

REVIEW

New insights on Alzheimer's disease diagnostic

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Abstract

One of the most common problems encountered in a world characterized by demographic ageing is Alzheimer's disease (AD) with an estimated number of 35.6 million people affected in 2010 to 65.7 million in 2030. Under recognition and delayed diagnose create problems for people diagnosed with dementia, for their families and for entire health system. Although there have been many breakthroughs and new insights into AD etiopathogeny in the last two decades, few steps have been made toward an accurate diagnosis but all steps point into one major direction namely "personalized medicine" that could represent a future perspective for AD patients. Starting with a more accurate diagnosis not of the clinical syndrome, but of underlying molecular defects, that may eventually lead to a personalized, more effective treatment.

Keywords: Alzheimer's disease, diagnostic, amyloid precursor protein, A β peptide, tau, phosphotau.

Introduction

The world population is ageing [1], adding an increased burden upon medical and social services addressed to elderly patients. Defined by OMS as patients over 65 years of age [2], the elderly are not a homogeneous group, with the same pathology. They may be in good health, active and self contained or they may have multiple disabilities and be frail and in need of special care. One of the most common problems encountered in elderly is Alzheimer's disease (AD). In 2010 there was an estimated number of 35.6 million people diagnosed with various forms of dementia and this number doubles every 20 years, up to 65.7 million in 2030 and 115.4 million in 2050. Unfortunately, under recognition and delayed diagnostic are the two major current problems that complicate the outcome and the familial context [3].

Alzheimer's disease is the most prevalent form of dementia and represents an increasing problem of modern society because the evolution is progressive until exitus. In 1906, German psychiatrist, neurologist and neuropathologist Alois Alzheimer diagnosed the first patient with AD Alzheimer's Disease. The patient presented cognitive, language and behavior impairment in 1901 and after her death, in 1906, the physician performed brain autopsy, describing the two anatomopathological features that continue to be the "golden diagnosis" of AD in present days [4]. The disease was not taken into consideration until 1970 and since then it become considered gradually being a disorder and not a normal cognitive decline with aging [5].

Molecular pathogeny of AD

Gene mutations and polymorphisms

Alzheimer's disease mutations are significant for familial forms and are transmitted to offspring in an autosomal dominant manner. Interestingly, most mutations involving amyloid precursor protein (APP) affect the C-terminal of A β peptide, leading to either increased production, or increased aggregation and plaques formation [6]. APP mutation represents however, less than 1% of total AD forms and less than 15 % of familial AD (FAD) [7]. Most FADs are characterized by mutation of Presenilin1 gene, located on chromosome 14 and coding for the proteolytic enzyme of the γ secretase complex [8]. Although the structures of the PS-1 and PS-2 genes are remarkably similar, only 14 mutations have been identified so far for PS-2, as opposing to more than 150 for PS-1 [9]. PS mutation alter proteolytic amyloid cascade, leading to increased formation of A β peptide, especially of A β 42.

Searching for a genetic mark for sporadic AD, research was focused on polymorphisms of genes coding proteins intersecting amyloid or tau proteins metabolism pathways, such as: (i) LRP1 (Low density lipoprotein-related protein 1) [10]; (ii) MAPT (microtubule-associated protein tau) [11]; (iii) BDNF (brain-derived neurotrophic factor) [12]; (iv) IDE (insulin-degrading enzyme) [13]; (v) A2M (alpha 2-macroglobulin) [14] and (vi) ACE (Angiotensin I Converting Enzyme) [15]. One genetic event not related to APP or tau but significantly statistic for AD, is the presence of ϵ 4 allelic form of apolipoprotein E (apoE) which has

been shown to increase the risk for AD three to seven times (for homozygous form) [16] and may decrease the onset age with 7–9 years.

Post-translational mechanisms – evidence of microRNAs involvement in AD

MicroRNAs are single stranded RNA molecules (ssRNA) of approximately 22 nucleotides, partially complementary to the 3' untranslated region (UTR) of a messenger RNA (mRNA), that act as a posttranslational mechanism to down regulate protein expression [17]. There are several microRNA species, mostly brain-enriched or brain specific, that correlate with AD. By comparative studies between fetal, adult and AD hippocampus, miR-9, miR-125b and miR-128 have been shown to be statistically significant increased in the demented brains [18]. MiRNA-107 seems to be statistically significant decreased in AD brain vs. age-matched healthy brains. One of miRNA-107 targets

seems to be BACE1, explaining the acceleration of disease with microRNA downregulation [19]. Also targeting mRNA BACE1, the miRNA-29a/b-1 cluster is significantly decreased in the AD cortex, not restricted to a specific area but in a generalized manner, including in the cerebellum [20].

Protein alterations

Amyloid precursor protein (APP)

APP is a protein constitutively synthesized, that suffers post-translational modifications by N- and O-glycosylation, phosphorylation and tyrosin-sulphatation and subsequently enzymatically cleaved into secreted products [21]. Depending on enzymes, the final result may be either p3 (a 3-kD soluble peptide), resulting from initial α cleavage, or the Aβ peptide (the hydrophobic form found in senile plaques of AD brain), from β cleavage (Figure 1).

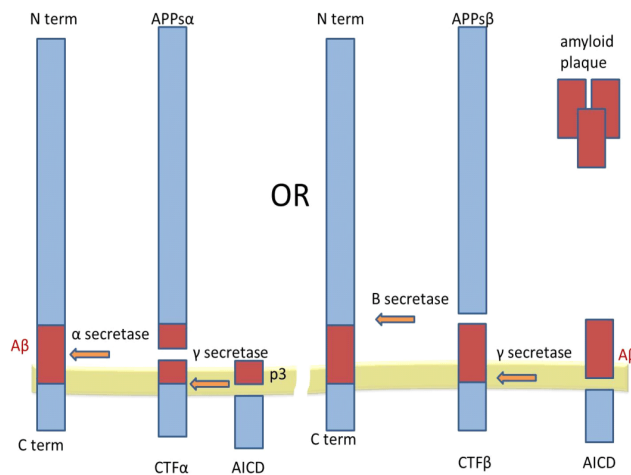


Figure 1 – The two enzymatic pathways responsible for APP processing are the non-amyloidogenic pathway (left) and the amyloidogenic pathway (right). The non-amyloidogenic pathway starts with α secretase cleavage that cuts through Aβ sequence, preventing peptide generation by the subsequent γ secretase cleavage. The amyloidogenic pathway starts with β secretase that cleaves the large extracellular domain of APP into a suitable position for Aβ peptide generation by the subsequent γ secretase cleavage. The peptide will aggregate into the extracellular environment to generate amyloid plaques. APP – amyloid precursor protein; C term – C-terminal domain; N term – N-terminal domain; APPsα – APP soluble α cleavage-generated fragment; CTFα – C-terminal fragment following α cleavage; APPsβ – APP soluble β cleavage-generated fragment; CTFβ – C-terminal fragment following β cleavage; AICD – APP intracellular domain.

Aβ peptide exists at very low concentrations in the plasma and cerebrospinal fluid (CSF) of healthy persons, at nanomolar concentrations (10⁻¹⁰–10⁻⁸ M) [22]. However, increased concentrations have neurotoxic effects, in both *in vitro* [23–25] and *in vivo* animal models [26–28].

Tau protein

The other hallmark of AD is the presence of hyperphosphorylated filaments of tau protein – a microtubule associated protein, with role in microtubules stabilization [29]. The microtubule-binding occurs through several repetitive domains in the C-terminal sequence, named carboxyl-terminal microtubule (MT)-binding repeats. Depending on the alternative splicing, some tau isoforms contain three such repeats (3R), while others, 4R. Certain tauopathies are characterized by an increased 4R/3R ratio, suggesting that either over-stabilization of cytoskeleton might result in disease, or that the 4R form has a higher propensity to aggregate [30].

AD diagnostic

Former diagnostic of AD – DSM IV clinical criteria and current diagnostic NINDS ADRDA criteria

There are many types of dementia and there is no

singular test for dementia diagnosis. Nowadays, there are two types of diagnosis criteria for Alzheimer’s Dementia: DSM IV Clinical Criteria and NINDS ADRDA Criteria [31, 32].

Table 1 – Diagnostic criteria of Alzheimer’s disease

DMS IV Diagnostic Criteria:	NINDS ADRDA Criteria revised 2007
Multiple cognitive deficits	
A – 1 Memory impairment.	A – Memory impairment.
A – 2 One or more of the following: aphasia, apraxia, agnosia, disturbance of executive functioning (planning, organizing).	1 – Gradually and progressive in the last six month. 2 – The deficit must be confirmed by neuropsychological tests. 3 – Memory impairment may be isolated or associated with other cognitive deficits.
B – The above mentioned cognitive deficits cause significant social, occupational and familial functioning impairment.	B – Temporomedial lobe atrophy objectified by MRI measurements.
C – The cognitive decline has gradual onset and the evolution is progressive and continuing.	C – CSF analysis: evidence of low Aβ 42 and high τ și P-T.

DMS IV Diagnostic Criteria:	NINDS ADRDA Criteria revised 2007
Multiple cognitive deficits	
D – The cognitive deficits mentioned in A1 and A2 criteria are not caused by organic systemic conditions, other central nervous conditions or substance-induced conditions.	D – PET-Scan: reducing glucose metabolism temporoparietal bilateral.
E – The deficits do not occur only during delirium.	E – Presence of autosomal dominant mutation involved in Alzheimer's disease: chromosomes 1, 14, 21.
F – Psychiatric conditions must be excluded (major depressive episode, schizophrenia).	

Anatomopathologic diagnostic of AD

Alzheimer's disease is a neurodegenerative disease diagnosed by criteria set forth by Alois Alzheimer one hundred years ago [4]. Microscopic examination of cortical sections from Alzheimer's disease brains shows neuronal loss due to extracellular amyloid plaques [33] and intracellular neurofibrillary tangles of abnormally hyperphosphorylated tau protein [34]. Neuronal loss may lead to vacuolizations of gray matter, accompanied by secondary astrogliosis, possibly as an attempt to reconnect lost neuronal pathways (Figure 2).

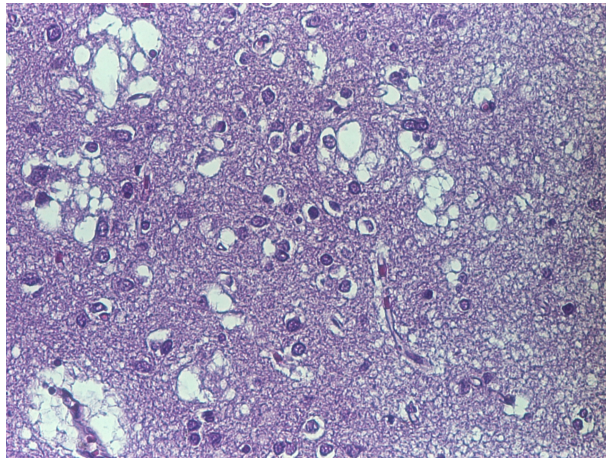


Figure 2 – Vacuolizations of gray matter, accompanied by secondary astrogliosis (courtesy of George Simion, MD, Senior Physician, Department of Pathology, Emergency University Hospital, Bucharest).

Future perspectives

Imagistic diagnostic – PET Scan, MRI

PET Scan, unfortunately cannot be used on regular basis because of its cost and poor availability. PET scanning reveals hypoperfusion or hypometabolism of the central nervous system and can be used to differentiate Alzheimer's disease from fronto-temporal vascular dementia [35, 36]. 18-Fluorodeoxyglucose PET shows metabolism changes like hypometabolism or decreased signal associated with AD [37].

PIB (Pittsburg Compound-B), an agent labeling amyloid is used for amyloid deposits in the brains of

patients with Alzheimer's disease. However, 10% to 20% of normal elderly by 65 years of age are PIB positively and the percentage increases until 50% of normal elderly in their mid 80s [38].

Structural MRI shows hippocampal atrophy and whole brain volume loss, these changes correlating with disease progression and clinical symptoms [39].

In our current practice, we use computer tomography, which shows cortical atrophy (Figure 3).



Figure 3 – CT image of dementia patient with large ventricles and cortical atrophy (courtesy of Gabriel Ioan Prada, MD, PhD, Associate Professor, Geriatrics and Gerontology, "Carol Davila" University of Medicine and Pharmacy, Bucharest).

Molecular diagnostic

Investigation of amyloid products and tau and P-tau levels in CSF are nowadays used as base for a molecular diagnostic in AD. According to numerous reports, CSF of AD patients presents low $A\beta$ 1–42 and high tau and P-tau [40–44]. Correlation of all three factors yielded a higher diagnostic accuracy than each of them separately, or paired two by two. Important for clinical practice is that correlation between decreased $A\beta$ and increased tau levels offers a strong prediction of MCI progression into AD [45]. There are however, few drawbacks in current use of this method, the most notable being the significant cut-off levels differences between laboratories [46], which are hindering its use when is needed the most – in the mild cognitive impairment.

Although there have been many breakthroughs and new insights into AD etiopathogeny in the last two decades, few steps have been made toward an accurate diagnosis. The complexity of the molecular mechanisms and anatomopathologic findings in patients with dementias make more difficult the delineation of one dementia type from the rest and hinder the development of targeted therapies. Already a reality for cancer patients, "personalized medicine" could represent a future perspective for AD patients also, starting with a more accurate diagnosis not of the clinical syndrome, but of underlying molecular defects, that would eventually lead to a personalized, more effective, treatment.

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