Genetic and biochemical markers for Alzheimer’s disease: recent developments

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Alzheimer’s disease (AD) is a progressive neurodegenerative disorder of unknown aetiology, characterized by irreversible cognitive and physical deterioration. It is a major cause of morbidity and death in the elderly and a growing public health problem as life expectancy in the general population increases. AD is both genetically and phenotypically a heterogeneous disorder. An early-onset, familial type is recognized, as well as a later-onset, sporadic type. The diagnosis is made on clinical grounds, with the aid of a small number of additional investigations, using consensus criteria. However, at autopsy about 10–20% of clinically diagnosed AD patients are found to have conditions other than AD. Therefore, genetic and/or biochemical markers that support the clinical diagnosis and can distinguish AD from cognitive symptoms attributable to ageing and from other dementias will be of great value. The identification of such accurate markers for the early diagnosis of AD is mandatory for the development of efficient pharmacological treatment, since therapy should be initiated at an early stage of the disease, before extensive and irreversible brain damage has occurred.

According to a recent consensus report,2 the ideal biomarker for AD should fulfil the following criteria:
- detect a fundamental feature of the neuropathology
- be validated in neuropathologically confirmed cases
- have a sensitivity >85% for detecting AD
- have a specificity of 75–85% or more for distinguishing AD from other causes of dementia.

Moreover, a useful biomarker should be precise, reliable and inexpensive; it should be convenient to use and not harmful to the patient.

An ideal biomarker would be helpful in confirming the diagnosis of AD in epidemiological screening, in predictive testing, in monitoring progression and response to treatment, and in studying brain–behaviour relationships.

The diagnostic utility as biomarkers of more than 60 substances in serum and cerebrospinal fluid (CSF), such as trace elements, metals, neurotransmitters, (neuro)peptides, proteins, amino acids and purines, has been reviewed by Basun3 and by van Gool and Bolhuis.4 None of the parameters described by these authors appeared to be useful to support the diagnosis of AD. Here, we review current hypotheses regarding the pathogenetic mechanisms in AD and describe new genetic and biochemical markers for the disease.

NEUROPATHOLOGICAL CHARACTERISTICS OF ALZHEIMER’S DISEASE

The neuropathological hallmarks of AD are senile (neuritic) plaques (SPs) and neurofibrillary tangles (NFTs). Several types of SPs can be distinguished, but all plaques contain extracellular deposits of amyloid-β peptide (Aβ) that include abundant amyloid fibrils with non-fibrillar forms. Aβ is generated during proteolytic processing of amyloid precursor protein (APP) (Fig. 1). For that reason, APP and its derivatives could provide good biological markers.

NFTs are intraneuronal lesions occurring in large numbers in the AD brain. The major components of NFTs are hyperphosphorylated, insoluble forms of tau protein, associated with microtubules. The insoluble tau aggregates in the NFTs are often conjugated with ubiquitin, a feature that is also found in other intraneuronal proteinaceous inclusions in aetiologically diverse disorders such as Parkinson’s disease and diffuse Lewy-body disease. Furthermore, apolipoproteins E and J (ApoE and ApoJ), as well as
glycosylated acetylcholinesterase (AChE), protein S-100 and neuronal thread protein (NTP) have been demonstrated in the deposits in AD brain. Therefore, measurement of these parameters in CSF or other body fluids as diagnostic markers for AD would appear promising.

GENETIC VARIANTS OF ALZHEIMER’S DISEASE

AD is the most common form of dementia in the elderly, affecting 5–10% of the population aged over 65 years. It is well known that some Alzheimer patients have a family history of AD and a genetic predisposition to the disease. Estimates of the proportion of genetically based AD cases vary widely, but an established AD-related gene mutation occurs in less than 1% of all AD patients.

Mutations or polymorphisms in specific genes (presenilin-1, presenilin-2 and APP genes) have been found in early-onset familial AD. In contrast to deterministic genetic mutations, genetic factors may modify the risk of developing AD. Alleles of ApoE are the most powerful risk factors.

BIOMARKERS

Both genetic and biochemical markers for the different types of AD have been described in recent years (Table 1). The utility of these and other markers and of the risk factor ApoE genotype will be critically evaluated in this review.

Genetic markers

Amyloid precursor protein mutations

It has long been known that AD in certain families can occur in a familial form that transmits as an autosomal dominant trait (familial early-onset AD). A major step in understanding the pathological process leading to AD was the discovery that mutations in the
TABLE 1. Summary of useful genetic and biochemical markers for Alzheimer’s disease

Early-onset, familial type
- Genetic markers
  - Presenilin-1 gene mutations
  - Amyloid precursor protein gene mutations
  - Presenilin-2 gene mutations
- Biochemical markers
  - Plasma/CSF Aβ1-42 peptide
  - CSF tau protein

Late-onset, sporadic type
- Genetic markers
  - APP mutations
  - Apolipoprotein E polymorphism

Late-onset, sporadic type
- Biochemical markers
  - Plasma/CSF Aβ1-42 peptide
  - CSF tau protein
  - CSF AD7C-NTP

Aβ1-42 peptide = amyloid β1-42 peptide; CSF = cerebrospinal fluid; NTP = neuronal thread protein.

APP gene on chromosome 21 are associated with the disease.5-8 This finding is in accordance with the observation that individuals with trisomy 21 (Down’s syndrome), who have a lifelong increase in APP expression, develop Alzheimer encephalopathy by their forties or fifties. Patients affected by Down’s syndrome develop SPs containing amyloid depositions as early as the second or third decade of life, which may be the result of an increased gene dosage of APP. In 1991, a missense mutation in the APP gene was detected in early-onset AD,9 and 1 year later a double mutation at the N-terminal of APP was found in an extended Swedish family.5 All eight reported APP missense mutations linked to AD result in chronically elevated levels of a specific APP degradation product, the Aβ1-42 peptide, in the brain. However, mutations in the APP gene are rare causes of AD, and only about 25 families have been found worldwide.8 Nevertheless, these mutations have proved to be highly informative about the pathogenic mechanisms of AD in general. For example, transgenic expression of mutant APP in mice provided the first reproducible animal models of AD.10

Presenilin mutations
A genetic linkage was found between early-onset Alzheimer families and chromosome 14.11,12 The gene product presenilin-1 (PS1), is a previously unknown protein containing 467 amino acids. To date,9,13 at least 35 different mutations in the conserved domains of the protein have been identified in families with early-onset AD and this number is increasing rapidly.

Shortly after the discovery of PS1, another gene was identified that codes for presenilin-2 (PS2). The DNA sequence, on chromosome 1, was found to be 67% homologous to that encoding for PS1. Until now, two gene mutations of PS2 have been described in AD patients.14,15 Finding a mutation in the PS1 or APP gene has a high predictive value for the development of AD. PS1 and PS2 are homologous, polytopic membrane proteins that have so far been localized in the endoplasmic reticulum (ER) and the Golgi apparatus in mammals. They are expressed in most cell types, including neurons. The presenilin holoproteins undergo endoproteolysis, generating stable N- and C-terminal fragments that associate into higher molecular mass complexes in Golgi-type vesicles.16

The most clearly identified functions of presenilins are in embryonic development17 for the proper formation of the axial skeleton, normal neurogenesis and survival of progenitor cells and neurons in specific brain subregions.18 Furthermore, PS1 regulates the intramembranous proteolysis of APP, thereby promoting the formation of Aβ.19 Different investigators have found that presenilin mutations increase γ-secretase cleavage of APP, so increasing the formation of the Aβ peptides, particularly Aβ1-42.13,20,21

Tau mutations
The tau gene has so far not been found to be a site of mutations in familial AD.

Apolipoprotein E polymorphism
An important chapter in AD concerns the role of ApoE isoforms in the disease. ApoE is a plasma protein involved in the transport of cholesterol. In the central nervous system (CNS), ApoE is produced by astrocytes and is implicated in growth and repair of the nervous system during development or after injury.

The ApoE gene is localized on chromosome 19 and presents three alleles (ε2, ε3 and ε4) determining ApoE polymorphism. Pericak-Vance et al.22 described evidence for a linkage in late-onset familial AD. Analysis of ApoE alleles in AD patients and controls demonstrated a highly significant association of ApoE ε4 and late-onset familial AD.

Approximately 40–50% of all patients with AD carry the ε4 allele, compared with 15–25% of controls.23,24 Individuals heterozygous for ε4 have a 3–4-fold increased risk of developing AD and in ε4 homozygotes there is a 6–8-fold increased risk. The disease-promoting effect of inheriting one or two ε4 alleles seems to involve enhanced aggregation and/or decreased clearance of Aβ.25-28
The e4 allele is a risk factor for, but not an invariant cause of, AD. In a meta-analysis of 5930 patients who met the criteria for probable or definitive AD and 8607 controls, the ApoE e4 allele was identified as a major risk factor in all ethnic groups studied (Caucasian, African-American, Hispanic and Japanese), across all ages between 40 and 90 years, and equally in men and women.29

To evaluate the usefulness of the ApoE genotype as an adjunct in the diagnosis of AD in persons with dementia, the data of clinical diagnoses and diagnoses obtained at autopsy in 2188 patients from 26 AD centres in the USA were pooled.30 The clinical criteria for the diagnosis of AD were highly sensitive (93%), but the specificity was low (55%), resulting in a high false positive rate.

By contrast, the sensitivity and specificity of the ApoE e4 allele were 65% and 68%, respectively. Thus, these test characteristics are not sufficient to allow the sole use of ApoE genotyping as a diagnostic test for AD, especially not in presymptomatic individuals, but should be reserved for demented patients.31 However, sequential use of ApoE genotyping in patients who fulfil the clinical criteria for AD significantly improves the specificity of the clinical diagnosis, reducing the false positive rate but also decreasing the sensitivity.

A recently found genetic polymorphism in the transcriptional regulation region of ApoE may also be linked with AD risk.32,33

Biochemical markers
Table 1 summarizes useful biochemical markers. First we describe APP metabolism in relation to the working hypotheses on the pathological mechanisms in AD, as this information is essential in evaluating the different biochemical markers.

Amyloid precursor protein metabolism
APP is a membrane-bound protein encoded by a gene on chromosome 21. It comprises a group of ubiquitously expressed polypeptides whose heterogeneity arises from both alternative splicing and post-translational processing34 (Fig. 1). Neurons express an isoform of 695 amino acid residues; non-neuronal cells throughout the body express 751/770 residue splice forms. The difference between these forms is the presence of an exon encoding for a motif of 56 amino acids, homologous to the Kunitz type of serine protease inhibitors. Functions that have been postulated for APP include inhibition of certain serine proteases, enhancement of cell-substrate adhesion, neurotropic and other growth-promoting effects, and neuroprotective properties.

APP is composed of a large extracellular domain at the N-terminal site, followed by a transmembrane domain and an intracellular domain of 47 amino acids at the C-terminus.

The biosynthetic pathway of APP begins in the endoplasmic reticulum (ER), as with all membrane proteins.35 A putative role of presenilins is the regulation of the trafficking of APP in the constitutive secretory pathway leading to full maturation of the APP holoprotein in the trans-Golgi network.

Cleavage of the 770 amino acid residue after residue 687 by a protease designated z-secretase creates a large, soluble fragment (sAPP-z) that is released from the cell surface into the lumen of the vesicle and a membrane-retained C-terminal fragment of 83 amino acids (C83) (Fig. 1). In most cell types, a minority of all APP molecules undergoes z-secretase cleavage. An alternative cleavage by b- and g-secretases leads to Aβ formation (Aβ1–40 and Aβ1–42). The proteolytic action of b-secretase on APP is essential for the generation of Aβ peptides. Recently b-secretase has been definitively identified by several groups.36 b-Secretase is identical to the b-site APP-cleaving enzyme (BACE), also known as Asp-2. Most Aβ is extracellular, and only small amounts can be detected inside the cell. Aβ can be detected in plasma and CSF of humans and other mammals. Normally, the majority of Aβ released is Aβ1–40. Only about 10% of Aβ extends to amino acid 42, Aβ1–42.9,37 The routing of APP through these pathways is under the control of phosphorylation, in particular by protein kinase C, a mechanism that is defective in sporadic AD fibroblasts. The Aβ1–42 peptide aggregates far more rapidly into amyloid fibrils than does the Aβ1–40 peptide.

Finally, the endosomal–lysosomal pathway is activated for the degradation of the C-terminal fragment left over by secretase processing and for the degradation of the full-length APP molecules recycling from the cell surface or derived directly from the biosynthetic pathway.

Working hypotheses on AD pathological mechanisms
The relationship between the observed lesions in the brain and the AD disease process has long been debated. Two broad hypotheses about the
mechanism have emerged the amyloid cascade hypothesis and the inflammatory and neurotoxic cascade hypothesis. According to the amyloid cascade hypothesis, both familial and sporadic variants of AD are caused by amyloid accumulation, especially $\text{A} \beta_{1-42}$, in the brain. Overproduction of $\text{A} \beta_{1-42}$ or failure to clear this peptide leads to AD, primarily through amyloid deposition associated with cell death. Indeed, crossing mice transgenic for mutant APP with mice expressing a PS1 mutation results in a substantially accelerated AD-like phenotype with AD-like $\text{A} \beta_{1-42}$ plaques occurring early in life. Moreover, the ability of presenilin mutations to selectively enhance $\text{A} \beta_{1-42}$ deposition in the brain has been directly demonstrated in AD patients carrying these mutations. Evidence has recently emerged to support a direct involvement of presenilins in the $\gamma$-secretase cleavage of APP.

According to the inflammatory and neurotoxic cascade theory, $\text{A} \beta_{1-42}$ accumulation and diffuse plaque formation is associated with local microglial activation, cytokine release, reactive astrocytosis and a multi-protein inflammatory response including the binding of the Clq component of the classical complement cascade by $\text{A} \beta$ and the triggering of this cascade. There is evidence that this $\text{A} \beta$-initiated inflammatory and neurotoxic process includes excessive generation of free radicals and peroxidative injury to protein and other macromolecules in neurons.

It is possible that $\text{A} \beta$ accumulation triggers the hyperphosphorylation of tau protein which precedes the assembly of these molecules into filaments. In a recent study, Schenk et al. found that, in a mouse model of AD, immunization with $\text{A} \beta$ inhibits the formation of SPs and the associated dystrophic neurites, underlining the central role of $\text{A} \beta$ in developing AD. These results raise the possibility of future vaccination against human AD.

Amyloid precursor protein and metabolites

The discovery of disease-causing mutations in the APP gene has firmly established a key role for APP and $\text{A} \beta$ in the pathogenesis of AD. Therefore, it seems reasonable to detect and quantitate APP and its metabolites in plasma, serum or CSF and determine whether changes in concentration occur during development of AD.

Amyloid precursor protein. Since APP is a membrane-bound protein, assays for APP in CSF measure the soluble or secreted derivative, generated by $\alpha/\beta$-secretase cleavage. Henriksson et al. reported markedly lower concentrations of APP in lumbar CSF of patients with AD compared with healthy controls, whereas ventricular CSF did not show any difference. Other studies did not discriminate in the site of collection of the CSF sample. In AD patients, substantially lower APP concentrations were found in some studies, while other studies showed relatively small reductions. CSF APP does not seem to be a reliable biomarker for AD.

Amyloid-$\beta$ peptide. The central pathological event in Alzheimer's disease is the deposition of $\text{A} \beta$ as amyloid fibrils within the SPs and cerebral blood vessels. It has been shown that $\text{A} \beta$ is a normal component of plasma and CSF.

Another study identified soluble $\text{A} \beta$ forms of 4, 3 and 3.7 kDa in AD brains but not in control brains free of amyloid deposits. This indicates that, in healthy brain, $\text{A} \beta$ is normally removed or bound to other proteins. Failure of this protective mechanism might cause amyloid formation and deposition in AD.

There is no significant difference in CSF total $\text{A} \beta$ peptide concentrations between AD patients, healthy controls and neurological controls, and therefore measuring total $\text{A} \beta$ ($\text{A} \beta_{1-40} + \text{A} \beta_{1-42}$) has no clear diagnostic utility. However, Nitsch et al. as well as Hock et al. showed that CSF $\text{A} \beta$ concentrations were inversely correlated with a functional measure of dementia severity, indicating that determination of CSF $\text{A} \beta$ can be used to monitor the course of the disease in an individual patient.

$\text{A} \beta_{1-40}$ and $\text{A} \beta_{1-42}$ peptides. In the cortex of two familial AD patients with an APP mutation, a remarkable predominance of $\text{A} \beta_{1-42}$-positive over $\text{A} \beta_{1-40}$-negative plaques was found. Diffuse plaques, representing the earliest stage of $\text{A} \beta$ deposition, were exclusively positive for $\text{A} \beta_{1-42}$, but completely negative for $\text{A} \beta_{1-40}$. Further, during the disease, aggregation of the more soluble $\text{A} \beta_{1-40}$ into fibrils can occur.

As shown in Table 2, several studies have reported no difference in the CSF $\text{A} \beta_{1-40}$ concentration between AD patients, healthy controls and neurological controls. However, decreased levels of $\text{A} \beta_{1-40}$ were reported in early- and mid-stage AD in one study. In contrast,
many studies have shown that concentrations of CSF $\alpha_{\beta_{1-42}}$ are significantly reduced in patients with AD compared with age-matched normal subjects or patients with neurological disease.

Presumably, concentrations of soluble $\alpha_{\beta_{1-42}}$ in brain interstitial fluid decrease as the peptide becomes increasingly insoluble and forms deposits in the form of large numbers of diffuse and senile plaques. The drop in soluble $\alpha_{\beta_{1-42}}$ concentration in brain is reflected by a decline in the concentration of soluble peptide in CSF. CSF $\alpha_{\beta_{1-42}}$ seems to be a good biomarker for AD.

In a limited number of studies, the mean concentration of plasma $\alpha_{\beta_{1-42}}$ was reported to be consistently and significantly increased in subjects with each type of mutated gene known to be related to early-onset familial AD compared with age-matched controls. However, the mean plasma $\alpha_{\beta_{1-42}}$ concentration was not increased in subjects with late-onset sporadic AD. At present, there are insufficient data on plasma $\alpha_{\beta_{1-42}}$ concentrations to allow any firm conclusions about the potential diagnostic utility of measuring plasma $\alpha_{\beta_{1-42}}$ in late-onset AD.

Studies are in progress on the diagnostic utility of detecting $\alpha_{\beta}$ peptides in human urine, but no clear conclusion is yet available.

**Tau protein**

Tau protein is a human brain phosphoprotein that binds to microtubules in the neuronal axons, thereby promoting microtubule assembly and stability. Multiple tau isoforms are produced from a single gene through alternative mRNA splicing. In adult human brain six isoforms are found, ranging from 352 to 441 amino acids.

The NFTs found in brains of AD patients are neuronal inclusions consisting of paired helical filaments (PHF), of which the main protein components are the six hyperphosphorylated tau proteins. The increased phosphorylation causes lack of binding to microtubules and is believed to be responsible for self-assembly into PHF. Current evidence suggests that protein kinases or protein phosphatases are involved in the abnormal hyperphosphorylation of tau.

It has been demonstrated that CSF tau levels increase in the early stage of AD, with the highest concentrations found in the mid-stage of the disease. These results suggest that increases in tau can be detected even in patients with very mild impairment and short duration of symptoms. It is unclear whether the elevation of CSF tau is a result of dying neurons, dystrophic neurites or the generation of NFTs.

Using enzyme-linked immunosorbent assays (ELISAs), several studies have shown that the concentrations of CSF tau are significantly elevated in AD patients compared with normal elderly control subjects. However, elevated levels of CSF tau were also detected in patients with other dementias and acute or chronic neurological diseases.

Therefore, the value of the CSF tau in discriminating AD from other neurological diseases may be limited. In addition to its potential as a diagnostic aid, simultaneous measurement of $\alpha_{\beta_{1-42}}$ and tau in the same CSF sample may become useful as a predictor of the progression to...
AD in individuals with memory impairment who do not meet clinical criteria for dementia. Detecting elevated concentrations of tau in CSF is a promising ante-mortem marker for AD, and might possibly be useful for monitoring disease progression and response to treatment. The development of assays incorporating more specific anti-tau antibodies that can distinguish normally and abnormally phosphorylated tau may enhance the discriminative power of CSF tau assays.

Neuronal thread proteins

Neuronal thread proteins (NTPs) are a family of molecules that are expressed in brain and are immunologically related to pancreatic thread protein. They are normally present in neurons and are found in large amounts in association with NFTs. There are at least six NTP immunoreactive species. Increased CNS expression of a 41-kDa NTP is correlated with dementia in AD. The gene coding for this NTP has been cloned and sequenced, and the protein (produced by recombinant techniques) has been used to generate both monoclonal and polyclonal antibodies to NTP (AD7C-NTP). Ghanbari and Ghanbari developed an ELISA with a monoclonal antibody as catcher and polyclonal antibodies for detection. In post-mortem CSF, the mean concentration of AD7C-NTP in cases of confirmed AD (n=121) was higher than in 19 age-matched control cases. In CSF from individuals with early possible or probable AD (n=89), AD7C-NTP was also elevated in comparison to the concentrations in CSF from 18 age-matched controls. The concentrations in AD patients were also elevated in comparison to controls with other neurological diseases (n=41; specificity=98%) and ambulant patients with Parkinson’s disease (n=32; specificity=84%). Therefore, NTP in CSF appears to be a promising marker for the diagnosis of AD. Additional studies are required to establish the exact sensitivity and specificity of this marker.

Apolipoprotein E

Since ApoE is associated with Aβ in SPs, several groups have measured ApoE in CSF, in addition to ApoE genotyping. Plasma ApoE seems to have limited ability to cross the blood–brain barrier and ApoE in CSF is essentially derived from the brain. Therefore, the CSF concentration may reflect cerebral ApoE production. ApoE is known to have a general function in brain repair. After injury, ApoE is produced and secreted by astrocytes, to scavenge cholesterol and other membrane lipids from degenerating axons and myelin sheets. At the time of sprouting and remyelination, neuronal growth cones take up and re-use the lipids in membrane and myelin synthesis. This process of membrane lipid re-utilization may be an important repair mechanism in various degenerative brain disorders, and impairment of this mechanism might contribute to earlier presentation of degenerative disorders. Increased re-utilization of ApoE–lipid complexes in the brain may explain the lower concentration of CSF ApoE in AD. However, ApoE has also been found to bind to Aβ in vitro, and to be adsorbed onto the Aβ deposits in

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**Table 3.** Cerebrospinal fluid tau protein concentrations of Alzheimer’s disease (AD) patients, healthy controls and controls with neurological diseases (NC) or non-Alzheimer-type dementia (DNAT) as reported in different studies

<table>
<thead>
<tr>
<th>Ref.</th>
<th>AD</th>
<th>n</th>
<th>Controls</th>
<th>n</th>
<th>NC/DNAT</th>
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<td>190 (80)</td>
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<td>77</td>
<td>1430 (739)</td>
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<td>816 (355)</td>
<td>14</td>
<td>790 (579)</td>
<td>26</td>
</tr>
<tr>
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<td>37</td>
<td>212 (102)</td>
<td>20</td>
<td>168 (63)</td>
<td>32</td>
</tr>
<tr>
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<td>14</td>
<td>380 (120)</td>
<td>36</td>
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<tr>
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Results are given in ng/L and expressed as mean (standard deviation). n = number of patients.
SPs. Moreover, ApoE binds tau protein, the principal component of NFTs. Yamada et al. found increased mRNA levels encoding for ApoE in AD brain, which suggests increased ApoE production.

Various studies reported significantly reduced CSF ApoE concentrations in AD patients compared with healthy controls, irrespective of ApoE genotype. However, other investigators found no difference or a significant increase. The control patients with neurological disease generally demonstrated similar concentrations to those of the AD patients, thus limiting the usefulness of ApoE quantitation.

**Melanotransferrin**

In several neurodegenerative diseases, including AD and Parkinson's disease, large quantities of iron are deposited in the CNS. Iron depositions can directly harm tissues by catalysing the generation of oxygen radicals. Jefferies et al. have identified a novel pathway of iron uptake into mammalian cells, independent of the transferrin receptor. The melanotransferrin molecule, also known as p97 protein, is essential for this pathway. In brain tissue derived from AD patients, melanotransferrin was detected in a subset of reactive microglia associated with SPs, indicating that iron uptake through this alternative pathway plays a role in AD.

Kennard et al. have shown that p97 concentrations [mean (standard deviation)] are consistently elevated in the serum of AD patients [43.8 (11.6) ng/mL; n = 17] compared with controls [7.0 (3.3) ng/mL; n = 15]. There was no overlap between the groups, and the correlation between age and p97 serum concentration was not significant. However, a significant correlation was found between disease progression and increased p97 serum concentrations. Extrapolation of these data suggests that the p97 concentration may begin to increase about 2 years before the first clinical symptoms of AD. Quantitation of melanotransferrin in serum is a promising candidate biomarker for AD.

**Apolipoprotein J**

ApoJ (clusterin) is a lipoprotein present in most, if not all, physiological fluids, in particular in plasma and CSF. In normal CSF, ApoJ appears to be complexed with $\alpha$-amyloid, in particular with $\alpha$-amyloid 1-40. Using a well-characterized in situ perfused guinea pig brain model, it was demonstrated that ApoJ may facilitate receptor-mediated transport of $\alpha$-amyloid complexes across the blood–brain barrier and the blood–CSF barrier.

ApoJ is overexpressed in response to neuronal injury. Available information supports the view that its basic function is maintenance of membrane integrity in an environment that exposes cells to membrane-destructive factors that occur normally or are generated in abnormal, degenerative situations. ApoE and ApoJ may have complementary functions in lipid homeostasis during CNS degeneration and synaptic remodelling. However, the roles may be opposite with respect to $\alpha$-amyloid deposition in SPs, in which ApoE acts as a pathological chaperone, whereas ApoJ would be functioning as a physiological chaperone of $\alpha$-amyloid. ApoJ concentrations in the hippocampus of AD subjects were 60% higher ($P<0.001$) than in non-demented controls. In post-mortem human brains, ApoJ concentrations have been shown to be about 40% higher in AD patients than in controls.

As elevation of ApoJ may be indicative of tissue injury, it will be important to measure its concentrations in CSF. To date, no method for measuring ApoJ in CSF has been published, but Jenkins et al. presented a competitive ELISA for plasma ApoJ. It might be possible to modify this method for quantitation of ApoJ in CSF.

**S-100 protein**

S-100 is located in the cytoplasm and nuclei of cells that express it, as well as in synaptosomes and synaptic membranes. S-100 is a dimer comprising two separate subunits, $\alpha$ and $\beta$. The subunit S-100-$\beta$ has been implicated in development and maintenance of the nervous system and may also play a role in neuropathology because of its specific localization and selective overexpression in AD.

CSF concentrations of S-100 protein in AD, and also in patients with frontotemporal dementia and other non-neurological conditions, were found to be significantly increased in comparison with healthy controls, pointing to a lack of specificity of S-100. Nevertheless, further studies on the value of serum and CSF S-100 concentrations in the diagnosis of AD seem to be justified.

**Glycosylated acetylcholinesterase**

There is no consistent change in acetylcholinesterase (AChE) activity in the CSF of AD patients, and the large overlap with controls.
prevents the use of AChE as a diagnostic marker for AD.

As AChE in the AD brain is highly glycosylated, the hydrophobic property of anomalous AChE may serve as a seed for amyloid fibrils in senile plaques. Lectin-binding analysis of CSF AChE of AD patients showed a significant difference in glycosylation (P < 0·01) compared with controls. Preliminary results suggest that glycosylated CSF AChE may be a diagnostic marker for AD, with a sensitivity as high as 80% and a specificity of 97%. Combination of markers

The ApoE genotype does not influence CSF total Aβ concentration, as similar values of CSF Aβ are found in AD patients with ApoE ε3/3, ε3/4 and ε4/4 alleles. However, Galasko et al.68 demonstrated a negative correlation between CSF Aβ1–42 levels and the number of ApoE ε4 alleles.

It has been reported that increased concentrations of CSF tau protein are independent of ApoE genotype,79 as well as age of disease onset and clinical stage. However, in other studies,119,120 CSF tau concentration was found to be related to ApoE genotype. AD patients carrying the ApoE ε4 allele demonstrated higher CSF tau concentrations than AD patients without the ε4 allele, and the highest value of CSF tau was found in patients with two ε4 alleles. The ApoE genotype should be considered in interpreting CSF tau concentrations. Determination of the ApoE genotype can increase the specificity and sensitivity of the clinical diagnosis.

Combined analysis of Aβ1–42 and tau in CSF64 is interesting. In a plot of tau versus Aβ1–42, the high tau/low Aβ1–42 quadrant was highly predictive for AD, whereas the low tau/high Aβ1–42 quadrant contained control individuals only. It was found that patients with low CSF Aβ1–42 and high CSF tau have a strong likelihood of having AD (22/23 = 96%). Conversely, patients who exhibit low tau and elevated Aβ1–42 were free of AD (282/28 = 100%).

These results have been reproduced by Mulder et al. (unpublished data). Eighteen controls (18/20) and no patients (0/20) exhibited low tau/high Aβ1–42, and one control as well as 18 AD patients showed high tau/low Aβ1–42 values. Similar results were obtained in a Japanese multicentre study,67 and the sensitivity improved in a longitudinal evaluation.

Galasko et al.58 studied 82 patients with probable AD, including 24 with very mild dementia [Mini-Mental State Examination (MMSE) score ≥ 23/30], 60 normal elderly control subjects and 74 subjects with neurological disorders. High CSF tau and low CSF Aβ1–42 levels discriminated AD patients from elderly controls. However, in subjects in the neurological control group with a CSF protein profile suggesting AD, follow-up at autopsy would be required to decide whether the CSF results are false positives or whether AD is the primary or a concomitant cause of dementia. The multicentre study of Hulstaert et al.69 also confirmed the value of a combined determination of markers.

We conclude that the combined analyses of CSF Aβ1–42 and CSF tau can discriminate AD patients from normal elderly control subjects, supporting the use of these parameters to distinguish early AD from ageing.

To discriminate AD from other forms of dementia, especially vascular and frontotemporal dementia, further studies are needed to develop more sensitive methods for current and potential markers.

Other potential markers

AD involves profound biochemical and molecular alterations in the CNS. Increased phosphorylation of tau and other cytoskeletal proteins has been demonstrated in neurons,121 as well as aberrant expression of genes modulated with neuritic sprouting such as the growth-associated protein GAP-43,122 constitutive endothelial nitric oxide synthase,123 transforming growth factor-β124 and metallothionein-3.125 An increased expression of genes associated with glial cell activation, such as glial fibrillary acidic protein126 and z1-antichymotrypsin127 is also found. Moreover, there are alterations in expression of genes coding for proteins protecting neurons from either cytotoxic or programmed cell death, including calpain-2,128 cathepsin D,129 superoxide dismutase 1,130 mitochondrial cytochrome oxidase,131 α1Cq component of complement,132 Calbindin D28k133 and Bel-2.134

Advanced glycation end-products (AGEs) may play an important role in the pathogenesis of AD. These are mainly found in NFTs and in about 5% of the SPs. However, SPs and NFTs were also AGE-positive in non-Alzheimer neurodegenerative diseases.135 All these alterations in the CNS may lead to changes in CSF concentrations of AGEs.
Another recent finding is the presence of molecular misreading mutant forms of APP and ubiquitin in SPs and NFTs in AD brains.\textsuperscript{136} In this study, mutant APP was detected in 15/21 AD patients, while aberrant ubiquitin was present in all patients. CSF ubiquitin is largely of intrathecal origin, which indicates that mutant ubiquitin may also be present in CSF and might be a sensitive biomarker for AD.\textsuperscript{137}

**CONCLUSIONS**

Our present knowledge of the aetiology of AD mainly focuses on the amyloid cascade hypothesis. The genetic and the sporadic variants may have a common pathophysiology, where disturbances in APP metabolism occur as an early event.

Genetic markers such as PS1, PS2 and APP have been found to be reliable markers in diagnosing familial AD. A large polymorphism in the gene encoding for \( z_2 \)-macroglobulin has been associated with an increased risk for late-onset AD.\textsuperscript{138}

It can be predicted that, within a decade, a sizeable number of additional genes will be implicated, most of them probably acting as polymorphic risk factors in some populations.\textsuperscript{10} There is consensus that searching for PS1, PS2 and APP genetic markers should be limited to probands and families with a pattern of early-onset AD, in a strict research setting.

Concentrations of CSF \( \text{A}\beta_{1-42} \) are significantly reduced in patients with familial AD in comparison to controls with neurological disease and normal subjects. In sporadic AD, significantly decreased levels of CSF \( \text{A}\beta_{1-42} \) are found in many patients, but there is some overlap with the control groups.

Measuring CSF \( \text{A}\beta_{1-42} \) in conjunction with other parameters, particularly CSF tau protein and ApoE genotype, could be potentially useful for supporting early diagnosis of AD. Commercial kits are available for quantitation of tau protein and \( \text{A}\beta_{1-42} \) as well as determination of the ApoE genotype.

AD\textsuperscript{7}C-NTP is elevated in CSF samples from individuals with early possible or probable AD, in comparison to the CSF concentrations of age-matched controls. The concentrations in AD patients were higher than those in controls with neurological disease and ambulant patients with Parkinson’s disease. Therefore, CSF AD\textsuperscript{7}C-NTP appears to be a promising marker for the diagnosis of AD. However, additional studies are required to confirm the sensitivity and specificity of this test.

ApoE genotyping might be reserved for patients who meet the clinical criteria for AD, as the ApoE genotype in those patients can significantly improve the specificity of the clinical diagnosis, reducing the false positive rate but also decreasing the sensitivity.

Serum melanotransferrin concentrations and CSF glycolysated AChE are elevated in AD patients. Preliminary studies of these markers seem promising, but further work is necessary.

Age-related increase in CSF S-100 concentration may be important in the pathogenesis of AD. However, increased concentrations of S-100 are also found in the CSF of patients with frontotemporal dementia or following cardiac operations or head trauma, stressing the lack of specificity of this marker.

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