = 100)</th>
<th>NINCDS-ADRDA Possible AD (n = 55)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NIA-AA subgroups %*</td>
<td>IWG-1 subgroups %</td>
</tr>
<tr>
<td>Probable AD</td>
<td>93%</td>
<td>76%</td>
</tr>
<tr>
<td>Possible AD</td>
<td>11</td>
<td>Dementia due to AD 71</td>
</tr>
<tr>
<td>MCI</td>
<td>7</td>
<td>Not classified as AD Dementia 24</td>
</tr>
<tr>
<td>Probable AD</td>
<td>96%</td>
<td>13%</td>
</tr>
<tr>
<td>Possible AD</td>
<td>13%</td>
<td>Dementia due to AD 15</td>
</tr>
<tr>
<td>MCI</td>
<td>2</td>
<td>Not classified as AD Dementia 48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not classified as AD Dementia 40</td>
</tr>
</tbody>
</table>

Note: Percentage agreement based on diagnosis of AD aetiology, defined as: (1) for NIA-AA criteria – probable and possible AD; (2) IWG-1 criteria – probable AD; (3) for ICD-10 criteria – dementia due to AD; (4) DSM-5 criteria – major neurocognitive disorder due to probable or possible AD.

*“%” refers to percentage of agreement with NINCDS-ADRDA criteria.
Figure 5.1: Pair-wise comparison of concordance among major consensus criteria (κ and p-values)

Note: This comparison defines “possible AD” under the NINCDS-ADRDA criteria, NIA-AA criteria, and DWM-5 criteria as being equivalent to “not AD” under the IWG-1 criteria and ICD-10 criteria. This was done to represent the conservative case, wherein lower probability disease is excluded. Such exclusion generally occurs when specificity considerations trump sensitivity considerations, such as during case selection for a clinical trial.
6. General Discussion

These studies demonstrate that AD is variable. This variability follows consistent patterns – giving rise to AD subtypes, even as it defines a typical prototype. This variability can be extreme – sufficient to lead to misdiagnosis through syndromic mimicry. This variability is common – complicating attempts to define AD through consensus criteria. In order to address this issue, we must first acknowledge, then define, and then finally, anticipate variability and its impacts.
6.1. Discussion section 1: Immediate implications of AD Heterogeneity

It follows from AD heterogeneity that certain key actions should immediately be taken. First it must be **recognized**, being brought in from the margins of the AD diagnostic and nosological framework; second it must be **defined**, so that heterogeneity exits the realm of vague definitions to specific, and testable criteria; and third it should be **anticipated**, so that as new information becomes available it can be properly integrated into the evolving AD framework.

The failure to properly address these implications may have contributed to the field’s continuing inability to provide an effective disease modifying therapy for AD, and failure to take immediate action to redress this gap may contribute to perpetuating this inability. For example, the failure to take into account course (namely fast vs. slow progressors) may have contributed to recent failures among phase 3 clinical trials aiming to develop a treatment of AD. Although one might expect a double-blind randomized design to account for any such variation, unaccounted variation remains a source of noise, degrading observable treatment effect. Failure to account for such variation at the start of a trial also often precludes meaningful post hoc subgroup analysis which might otherwise elucidate such treatment effects.

6.1.1. Recognizing that AD Variability Exists

Recognition of AD heterogeneity requires a shift in our conceptualization of AD, away from it being a unitary syndrome-pathology relationship and towards it being a syndrome cluster related to an array of inter-connected processes. This re-conceptualization should include endophenotypes. Although less easily used in the positivist paradigm of scientific inquiry, and requiring greater care in the design and statistical execution of future studies, it is increasingly
clear that AD is not a simple disease and that its complexity must be accounted for in order for the field to reach the next level of understanding.

Although it may be desirable and justified to exclude atypical cases from treatment trials where resource use is intense and capabilities therefore limited, an argument can be made for the incorporation of atypical AD cases in less resource-intensive observational studies. Failure to do so not only excludes a significant portion of the syndrome spectrum and thus obviating the study’s generalizability, but risks \textit{a priori} bias whereby only prototypic mechanisms of disease are observed because only prototypic disease was included. As useful as the amyloid hypothesis is, over-reliance on this or any single paradigm of pathophysiology hijacks our ability to elucidate complementary disease mechanisms, and may be narrowing our field of view by selecting carriers of APOE ε4 alleles. Although it may be unwise to abandon the amyloid hypothesis as some have advocated, looking beyond its confines seems increasingly necessary. AD itself is telling us, through its variability, that it is not a straightforward disease. Why should we try to force it into a shape it is not, as Alzheimer himself warned against doing?

There is a definite sea change in progress, with prominent researchers in the field advocating for a re-conceptualization of AD. In part this is driven by the continuing failures of the field in achieving the goal of novel, let alone disease modifying or curative therapies. Due to this impasse, such authors advocate for a shift away from what they cite as a reductionist model of AD to one that is dynamic and “systems based”. In this new conceptualization, AD is de-coupled from its clinical presentation, and seen as a series of biological failures that accrue to eventual expression as symptomatic disease. The primary
advantages to this conceptualization are (1) the allowance for pre-clinical diagnosis, (2) the
closer linkage between diagnosis and mechanism, and (3) full recognition of the dynamic
complexity of AD – i.e. its emergent nature from component endophenotypes. Individually
these ideas are not new, and like the AD heterogeneity concept can and should be viewed as
the iterative outcome of past decades of work and experience. It is, however, representative of
the ripeness of the time for a new chapter in AD research and care to be written. The question
going forward is how this should be done.

6.1.2. Defining AD Variability

Having recognized and acknowledged the heterogeneity of AD, be it with our current
conceptualization or the increasingly biomarker- and system-based-approach, careful attention
must be paid in how heterogeneity is defined. Such notions touch upon and borrow from
similar ideas in the re-conceptualization of AD as “a failure of systems”.

6.1.2.1. From Syndrome Dimensionality and the Axes of Heterogeneity to Disease Subtypes

As advocated by some, a necessary first step is the uncoupling of the clinical syndrome of
Alzheimer’s dementia from the biology of Alzheimer’s disease\(^{32,273}\). This notion lies at the heart
of the IWG criteria, and is hinted at within the pre-clinical diagnostic framework of the NIA-AA
(although it is less apparent in the NIA-AA’s approach to the diagnosis of Alzheimer’s dementia
itself). In many ways, this is the precise goal of the axes of heterogeneity framework – the de-
convolution of the overall syndrome into its component clinical dimensions. The axes of
heterogeneity concept and systems based model are complementary. Whereas the systems-
based model addresses the core phenotype of AD, and focuses on the presence of AD biology
through biomarkers (i.e. the yes/no of whether AD is present), the axes of heterogeneity address the nature and nuance of that AD biology (i.e. the “flavouring” of AD that is present). A recent study using this dimensional approach to organizing AD features, coupled with machine learning, elucidated four distinct atrophy patterns from among ADNI participants\textsuperscript{281}. These patterns correlated with distinct cognitive sub-syndromes (near normal, typical, dysexecutive, and slow progressing).

As currently discussed, the axes of heterogeneity are defined primarily on clinical grounds – young vs. old, by cognitive domain, by cortical region of predominant neurodegeneration etc.; however, this approach melds well with the use of biomarkers and other disease surrogates. It has, for instance, been identified that not only is cognition in general closely linked to DNA methylation\textsuperscript{282}, but that methylation varies across regions and between individuals\textsuperscript{283,284}, and specific variations in methylation correlate with specific cognitive measures, such as of memory\textsuperscript{285}. Epigenetic surrogates are thus but one example of how the axes of heterogeneity can be enriched beyond just clinical measures. The use of biomarkers, however, is not straightforward (as discussed below).

Feasibility is another hurdle in applying the AD heterogeneity concept in practice. While de-labelling avoids \textit{a priori} bias, sub-syndromic designations must eventually be assigned. The axes of heterogeneity serve well in theory and research, but are too cumbersome for use in clinical care. Further, diagnostic labels provide a common lexicon for research and thus cannot be abandoned completely. What then constitutes (or what \textit{should} constitute) a syndrome in this
sense? Using the pathobiological framework, reasonable definitional requirements for a sub-
syndrome may include:

1. **Reproducibility**: A syndrome should occur repeatedly and be recognisable in a variety of
   patient and environmental contexts.

2. **Unique biological linkage**: A syndrome should reflect some underlying biology, be it
   elements unique to the syndrome, or unique interplays of common elements.

Reproducibility means that a syndrome can be recognized as a distinct entity, and that it recurs
such that future cases are expected to arise and require treatment. Unique linkage to biology
ensures that a syndrome reflects something that would benefit from tailored therapy (to the
extreme of personalized medicine, in the case of very rare syndromes). Unique linkage to
biology also means that the syndrome provides a singular vantage, as it is the only means for
which to interrogate, by definition, those unique elements or element interactions that
underlie its mechanistic core.

An approach taken thus far has been to assign syndrome names based on the most
prominent deficit. This is the approach taken largely by the NIA-AA. However, naming
syndromes simply based on their primary cognitive deficits, while a useful first step, may
ultimately be insufficient, and in some respects reflects 19th century concepts of brain centres,
versus functional network disorders. Such labels lack the specificity, and lose out on the
richness of clinical and pathological correlates. Another option is to name based on region. This
is also not without pitfall. Consider the case of Behavioural-Dysexecutive AD, which was
previously designated the frontal variant. On subsequent analysis, this variant was not actually
frontal at all. Perhaps the best approach is to designate syndromes based on their primary cognitive or behavioural syndrome (or even functional syndrome), applying regional localization for additional information, and commenting on underlying pathology if such correlates are available. However, such a priori classification cannot entirely avoid biasing nosology in favour of existing knowledge, lacking the objectivity to identify novel syndromes, and the flexibility to incorporate them. It is for this reason that the unbiased, data-driven approaches such as CHIMERA for regional atrophy, and A/T/N for biomarkers in general, have been proposed.

While the nosology is being clarified, it would be helpful if there were a way to signal the provisional nature of a syndromic classification. Perhaps “macrophenotype” could serve. A macrophenotype would be defined as satisfying the condition of recurrence, but has yet to demonstrate the unique linkage to biology. Eventually, the most robustly established sub-syndromes may deserve eponyms, or at least names that go beyond mere descriptors. Perhaps a call-out to the underlying mechanism could be considered. This is the approach taken to some extent by the IWG (e.g. Trisomy 21-related amyloidosis).

6.1.2.2. Anchoring the Diagnosis through the Clinicobiological Paradigm: Objective Classification Using Biomarkers

It is clear that we are entering an era where clinical definitions alone are insufficient. This is why new consensus criteria place emphasis, to varying degrees, on biomarkers. Biomarkers also permit the establishment of the unique linkage to biology necessary for this proposed definition of syndrome. Indeed, some equate the use of biomarkers with mechanism-based therapeutics. However, biomarkers are a double-edged sword.
Although a full discussion is beyond the scope of this manuscript, it must be briefly noted that biomarkers must also be used with caution from the standpoint of causality. Although the terms surrogate endpoint and biomarker are often used synonymously, disease causality has been established in the case of surrogate endpoints whereas it has not in the case of biomarkers\textsuperscript{287}. Taking biomarkers as being equivalent to disease (see below) may therefore be vulnerable to scientific fallacy\textsuperscript{288}.

Within this limitation, biomarkers are nevertheless immensely powerful tools both for diagnosis and research. Key among the promises and opportunities offered by the use of biomarkers is detection of disease pathology in asymptomatic individuals. Extending on this, some researchers go on to define such cases, granting them nosological standing on par with diseases – designations traditionally reserved to those with symptoms. This shift from diagnosis-based on clinically-evident manifestations, to investigational detection of biology, regardless of its expression or lack therefore, represents a fundamental shift in the medical paradigm.

The advantages are self-evident. Many diseases represent the long culmination of complex processes, with symptomatic expression being only the proverbial tip of the iceberg. Pre-symptomatic diagnosis, essentially an extreme of early diagnosis, holds both clinical and economic promise. Whereas the up-front costs may be high, when coupled with effective therapy, evidence suggests this strategy would save money overall\textsuperscript{289–291}. It is noteworthy that such analyses are based on the use of cholinesterase inhibitors, symptomatic treatments not
generally viewed as being particularly effective. When factoring the potential of hypothetical disease modifying therapies, the benefits are great\textsuperscript{280}.

### 6.1.2.3. Ethical Considerations of Increased Biomarker Use: The Social Justice of Biomarkers

However, the pitfalls are also as glaring. As promising (and as seemingly necessary) as biomarkers are, is their widespread implementation of biomarker feasible? The early “diagnosis” they permit depends largely on cost-intensive technologies, risking such technologies becoming the privilege of the wealthy few.

#### 6.1.2.3.1. Age-based Discrimination

Geriatric populations are frequently marginalized as a result of age-based discrimination, resulting in inequitable apportionment of health care resources. For instance, cancer care is more likely affected by a patient’s age than their co-morbidities, and age alone reduces the likelihood of an individual receiving care\textsuperscript{292}. Ageism in research can be seen in the exclusion of elderly from clinical trials targeting the very diseases most relevant to their age group\textsuperscript{293,294}.

The shift from symptomatic to pre-clinical and at-risk states of Alzheimer’s disease has the effect of taking the research and care focus away from the elderly, abandoning this population for younger, less-afflicted individuals who are more likely to (i) provide economic returns following effective disease modification, and (ii) respond to our limited armamentarium of biological manipulation. Although this shift will, in the long term, benefit the aging population by allowing individuals to age better and healthier, it will likely come too late for those already suffering from dementia.
6.1.2.3.2. Cost Effectiveness and Income Disparity

The cost of AD is high and variable, largely due to the need for informal care\textsuperscript{295}. The expense of novel therapies may inflame this already precarious situation. For this reason, the International Psychogeriatric Association has advocated to include health economic measures into Phase 3 studies\textsuperscript{296}, recognizing the potential impact arrival of new therapies might have on health care systems, especially as not all such systems globally are equally equipped.

Similar concerns exist for biomarkers, increasingly required for inclusion in clinical trials for such potential therapies. The added cost is not trivial. Diagnostic strategies can incorporate a variety of methods, including clinical examination by primary care physicians or by specialists, neuropsychological assessment, structural imaging, CSF biomarkers, and molecular-PET imaging. The choice of which and in what combination these strategies are used can lead to as much as a five-fold increase in the cost of the diagnostic process\textsuperscript{275}. Very clearly, the extreme cost of biomarkers, especially molecular-PET imaging, cost-effectiveness is not only an economic consideration, but becomes an ethical one as well.

As an additional consideration, even when the cost-benefits are justified, one must consider that costs and benefits may be incurred (and granted) to different aspects of the health care system. For instance, costs are incurred by the medical system (drug provision and diagnostic test costs), but benefits enjoyed by the long term care (long term care facilities) and informal care (caregivers) sectors. This imbalance presents an additional layer of challenge vis a vis the social justice of high cost treatments and biomarkers\textsuperscript{275}. 
6.1.2.3.3 Ethical Considerations of Increased Biomarker Use: Lack of Therapy

Although beyond the scope of this manuscript, it bears mentioning that another key ethical consideration is the prospect of being able to detect a pathological process (or label someone with a disease) for which no effective treatment, let alone cure, exists, raises significant ethical concern\textsuperscript{297,298}.

6.1.2.3.4. Summary of Ethical Considerations

Whereas the need for biomarker verification (or “enrichment”) is acute, research hopes and clinical realities must be balanced. Clinicians on the front lines of dementia care must still be provided the guidance necessary to diagnose AD; if consensus criteria become overly focused on costly biomarkers, AD would become essentially non-diagnosable by doctors at large. This consideration is especially acute in economically disadvantaged jurisdictions, where access to any investigation, let along CSF or molecular-PET imaging, is already an economic strain.

One possibility in the short term, as discussed in Chapters 3, is a layered approach, using lower-cost, clinically-focused criteria, followed by judicious application of more costly biomarker verification. In the longer term, the field must grapple with the dilemma of wanting biological and mechanistic clarity, with the absurdity of defining a disease in such a way that it cannot even be diagnosed without the use of resources greater than the average annual income of the majority of the world’s population. One option would be to rely more heavily on CSF biomarkers, which are cheaper than amyloid imaging. There is evidence this is useful, especially when combined with neuropsychological testing\textsuperscript{280,299}. 

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Another strategy would consider how we use biomarkers. One could strive for universal biomarker use just as we should attain universal investigational tool access but to adopt a graded approach whereby less expensive biomarkers fall closer towards the “required” category of diagnostic verification, and more expensive technologies be clearly placed in the “optional” realm. The conduct of studies using such a graded approach already exists\textsuperscript{300}, and it would be a matter of incorporating cost-effectiveness as a secondary or exploratory endpoint. Efforts to reduce the cost of such technologies should be given significant priority.

6.1.3. Anticipating Heterogeneity

Before AD heterogeneity can be harnessed, it is necessary to establish a conceptual framework, preferably facilitated by regulatory mechanisms, which would allow for atypical subtypes and macrophenotypes to be formally diagnosed, treated, and funded. There would be four key elements in this approach:

- **Establish Criteria for Subtypes and Macrophenotypes**: Clear, consistent definitions for AD subtypes and macrophenotypes would permit recruitment of atypical cases into existing studies. Such definitions might first be applied to observational studies, where per-subject costs are lower and if proven valid, later applied to clinical trials where per-subject costs are higher.

- **Enact De-Labelled Syndrome Exploration**: While useful, diagnostic labels can hinder the identification of novel pathophysiologic mechanisms, by biasing one towards accepting the existence and nature of presupposed phenotype-mechanism relationships. Data-driven, systems-approaches avoid such bias.
• **Ensure Precise Operationalization**: Consistent and well-defined operationalization of criteria will help reduce variability introduced through ad hoc clinician and researcher judgement\(^\text{301}\).

• **Regulatory Support**: Without allowance from funders and regulatory stakeholders, any study into sub-syndromes or macrophenotypes would be under-resourced and less likely to succeed as a result. Pharmaceutical companies also require some re-assurance that this by definition high risk venture has a competitive chance of bearing returns proportionate to the currently much more attractive goal of treating prototypic disease.

### 6.1.3.1. Criterion Definition: Integration of Atypical Subtypes and Macrophenotypes

**Into the Diagnostic Lexicon**

Key to the proactive accounting for atypicality is the integration of atypical subtypes into consensus criteria, and potential pre-specification of “atypical sub-studies” in clinical trials as a means of improving (1) generalizability, (2) accountability (to subtypes), and (3) scientific value of clinical trials. Recognizing that the costs associated with conducting clinical trials is already high, this section advocates for a blended funding model, wherein government and non-governmental agencies might support these additional arms, creating blended “public-private partnerships” such as seen in the A4 Study (ClinicalTrials.gov NCT02008357)\(^\text{302}\). Blending also addresses the reality that both industry and the public are equal stakeholders here. The ultimate goal is success in Phase III clinical trials, which benefits both parties.
6.1.3.2. De-Labelled Syndrome Exploration: “Phenobanking” As a Method for Identifying Novel Subtypes and Macrophenotypes

Phenotype banking, or “phenobanking”, is a proposed variation on the idea of biobanking, incorporating additionally ideas involving endophenotypes but expanding on it. In this proposed approach, researchers would systematically record large data sets of ostensibly non-relevant or only possibly-relevant clinical data for the express purpose of association discovery at a later date through large data networks. This can be a relatively low-cost way to capture existing and potentially discovery novel subtypes. Along these lines, phenobanking is well placed to leverage the advent of wearable technologies (such as will be incorporated in Ontario Neurodegenerative Disease Research Initiative 2303 and Canadian Consortium on Neurodegeneration in Aging304), or portable monitoring (such as with portable sleep apnea monitors). Although risking overwhelming complexity, macrophenotypes might also be incorporated, albeit never beyond the level of exploratory analysis or exploratory endpoints. Again, the goal here is to provide the unique biological linkage that elevates a macrophenotype into the realm of sub-syndrome.

Phenobanking may thus be a way to get the best of both worlds, providing a mechanism to anticipate as yet unrecognized sub-syndromes, while simultaneously gathering the necessary information to hint at novel mechanism discovery. Because phenobanking, by definition, captures information where relevance has yet to be established, extending beyond the standard of care expressly for the purpose of research, the research ethics must be stringently applied to its use.
There are several advantages to phenobanking. This recording in a systematic way of ostensibly non-relevant or only possibly-relevant clinical data for the express purpose of association discovery at a later date through large data networks may eventually prove a relatively low-cost way to capture existing and potentially discovery novel subtypes, endophenotypes, and disease mechanisms. Further, the breadth of the phenobanking net would be limited only by feasibility. Anything that might be measured could be, so long as costs allow.

This approach is necessary because it is not possible, and potentially dangerous to pre-specify relatedness of observed phenomena with disease. Although a “shotgun approach”, this exploratory method avoids such a priori traps, and has become feasible in the internet era with the ability to store large datasets (big data) and use data analytic technologies such as machine learning to detect relationships.

Despite its apparent lack of sophistication, at least when compared to molecular techniques such as gene chips or molecular-PET imaging, the need for collecting seemingly unrelated clinical data is no different than its highly regarded cousins. Whereas we feel there is justification to blanket collect blood samples for subsequent genetic screening, there remains reluctance to widen the collection of “non-relevant” patient data. This likely arises from the intuitive understanding that we do not yet fully understand the content or the interaction of the components of the human genome. However, one might argue that such a gap in understanding exists with our recognition of the clinical components of AD and the interplay of its endophenotypes. Consider for example the case of white matter disease, which was
discussed in the Introduction, briefly in Chapter 1, and again in Chapter 3. Central to the
discussion in all cases was to what degree white matter disease either acted as an innocent
bystander (unlikely), interacted with “true” AD pathology to enable disease expression (almost
certainly), and was perhaps even a central mechanism of AD itself (possible, though a bold
claim). Recent work within the DIAN cohort suggests that the last may in fact be true, with the
authors going so far as stating in their title that “White Matter Hyperintensities Are a Core
Feature of Alzheimer’s Disease”\textsuperscript{104}. If as a field we can be uncertain about the role of what is
now appearing to be a central mechanism of disease, it stands to reason we could be similarly
mistaken about... just about anything. While the situation is unlikely this extreme, SVD and
WMH’s story (which is ongoing) provides good justification for us not to discount anything as a
potential endophenotype. Only systematic, unbiased collection and analysis of clinical features
can serve to provide us the accurate and precise nosological classifications.

Phenobanking represents a transition from acknowledging heterogeneity as a challenge,
to taking advantage of it for mechanism discovery.

\textit{6.1.3.3. Precise Operationalization: Reducing Uncertainty in a High-Uncertainty
Endeavour}

By definition, we are seeking the marginal cases, where atypicality and variability will be
greatest. In order to optimize the likelihood that sub-syndromes can be identified and properly
integrated into the AD syndrome framework, care must be taken in the design not only of
individual studies, but the methodologies governing all studies in this area. We saw in Chapter 3
how differences, at times seemingly minor ones, among classification approaches can lead to
radically different endpoints. Every effort must therefore be given to employing standardization
in methodology and operationalization so as to minimize the contribution of inconsistent approaches to the already complex AD syndrome issue\textsuperscript{301}.

The use of standard methodologies, along the lines of standard operating procedures, and consensus statements on research approach similar to the STRIVE\textsuperscript{305}, or Harmonization Standards\textsuperscript{306}, could go a long way to allowing cross centre, cross network, and cross national collaboration at every stage of the research endeavour. Most crucially, it would allow for easy aggregate analysis of the findings of all studies conforming to such standards. When combined with the framework of open science along the lines of ADNI\textsuperscript{101}, or the Tanenbaum Open Science Institute at McGill University\textsuperscript{307}, the potential arises for every project, regardless of size, to contribute towards the big data repositories that appear increasingly necessary and useful.

6.1.3.4. Regulatory Support: Funding Research and Enabling Industry and the Case for Using the Orphan Disease Model

As already noted, anticipatory strategies can be costly, especially when incorporated into clinical trials (e.g. subtype cohorts). A potential solution might be to model after the orphan disease system. In essence, a rare AD subtype would be treated as an orphan disease\textsuperscript{308}. A single drug trial, wherein no benefit was demonstrated in the main study, but demonstrated in an atypical sub-syndrome arm, could set a key precedent for atypical cases salvaging an otherwise failed agent. Including atypical cohorts would carry financial incentive, rather than being seen as a nuisance. The shift in paradigm would thus be to pursue treatment not for the entire AD syndrome spectrum at once, but to cure each subtype individually. We know these subtypes exhibit distinct deviations in many of the axes of heterogeneity. Should it not follow that the mechanism-based therapies for them would likely diverge between subtypes as well?”
6.1.4. Summary to Discussion Section 1

Once heterogeneity is recognized, defined, and anticipated, it may become possible to harness it as well. Every divergent case hints at additional mechanisms, or an as yet unknown interaction between known causes. Every subtype suggests a specific neurobiology, be it pathology, host factors, or the interplay of these with specific co-morbidities. Already this approach has revealed the existence of distinct amyloid fibrillation patterns, and that differences among patterns in Aβ1-40 segregate between typical AD, posterior cortical atrophy, and rapidly progressing AD\textsuperscript{130}. 
6.2. Discussion Section 2: Harnessing AD Variability to Probe Mechanisms of Disease

With the recognition of phenotypic variability, attention can be turned to elucidating the underlying mechanisms. This in turn increases our understanding of the processes underlying AD itself. In one sense, the subtypes and macrophenotypes that comprise the AD syndrome spectrum can be likened to subgroups of an extremely large, observational, longitudinal study that nature has fashioned in the human species. By looking at “between group differences”, it becomes possible to tease out the mechanistic distinctions that lead to clinical distinction and AD mechanisms thereby (see future directions). Heterogeneity can thus become a boon – a means to probing underlying disease mechanisms, with mechanism-based treatment being the ultimate goal.

6.2.1. Variability as a Lens: Probing Genetic and Epigenetic Mechanisms of AD and AD Heterogeneity

Genetic effects (e.g. location of PSEN1 polymorphism), epigenetic effects (e.g. promoter methylation), and modulator gene effects (e.g. influences from APOE ε4, and causative mutations for related disorders) have all been implicated as contributors to the intrinsic variability of AD.

A specific example is the notion of genetic resilience. The ADNI cohort demonstrates variation in rates of clinical decline. Homan and colleagues examined gene expression across a number of tissue types within the heart and brain. Interestingly, associations could be demonstrated between levels of expression in genes related to cell-cycling, angiogenesis, heme
biosynthesis, and longitudinal decline on clinical endpoints, or on incremental amyloid burden on biomarker imaging \textsuperscript{296}.

Adding to the complexity of genetic mechanisms, emerging evidence implicates epigenetic determinants in the pathogenesis of AD, and thereby AD heterogeneity. Unlike genes, the epigenome can change over time and in response to the environment. It therefore not only contributes to AD variability, but is itself variable. Like pathology, the epigenome demonstrates topographic variation. For instance, epigenetics changes in brainstem, potentially alter function of specific nuclei\textsuperscript{311}. As discussed in Chapter 2, it is necessary to look at the epigenome in addition to the genome, because even when identical causative genetic culprits are present, phenotypic variation can occur.

Another reason why there is great value in exploring the genetic and epigenetic elements of AD heterogeneity is that such mechanisms have influence beyond AD. Proteopathic strains, or the dynamic sequence of protein self-catalyzed misfolding that underlies not only AD but other “proteinopathies” including DLB and ALS are, as summarized by Walker, directed by genetic and epigenetic factors\textsuperscript{312}. Indeed, 5-methylcytosine and 5-hydroxymethylcytosine levels within the epigenome have been shown to change not only in AD, but also DLB and FTD\textsuperscript{313}. Could it be that epigenetic direction underlies the common observation of co-occurring neurodegenerative processes?
6.1.2.1. An Interesting Aside: The Specific case of Epigenetic Effects on Circadian Dysregulation

Disruption of the biological clock is a hallmark of AD, and represents a special case for considering a potential approach for harnessing heterogeneity to probe epigenetic effects. There exists a genetic core, the variably periodic expression of which generates and maintains the circadian rhythm at a cellular level as summarized by Cronin et al\textsuperscript{314}. Control is exerted at least in part through modifications of the epigenome, such as in a drop in promoter methylation in the 1-3 hour period preceding a subsequent burst in transcription activity\textsuperscript{315}. Methylation patterns change during the progression of AD, with attenuation in this variability in methylation along with loss of temporal phase-locking of methylation peaks with the 24-hour cycle\textsuperscript{314,315}. In addition to association with dysregulation of day-night cycles, these alterations have been linked to Alzheimer’s pathology burden\textsuperscript{316}.

Importantly and similarly, that circadian cycles change with aging and that methylation of the circadian “genetic regulator” likewise changes with age. Might EOAD vs. LOAD demonstrate variability within this regulator’s epigenome, which in turn could then be looked for in individuals of the same subtype (e.g. LOAD), who exhibit different patterns and degrees of sleep disturbance? Can direct modulation of this epigenome serve as a novel disease target? Is there an association between APOE ε4 carrier status and these changes? Given the regional nature of at least some the methylation oscillators (e.g. within the suprachiasmatic nucleus, or the prefrontal cortex), are such patterns correlated with focal AD syndromes? Given that epigenetic mechanisms span disease, do similar changes occur in DLB, where parasomnic
behaviour is more common? Given that the epigenome changes in response to environmental challenges, are there changes that occur in response to conventional therapies for circadian disruption, such as trazodone, neuroleptics, and zopiclone?

6.2.2. Variability as a Lens: Probing Host Adaptation to Dementia and the Concept of Reserve

Host factors must always be considered, especially in chronic conditions that develop over many years. This protracted course permits a period during which disease and host factors interact and result in further variation in phenotype. In AD, there is the additional reality that the very pathological processes are host processes. Rather than a foreign chemical agent or pathogen (setting aside the slow infection hypothesis for the moment), AD arises from apparent dysregulation of an individual’s own biology. What causes this dysregulation, and how does this maladaptive response by an individual’s own biology eventually lead to AD neurodegeneration? Phenotypic variation may offer us a clue, and allow for the probing of host factors governing an individual’s resistance or susceptibility towards these Alzheimer’s mechanisms, the fortification or replication of which might open up equally potent therapeutic modes and targets.

Reserve is perhaps the best described and understood host factor that may describes both adaptation in face of AD, and pre-morbid factors that may aid in adaptation. Reserve is broken down into two elements: (1) passive elements or “brain reserve”, and (2) active elements of “cognitive reserve”. Brain reserve encompasses relative static, physical elements such as brain size, neuronal count, and synaptic density. Brain reserve theory posits that a static threshold of such elements needs to be injured in order for clinically apparent outcomes to
manifest. By contrast, cognitive reserve encompasses the processes that the brain’s physical elements subsume – speed of processing, network integrity, and the ability to recruit still-functioning elements to compensate for diseased elements in the face of disease. Using the NART as a proxy for cognitive reserve, it was found to explain part of the variance in cognitive decline, independently of biomarkers for amyloid/tau and for neurodegeneration (i.e. atrophy). Furthermore, the authors concluded the effects were additive, rather than interactive\textsuperscript{318}. Individuals with high cognitive reserve show more advance amyloid deposition on PIB imaging\textsuperscript{319}.

**Functional reserve** is a new proposed form of host adaptation that may be important particularly in those settings where an individual has not had a chance to obtain cognitive reserve through the traditional methods (i.e. formal education, as in the case of many women from prior generations)\textsuperscript{320}. **Social reserve** represents a third form of host compensation in the face of, in this case, loss of theory of mind\textsuperscript{321}. The idea of reserve can be extended theoretically to any element of the core features of dementia (see Introduction) that govern the axes of heterogeneity. For instance, the idea of **behavioural reserve** has recently been proposed in the setting of FTD\textsuperscript{322}, underscoring the inescapable link between disease and host factors in every aspect of AD.

**6.2.3. Summary: Heterogeneity as a Lens: Stage vs. Subtype vs. Compensation Revisited**

As alluded to in Chapter 1, the controversy surrounding the root cause of AD heterogeneity has been intimately associated with our conception of AD itself. The three competing theories this gave rise to – the stage, subtype, and compensatory hypotheses – are a direct result of this.
The **subtype hypothesis** proposes that different AD syndromes reflect different underlying pathologies, such as genetic and epigenetic variations.

The **stage** or **phase hypothesis** states instead that observed variation merely reflects one unified disease being observed at different stages of progression, similar to the Sufi story of the blind men and the elephant. Arguments have been made for the related disorder(s) of Dementia with Lewy Bodies versus Parkinson’s Disease Dementia. At some point, the syndrome of both diseases is effectively the same. The distinctions are artificial.

The **compensatory hypothesis** can be best thought of as the mirror complement of the subtype hypothesis. Whereas the subtype hypothesis emphasizes disease factors to explain variation, the compensatory hypothesis favours host factors; variation is the result of the same or similar pathology, interacting with markedly different brains (such as with different degrees of reserve) and in turn producing sometimes markedly different disease patterns, at least until the final disease stage.

Increasingly, it is evident that although the subtype hypothesis is currently in vogue, given its seamless integration into the disease-mechanism/disease-phenotype linkage, the shifting conceptualization of AD which includes broader acceptance of the heterogeneous nature of the AD syndrome will almost certainly breathe new life into all models. The association between stage and phenotype remains weakest. However, acknowledging that disease course is intimately linked to atypicality, defining one axis of heterogeneity, it may eventually be that even the stage hypothesis will see at least partial acceptance within a new AD conceptual framework. Much stronger is the emerging evidence in support of the
compensatory hypothesis, as seen in the concept of reserve. To take only one of these three as true, and discounting entirely the other two, as we have seen in the previous discussion on reserve, may be a fallacy.

In a way then, as with the plethora of AD sub-syndromes, the field may be shifting towards a spirit of inclusion. It is not so much which of the three models is correct, but how can each explain what we observe clinically, and under which circumstances is a particular model most key. Only by accounting for the interaction of all three may we unravel the monolith of AD.
7. Future Directions

7.1. The Influence of Operationalization

As seen in Chapter 3, the very consensus criteria used to diagnose AD are themselves a source of potential ascertainment bias and non-equivalent classification. Their application to an already heterogeneous patient base can only further complicate matters by injecting noise into study results. This problem is compounded by a lack of consistent operational definitions for key elements within these criteria. For example, the presence of significant cerebrovascular disease serves to downgrade or exclude the diagnosis of AD, depending on the consensus criteria. However, what qualifies as significant cerebrovascular disease is inconsistently and in some cases poorly defined. This lack of consistent operational definitions forces each laboratory and clinic to use their own definitions on a center-by-center, even case-by-case basis. Such substitutions, well informed though they may be, are by necessity another exercise in individual judgement and thus a source of further variance, which can be observed\(^{324}\). This variance complicates criterion application\(^{325}\), with demonstrated effects on case ascertainment\(^{301}\).

Two key areas where operational definitions were found in our study to play a role included (1) determination of functional decline, and (2) the adjudication/diagnosis of co-morbidities, with cerebrovascular disease being foremost (e.g. how much white matter disease matters)?
7.2. Pathological and Biomarker Confirmation

An unanswered question from our studies is how these observations of clinical variability and clinically-defined cognitive sub-syndromes (Chapter 1), syndromic mimicry (Chapter 2), and diagnostic classification (Chapter 3) potentially change in light of pathological confirmation. A subset of individuals from the Sunnybrook Dementia Study has such pathological confirmation, with additional cases being ascertained. In parallel, an increasing number of individuals now have CSF biomarkers, while overlap with studies using amyloid and, more recently tau imaging, will allow for a re-examining our findings, providing definitive validation of our work.

7.3. The Prevalence of Atypicality

The richness of the SDS cohort, most crucially its inclusion of atypical, mixed, and non-AD cases, uniquely positions the dataset for studies into the prevalence of atypicality. Current estimates put the rate of subtype AD at roughly 13-15%\textsuperscript{165}. Using this cohort we should be able to not only provide an estimate of the prevalence of atypical AD, but also allow through a systemic application process as done in Chapter 3 to test both published criteria as well as our own hypothetical diagnostic and operational definitions. An accurate estimate of the prevalence of atypicality, as well as a probe of potential confounds to this estimate through the use of different constructs and operational definitions (as in the diagnosis of typical AD), is a key initial step in anticipating AD heterogeneity.
7.4. Proof of Concept in Using Heterogeneity and Atypical Subtypes to Interrogate AD Mechanisms

The dominant (left) temporal selectively demonstrates severe atrophy in semantic dementia. However, focal left temporal polar involvement can also be seen in some cases of AD. Also, while semantic language disruption is common in AD, predominant semantic language disruption is rarer. Temporal polar atrophy and a pattern of semantic language-predominant decline represents a test case for the idea of using non-amnestic, atypical AD subtypes in interrogating AD and AD heterogeneity mechanisms.
8. Conclusions

There is significant phenotypic variation in AD. It is consistent enough to merit the definition of subtypes, it is extreme enough to lead to misdiagnosis, and it is common enough to complicate broadly-defined criteria, as is often the case with consensus guidelines.

Therefore, it is necessary to acknowledge and account for this variation if we are to sharpen our ability to diagnose AD in the clinic, interrogate its mechanisms in the laboratory, and cure it through our clinical trial methodologies and networks. To do this, we must define its subtypes, recognize their presence, and anticipate their potential effects at every stage in our dealings with AD from the bench to the bedside. This must begin with the language we use in our discussions of AD—i.e. we must develop a new lexicon for the Alzheimer spectrum of disorders, in accord with recommendations by Dubois, Hampel, and others\textsuperscript{2,273}.

By shifting our way of thinking away from AD as a singular disease, but as a disease spectrum, we furthermore allow ourselves points of leverage as yet unexploited to their full potential. Variability is a lens for interrogating AD mechanisms—a set of ready-made comparator groups awaiting deep endophenotyping and study. Variability is a treasure map leading us to potential new treatments. Variability is a caution against the temptation to be all too comfortable that we are seeing all that needs to be seen—in 50 years, it is with high hopes that future researchers will wonder at the naivety of this thesis.
9. References


47. The ICD-10 Classification of Mental and Behavioural Disorders. World Health Organization; 1993.


John B, Lewis KR. Chromosome variability and geographic distribution in insects. Science


155. Seltzer B, Sherwin I. A comparison of clinical features in early- and late-onset primary


205. Holland CM, Smith EE, Csapo I, et al. Spatial distribution of white-matter hyperintensities


233. Misch MR, Mitchell S, Francis PL, et al. Differentiating between visual hallucination-free


