IN DEPTH REVIEW

Biological markers in Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is the most common type of dementia occurring in human population. The disorder is characterized clinically by memory loss and histopathologically by the presence of neurofibrillary tangles and senile plaques in patient's brain. Accuracy of the clinal diagnosis of AD is quite variable (~60 to 95 %), leaving a significant number of AD patients undiagnosed or falsely positively diagnosed. Therefore there is a requirement for biological markers, which would unambiguously discriminate living AD patients from other non-AD individuals. Until now a few diagnostic biomarkers for AD have been identified, which can be divided in two groups: protein markers and genetic markers. The most significant protein biomarkers are levels of tau proteins, ubiquitin and amyloid ß-peptides in cerebrospinal fluid (CSF). Among genetic AD markers, the most relevant are allelic variants of gene for apolipoprotein E and point mutations in genes coding for amyloid precursor protein and presenilin 1 and 2. Nevertheless, neither of recent biomarkers allow the ultimate AD diagnosis, because the disease is multifactorial and heterogenous. Identification of various subgroups of AD will help improvement in diagnoses and development of potent therapeutic drugs *(Tab. 2, Fig. 2, Ref. 53)*.
Key words: Alzheimer's disease, biological markers, tau, beta amyloid, ubiquitin, cerebrospinal fluid.

Alzheimer's disease (AD) is the leading cause of dementia in elderly people (Tapiola et al, 2000). This progressive neurodegenerative disorder is characterized clinically by memory loss, language deterioration, impaired visuospatial skills, poor judgment, and often indifferent attitude. At the histological level, the hallmarks of AD are abnormal structures that occur in the brains of suffered individuals – neurofibrillary tangles (mainly composed of aberrant tau proteins) and senile plaques (with amyloid α-peptides as the main component) (Vickers et al, 2000).

Until now the diagnostic of AD in living persons is based on first clinical signs, such as memory loss. At present 80–90 % of AD-cases can be correctly diagnosed by using patient's history, brain imaging, and psychosocial testing. Since the clinical diagnosis of AD is difficult, especially in early stages of dementia, there is a requirement for some biological markers, which will unambiguously indicate the presence of AD and allow to distinguish AD from other non-AD dementing disorders (Kennard, 1998).

Diagnostic markers of AD are entities, whose presence, concentration or activity reflects some morphological, biochemical or genetic alternations that are associated with the disease (Robles, 1998). The search for a specific biomarker is essential for the assessment and management of AD, especially in the areas of:

1) diagnosis, in particular early onset of the disease,
2) monitoring the effect of therapeutic intervention,
3) routine screening in human population, i.e., those over 50 years of age,
4) false diagnosis of AD (Kennard, 1998; Boss, 2000).

In general, two types of biological AD-markers are recently considered for diagnosis:

1) protein markers (i.e. tau protein, ubiquitin and amyloid ß-peptides) (Tab. 1),

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Tab. 1. Protein markers for Alzheimer's disease (Robles, 1998). The most significant AD-markers are bolded. Other protein markers have only limited relevance for AD-diagnosis. They are not specifically associated with AD, and reflect also another immunological or pathological conditions in the body (i.e. inflammation).

Source	Marker	Level
Cerebrospinal fluid (CSF)	tau	↑
	phospho-tau	↑
	amyloid ß-peptide 1–42	↓
	amyloid ß-peptide 1–40	↓
	soluble amyloid ß-proteinprecursor	↓
	cyclic AMP	↑
	S 100 B	↑
	interleukin-6	↑
	p97	↑
	cathepsin D	↑
	pyruvate	↑
	ceruloplasmin	↑
	17ß-estradiol	↓
Plasma	amyloid ß-peptide 1–42	↑
	p97	↑
Blood cells:		
lymphocytes	Acetylcholinesterase (ACE)	↓
erythrocytes	Butyrylcholinesterase (BCE)	↓
platelets	Cytochrome oxidase	↓

2) genetic markers (allelic variant of Apolipoprotein E, amyloid precursor protein, presenilin 1 and 2) (Tab. 2).

Candidate protein biomarkers for Alzheimer's disease

Protein AD – markers are polypeptides, which could be detected in different body fluids and their respective concentrations were found to correlate with Alzheimer's dementia (Tab. 1). Progress in identifying biomarkers of AD has recently focused on analytes in cerebrospinal fluid (CSF). Many biochemical changes in the brain are reflected in the CSF and therefore it was considered as an obvious source of relevant biomarkers for AD. CSF biomarkers can be used for the clinical diagnosis (diagnostic markers), for the prediction of the disease prognosis (prognostic markers) and for the monitoring of the biochemical effect of the testing drugs (marker for the treatment responses) (Blenow, 2005). At present the most valuable biomarkers identified in CSF are tau protein, ubiquitin and amyloid – peptides. These three proteins are directly involved in formation of pathological hallmarks of AD in brain – neurofibrillary tangles (composed mainly of tau proteins) and senile plaques (formed by amyloid ß-peptides) (Boss, 2000). Furthermore, both mature tangles and Lewy bodies which are seen in varying numbers in AD brain are ubiquitinated (Grundke-Iqbal et al, 1988; Galloway et al, 1988; Morishima-Kawashima et al, 1993).

Tau protein

Tau protein belongs to the family of microtubule-associated proteins. The human tau gene is located on the long arm of chromosome 17. In central nervous system, six tau isoforms (ranging from 352 to 441 amino acid residues) are produced by alternative splicing from a single gene (Godert et al, 1989). Each of these isoforms is likely to have particular physiological roles (Buee et al, 2000).

Tau synthesized within the neuron is physiologically localized in the axon where it promotes stability and assembly of the microtubules (Blomberg et al, 1996). Since the tau is intracellular protein, only a small amount of it would be detected in CSF of individuals with intact neurons. In contrast, during AD progression tau underlies posttranslational alterations such as truncation and hyperphosphorylation, which result in dissociation of aberrant tau species from microtubules and their polymerization into insoluble pathological filaments (Grundke-Iqbal et al, 1986 a, b; Buee et al, 2000). Intracellular accumulation of non-degradable protein complexes results in death of neurons. Upon significant disruption of neuronal architecture would one anticipate appreciable amounts of aberrant tau released into the CSF. It was therefore deduced that increased level of tau in CSF could correlate with onset of neurodegeneration. This is well documented in many studies which have come to the same conclusion that CSF tau levels are elevated in mild cognitive impairment or very early stages of AD, when its diagnosis on clinical bases only may be questionable (Maruyama et al, 2001). Simultaneous screening for some other biomarkers such as ubiquitin and amyloid ß might increase the diagnostic performance and the reliability of prognosis of AD. Iqbal and coworkers suggested that Alzheimer's disease could be subdivided into at least five subgroups based on CSF levels of tau, ubiquitin and Aß1-42. Each subgroup presented a different clinical profile. These subgroups might represent different causes and mechanisms of neurodegeneration (Iqbal et al, 2005).

Tab. 2. Genetic markers for Alzheimer's disease.

Gene encoding for	Chromosome No.	Marker	Incidence
Apolipoprotein E (ApoE)	19	allele ε4 (112Arg/158Cys)	incidence in AD 67.6 % versus incidence in non-AD group 30.9 % (Ganzer et al, 2003)
Amyloid ß-protein precursor (APP)	21	point mutations	25 mutations in 71 AD-families
Presenilin 1(PS1 or S182)	14	point mutations	115 mutations in 315 AD-families
Presenilin 2 (PS2 or STM2)	1	point mutations	10 mutations in 18 AD-families

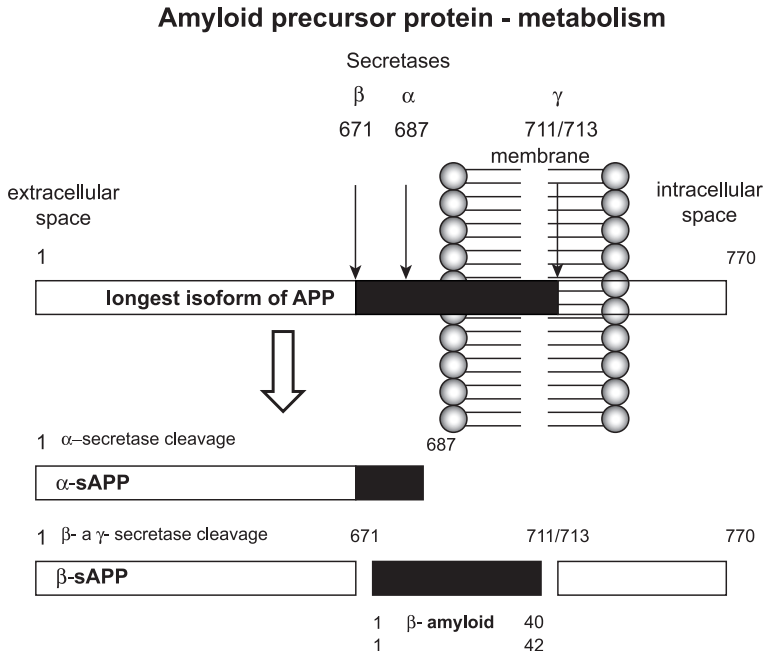

Fig. 1. The structure and metabolism of the amyloid precursor protein. In APP metabolism three different secretases participate on proteolytic processing of transmembrane glycoprotein amyloid ß-precursor, giving rise to peptides with different length.

Since the phosphorylation state of tau in AD is considered to contribute significantly to disturb cytoskeletal integrity, it would seem that the concentrations of hyperphosphorylated tau should be more sensitive marker of disease progression than total tau levels (Kaye, 1996). A marked increase in hyperphosphorylated tau in CSF of AD-patients has been found using different ELISA methods (Vanmechelen and Blennow, 2001, Hu et al, 2002, Blenow, 2005).

Amyloid ß-peptides

Although still controversial, a large number of studies implicate amyloid ß-peptides (Aß) as an early causative agent in AD (Torreilles and Touchon, 2002). These peptides composed of 39–42 amino acid residues are derived through proteolytic processing of a larger membrane-bound glycoprotein, encoded by a gene on chromosome 21 (Blenow and Galasko, 2000) – the amyloid ß-precursor protein (AßPP) (Fig. 1). Secreted, soluble Aßs are products of normal cell metabolism and are found in various body fluids, including plasma and CSF (Vickers et al, 2000). It was shown that the levels of Aß (Aß40 and Aß42) are decreased in AD patients (Tapiola et al, 2000, The Ronald and Nancy Reagan Research Institute, 1998, Okamura et al, 1999, Hock, 1998). The low level of Aßs in CSF is explained due to the deposition of these peptides in senile plaques and vascular amyloid deposits in the extracellular space of AD brain (Arai et al, 1998). Aß terminated at residue 42 (alanine) is mainly found in senile plaques; whereas Aß ending at residue 40 (valine) is prominent in vascular amyloid deposits (Mehta et al, 2001). The most convincing evidence is the presence of abnormalities in Aß metabolism (primarily production of the longer 1–42 form) in every autosomal-dominant form of familial AD (FAD) (Klunk, 1998). Some reports analyzed the difference in levels of different Aßs between AD patients with very mild dementia and patients with mild to moderate dementia. Authors found that Aß42 is most likely increased early in the disease process and levels of Aß42 and Aß40 were decreased during progression of the disorder (Lannfelt, 1998, Riemenschneider et al, 2002).

In recent years, the soluble globular Aß oligomers, known as amyloid-beta-derived diffusible ligands (ADDLs), were found to be present in CSF. Using an ultrasensitive, nanoparticle-based, protein detection strategy known as bio-barcode assay it was shown, that the ADDLs concentrations for the subjects diagnosed with AD were consistently higher than the levels in the CSF taken from nondemented age-matched controls. Although the number of AD patients and controls studied was low, these data are very promising (Georganopoulou et al, 2005; Blenow, 2005).

Ubiquitin

Ubiquitin (Ub) was first described in 1975 as a highly conserved ubiquitous protein. It has been shown that ubiquitin is involved in the degradation of unwanted proteins including the proteins that are affected by abnormal folding or other aberrant proteins (deVrij et al, 2004). Degradation cascade by ubiquitin-proteasome system (UPS) involves two separated steps: ubi-

quitation and protein degradation. Ubiquitination is characterized by the conjugation of ubiquitin to a target protein by the covalent attachment of multiple ubiquitin molecules to synthesize the polyubiquitin-chain degradation signal. This signal of a Lys48 polyubiquitin chain is recognized and degraded by the 26 S proteasome complex (Song and Jung, 2004). In the last years, evidence has accumulated that supports the premise that the ubiquitin-proteasome system might be an essential factor promoting AD pathogenesis (deVrij et al, 2004). In the cerebral cortex of Alzheimer's disease cases ubiquitin levels were increased many fold and the increase correlated strongly with the degree of neurofibrillary degeneration in the brain tissue (Wang et al, 1991). Furthermore, the level of ubiquitin in the CSF was observed to be elevated in AD, which might reflect the increased amount of the protein in the brain. Therefore, the ubiquitin in the CSF was suggested as a prospective biomarker of the neuropathology in this disease (Kondo et al, 1994).

Genetic AD-markers

The majority of cases of AD correspond to the sporadic form of the disease. Approximately 5–10 % of patients present an autosomal mode of transmission and account for cases called familiar Alzheimer's disease (Tanzi et al, 1996). Genetic factors contribute to AD susceptibility, and those known seem to increase the production or aggregation of Aß in the brain (Tanzi et al, 1996). It has been published, that mutation in APP (Goate et al, 1991; Mullan et al, 1992), presenilin 1 (Sherrington et al, 1995) and presenilin 2 (Levy-Lahad et al, 1995; Rogaev et al, 1995) genes were associated with familial AD (Tab. 2). The risk factor for late AD is considered the Apo E4 allele of apolipoprotein E (Saunders et al, 1993 and 1996; Strittmatter et al, 1993 a, b).

Genetic AD-markers are genes carrying specific point mutations or representing specific allelic variants, which are linked with cases of FAD. Respective gene mutants or allelic variants are identified by molecular biology techniques based on PCR, DNA restriction fragment length analysis or direct DNA-sequencing. Such genetic screening can be usefull for elimination of inheritance of such deleterious mutations in next generation and for starting with adequate treatment at right time.

Apolipoprotein E gene

Apolipoprotein E (ApoE), a 34 kDa protein composed of 299 residues, plays an important role in cholesterol and phospholipid transport, uptake and redistribution. Within the nervous system, ApoE might be involved in maintaining synaptic integrity after injury and during ageing. Moreover, involvement of ApoE in formation of senile plaque formation in AD-brain has been demonstrated (Riemenschneider et al, 2002). The ApoE gene (Fig. 2), located on chromosome 19, occurs in three allelic variants (designated ε2, ε3 and ε4), differing in coding for two amino acids at position 112 and 158 (Vickers et al, 2000, Riemenschneider et al, 2002). ApoE2 contains a cysteine at both sites; apoE3 contains cysteine112 and arginine158, while apoE4 possesses arginine at both positions (Beffert et al, 1999). In AD the ε4 allele is overrepresented (incidence in AD 67.6 % versus incidence in non-AD group 30.9 %) and is considered to be a major genetic risk factor for late onset (Ganzer et al, 2003). Nevertheless, some individuals carrying this allele can live to an advanced age without developing dementia (Vickers et al, 2000, Riemenschneider et al, 2002). Conversely, inheritance of ε2 appears to be associated with reduced risk of AD and delaying the age of onset of the disease. The ApoE4 allele frequency is mark-

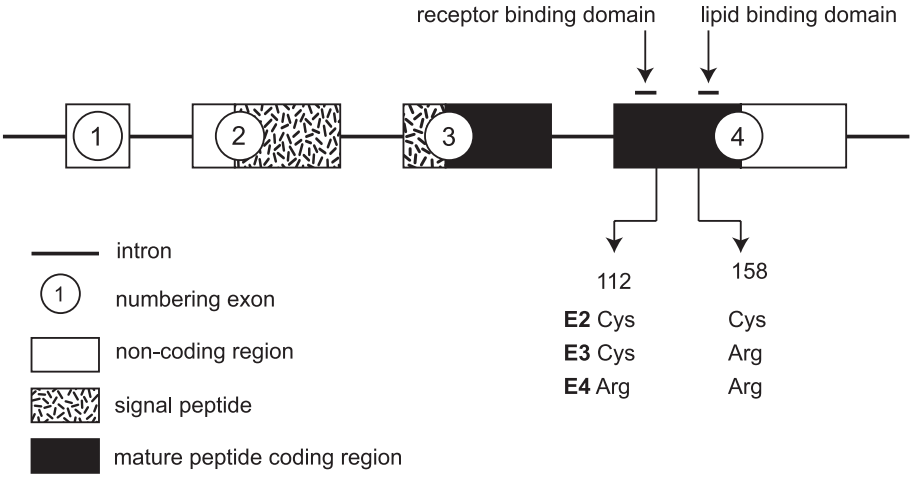

Fig. 2. The apolipoprotein E gene. ApoE gene – showing introns, exons and position of two codons for amino acids (Arg – arginin, Cys – cysteine) which differ in apoE variants apoE2, apoE3 and apoE4.

edly increased in both late-onset sporadic and familial AD (Arendt, 2001, Horsburgh et al, 2000).

Other genetic biomarkers

Another attitude to possible genetic diagnostic of AD focuses on mutations in three genes, which where discovered in families with early-onset of this dementia (Lannfelt, 1998). The gene coding for *amyloid precursor protein* (APP) is located on chromosome 21. To date, 20 missense mutations in 71 families with AD have been described for APP gene (available at URL: http://www.molgen.ua.ac.be/ADMutations/). Another causal gene for early-onset of familial AD is *presenilin 1* gene (PS1), localized on chromosome 14. Genetic analysis showed that PS1 mutations are responisble for 50–80 % of familial AD (Dugu et al, 2003). Until now, 155 missense mutations have been described in 315 families (available at URL: http://www.molgen.ua.ac.be/ADMutations/). In addition, an in-frame deletion of exon 9 associated with a missense mutation at the junction point and ~ 5000 bp deletion in intron 8 have been found (Tabira et al, 2002). Another deletion affecting the intron 4 splice-donor consensus sequence and resulting in a premature stop codon has been reported in one family (De Jonghe et al, 1999, Janssen et al, 2000). Mutations of a third gene for *presenilin 2* located on chromosome 1, have also been identified in some autosomal-dominant AD cases (Boss, 2000). These 10 mutations are very rare and only 18 families carrying respective mutations have been identified in the world (available at URL: http://www.molgen. ua.ac.be/ADMutations/).

Conclusion

Even though remarkable progress has been made during the past decade in diagnostic of AD, until now neither of biomarkers could identify unambiguously individuals suffering from or prone to this type of dementia. Monitoring of biomarkers is limited to estimation of potential risk of AD-onset or to consider the progression of disease. At present, the most relevant protein markers for AD diagnosis represent tau proteins, ubiquitin and amyloid ß-peptides. Among genetic AD-markers, the most relevant are allelic variants of ApoE-gene and point mutation in gene coding for PS-1.

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