Molecular misreading of the β-amyloid precursor protein (APP) gene generates mRNA with dinucleotide deletions in GAGAG motifs. The resulting truncated and partly frameshifted APP protein (APP\(^{+1}\)) accumulates in the dystrophic neurites and the neurofibrillary tangles in the cortex and hippocampus of Alzheimer patients. In contrast, we show here that neuronal cells transfected with APP\(^{+1}\) proficiently secreted APP\(^{+1}\). Because various secretory APP isoforms are present in cerebrospinal fluid (CSF), this study aimed to determine whether APP\(^{+1}\) is also a secretory protein that can be detected in CSF. Post-mortem CSF was obtained at autopsy from 50 non-demented controls and 122 Alzheimer patients; all subjects were staged for neuropathology (Braak score). Unexpectedly, we found that the APP\(^{+1}\) level in the CSF of non-demented controls was much higher (1.75 ng/ml) than in the CSF of Alzheimer patients (0.51 ng/ml) (\(p < 0.001\)), and the level of APP\(^{+1}\) in CSF was inversely correlated with the severity of the neuropathology. Moreover the earliest neuropathological changes are already reflected in a significant decrease of the APP\(^{+1}\) level in CSF. These data show that APP\(^{+1}\) is normally secreted by neurons, preventing intraneuronal accumulation of APP\(^{+1}\) in brains of nondemented controls without neurofibrillary pathology.

Alzheimer's disease (AD)\(^1\) is a progressive neurodegenerative disease and the most common form of dementia in aged populations (1). The major constituent of the extracellular plaques in the brains of AD patients is amyloid β (A\(β\)), which is cleaved from the β-amyloid precursor protein (APP) by β and γ secretases. Patients suffering from the hereditary forms of AD either carry a mutation in the APP gene or in one of the presenilin genes. These mutations cause an alteration in the proteolytic processing of APP, resulting in the formation of more A\(β_{40}\) and A\(β_{42}\), which are both prone to aggregate and precipitate in the plaques (2, 3).

Genomic mutations are not the only source of aberrant APP proteins in AD. We (4) and others (5) have reported that small frameshift mutations occur in APP transcripts near short simple repeats. The observed dinucleotide deletions, such as ΔGA, in the GAGAG motif of exon 9 or 10 of APP, result in the translation of an aberrant APP protein, i.e. APP\(^{+1}\), which accumulates in the neurofibrillary tangles, neuropil threads, and dystrophic neurites of the neuritic plaques of AD and Down syndrome patients (4, 6). APP\(^{+1}\) is a truncated APP protein of 348 amino acids with a wild-type N terminus and an aberrant C terminus translated in the +1 reading frame. The different isoforms of full-length APP are type I transmembrane proteins, which are proteolytically cleaved by secretases (7, 8) to form secretory APPs and A\(β_{40}\) or A\(β_{42}\) and p\(3\) (9, 10). These secretory APP proteins, sAPP\(α\) and sAPP\(β\) (11–13), and A\(β\) (14) are detectable in cerebrospinal fluid (CSF) as well as in human brain homogenates (15). The present study focuses on APP\(^{+1}\), which consists of the N-terminal 329 amino acids of the APP\(_{695}\), including the signal peptide, and a unique 19-amino acid C terminus (Fig. 1). A major difference between APP\(^{+1}\) and APP, except for their distinct C termini, is the lack of the membrane anchor (amino acids 625–648 of APP\(_{695}\)) and the lack of the Aβ sequence in APP\(^{+1}\). The presence of the signal peptide and the absence of the membrane anchor make it very likely that APP\(^{+1}\) is also a secretory protein. Indeed, it has been shown that an APP\(^{+1}\)-enhanced green fluorescent protein fusion protein is readily secreted from rat neuronal cell lines (16). The aim of the present study was to determine whether the endogenous human APP\(^{+1}\) is a secretory protein and can be detected in human CSF.

**EXPERIMENTAL PROCEDURES**

Antibodies—The AMY6 peptide (YNVPHERMGRRTSSKELA) represents the final 19 amino acids of APP\(^{+1}\) ΔGA exon 9 (see Fig. 1). The peptide was coupled to thyroglobulin by glutaraldehyde, and a rabbit was immunized with a mixture of the coupled peptide and Freund's complete adjuvant (1:1). Several bleedings were collected, and the immunoreactivity of the serum was tested with a spot blot. To the peptide was coupled to thyroglobulin by glutaraldehyde, and a rabbit was immunized with a mixture of the coupled peptide and Freund's complete adjuvant (1:1). Several bleedings were collected, and the immunoreactivity of the serum was tested with a spot blot. To the

This paper is available online at http://www.jbc.org
and Western blot. As this peptide contains an additional N-terminal tyrosine it can easily be iodinated.

22C11 is a monoclonal APP antibody directed against amino acids 66–81 (17). The antibody directed against the AMY6 peptide is used for the RIA and Western blot with pre-immune serum, the AMY6 antibody, and pre-adsorbed AMY6 antibody.

Cell Lines and Transfections—The human neuronal SH-SY5Y cell line (American Type Culture Collection number CRL-2260) (18) was cultured in high glucose Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal calf serum, 100 IU/ml penicillin and 100 µg/ml streptomycin (all media and chemicals from Invitrogen). For Western blot analysis the cells were seeded in 6-cm dishes (Nunc, Roskilde, Denmark). The next day, cells were transfected with 10 µg of plasmid DNA per 6-cm dish according to the calcium-phosphate transfection method. The human APP 695 isoform (kindly provided by Dr. T. Hartmann, Heidelberg, Germany) and APP-1 (APP 695 ΔGA exon 9) were cloned into pcDNA3 (Invitrogen). The full sequence of both constructs was confirmed by sequencing.

Human Cerebrospinal Fluid—Post-mortem non-hemolytic ventricular CSF was obtained from 50 non-demented controls and 122 neurologically confirmed AD patients. In addition, brain homogenates from four non-demented controls and eight AD patients were obtained. The CSF samples and brain tissues were collected by the Netherlands Brain Bank, Amsterdam (coordinator Dr. R. Ravid). Sex, age, brain weight, post-mortem delay, pH of CSF, and clinicopathological data of the patients described in this study can be found in the supplemental material in the on-line version of this article. The concentration of protein content of the CSF was determined with a Bradford assay (19).

Western Blotting—Approximately 24 h after transfection, the medium was collected and the cells were resuspended in 0.1 mM NaCl, 0.01 mM Tris-HCl, and 1 mM EDTA pH 7.6, containing the protease inhibitors phenylmethylsulfonyl fluoride (100 µM) and leupeptin (10 µg/ml). Fifteen microliters of human post-mortem CSF was directly mixed with 15 µl of loading buffer (50 mM Tris-HCl, pH 6.8, 100 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue, and 10% glycerol) and put on a gel. Cell culture medium, cell lysates, and CSF were fractionated on SDS-PAGE (7.5% gel) and transferred semi-dry to nitrocellulose (Protran BA85; Schleicher & Schuell). The blots were incubated overnight at 4 °C with a polyclonal rabbit anti-human APP-1 (AMY6 bleeding 050897; 1:10,000) and 10 µl of antiserum (AMY6; 1:10,000) and 10 µl (10,000 cpm) of 125I-AMY6 peptide. AMY6 peptide was iodinated by the chloramine T method. To precipitate the antibody peptide/protein complex, 50 µl of cellulose coated with a secondary antibody against rabbit IgG (Saccel; IDS LTD, Boldon, UK) was added and incubated at 4 °C for 1 h. The samples were centrifuged at 5,000 rpm for 15 min, and the pellets were counted in a Cobra γ-counter for 5 min. The sensitivity of the RIA is 15 pg of APP-1 per 50 µl.

Characteristics of APP-1 RIA—The AMY6 peptide was iodinated and subsequently purified on a Sepharose G25 column to separate free 125I-sodium, non-iodinated AMY6, and 125I-AMY6. The binding capacity of five different bleedings of the AMY6 antibody and the pre-immune serum to 125I-AMY6 was tested. A maximum binding of 80% could be reached with an antibody dilution of 1:1000. A clear increase in binding capacity was observed between the first bleeding (170697) and the later bleedings of the AMY6 peptide. Pre-immune serum did not bind to 125I-AMY6 peptide at all. All subsequent RIAs were done using AMY6 bleeding 050897.

The optimal antibody-peptide binding for the APP-1 RIA was reached with AMY6 bleeding 050897 at a dilution of 1:20,000. We performed displacement curves by adding an increasing amount of non-labeled AMY6 peptide or recombinant His6-APP-1 to AMY6 (dilution 1:20,000, bleeding 050897; results not shown). The slopes of the graphs obtained with peptide and the recombinant protein are identical, indicating that this assay can reliably measure full-length APP-1.

In our subsequent experiments we used the AMY6 peptide in the standard curves of the RIA.

Statistical Analysis—The Kruskal-Wallis nonparametric analysis of variance with multiple comparison of groups (20) was used to test differences between groups (program developed by J.M. Ruijter (Department of Anatomy and Embryology, Academic Medical Center, Amsterdam).

RESULTS

APP-1 Is Secreted by Human Neuronal Cells—To determine whether APP-1 is a secretory protein like sAPPα and sAPPβ, we transfected human neuronal SH-SY5Y cells with the APP and APP-1 pcDNA3 constructs. The expression of APP and APP-1 was driven by the cytomegalovirus (CMV) promoter to ensure high expression. The cells and their supernatants were harvested 1 day after transfection. Western blots of the cell pellets and the supernatants were probed with the 22C11 antibody (Fig. 2A) directed against residues 66–81 of APP as well as APP-1. The AMY6 antibody (Fig. 2B), directed against the unique C terminus of APP-1, was used to detect APP-1 specifically. The Western blot analysis revealed the presence of both APP (Fig. 2A, #) and APP-1 (Fig. 2A, ◊) in the cell lysate (Fig. 2A, lys) and the cell culture medium (Fig. 2A, med) with the 22C11 antibody. In addition, endogenous APP is visible in all transfection conditions (Fig. 2A, arrowheads). A second blot of the same experiment was probed with APP-1 specific antibody AMY6 and showed only staining of the APP-1 proteins (Fig. 1, left).
of APP the doublet consists of an nates, we measured significantly lower levels of APP Brain Bank. In contrast with the data on the cortex homoge-
tween non-demented controls and AD patients, we assayed
APP and sAPP are only detected by the AMY6 antibody (*,
the groups was found in the post-mortem delay; however, no
difference between the control subjects and the Alzheimer pa-
tients. The value of the pH of CSF is an indication of the agonal
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and the subject and pH of the CSF, there was no significant
proteins represent endogenous APP
proteins might be
the non-demented control group (Fig. 3
2C11 in CSF and determine the differences be-
quantitation of APP
APP 1 is present in the cell culture medium, which is likely
to be the fully processed, glycosylated form of APP 1. This band
does not run exactly at the same height as the higher APP 1
band in the cell pellet because of the presence of albumin in the
cell culture medium. In the lanes of the mock transfected cells
as well as the APP transfected cells, two bands, which repres-
ents proteins with an apparent mass between 50 and 60 kDa,
reacted with the 22C11 antibody. These proteins might be
degradation products of APP or N-terminal fragments. Because
AMY6 does not react with these bands, it is excluded that these
proteins represent endogenous APP
Quantification of APP 1—The RIA developed to measure
APP 1 in CSF was validated by analyzing temporal and frontal
cortex homogenates of four non-demented controls and eight
AD patients (Table I). The latter have a confirmed GA deletion
in either exon 9 or exon 10 in part of the APP transcripts (4). In
the cortex homogenates of AD patients a 3.4-fold increase in
intracellular APP 1 is present. In the cell culture medium, which is likely
on a Western blot. This protein is produced in a pro-
karyotic expression system, and, therefore, no glycosylation of
APP 1 takes place. Furthermore, six histidines are added to
the N terminus, and the signal peptide sequence is not cleaved
from His6-APP 1. Consequently, His6-APP 1 on a Western blot. This protein is produced in a pro-
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50–60 kDa band indicated with
APP 1 band, because this band has
been significantly reduced in the blot probed with pre-adsorbed
antibody (Fig. 4B, lane 1). In agreement with the findings in
the culture medium of the APP 1 transfected SH-SY5Y cells,
only the fully processed form of APP 1 protein is secreted. Fig.
4C provides a direct comparison between His6-APP 1, APP 1
in lysate, and APP 1 in a cell culture medium of APP 1 trans-
jected cells. On the same gel, the two different pre-stained
molecular mass markers used in the study were loaded, i.e. in
the left lane (Fig. 4C) with the Multimark® multi-colored
standard of Invitrogen and in the right lane (Fig. 4C) with the
Rainbow marker of Amersham Biosciences. It is clear from this

2B, *) in the cell lysate (Fig. 2B, lys) and the cell culture
medium (Fig. 2B, med). Recombinant APP (from the trans-
fected cells; Fig. 2A, #), endogenous APP and sAPP (Fig. 2A,
arrowsheads) was stained with 22C11 and not at all with the
APP 1-specific antibody AMY6. The intracellular APP 1 is
present in two forms, probably reflecting the non-glycosylated
and O-glycosylated forms of APP 1. Such a doublet has also
been observed by others for APP 695, and they also argue that
the doublet consists of an O-glycosylated and non-O-glycosy-
lated form (21). In addition, Hersberger et al. (16) have also
discussed the O-glycosylation of APP 1. Only the higher band
of APP 1 is present in the cell culture medium, which is likely
to be the fully processed, glycosylated form of APP 1. This band
does not run exactly at the same height as the higher APP 1
band in the cell pellet because of the presence of albumin in the
cell culture medium. In the lanes of the mock transfected cells
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in either exon 9 or exon 10 in part of the APP transcripts (4). In
the cortex homogenates of AD patients a 3.4-fold increase in
intracellular APP 1 levels could be measured compared with the
non-demented control group (Fig. 3A). To establish the
presence of APP 1 in CSF and determine the differences be-
 tween non-demented controls and AD patients, we assayed
post-mortem ventricular CSF collected by the Netherlands
Brain Bank. In contrast with the data on the cortex homoge-
nates, we measured significantly lower levels of APP 1 in CSF
samples of AD patients (Fig. 3B). CSF of non-demented
controls contained 3.4 times more APP 1. With respect to the age
of the subject and pH of the CSF, there was no significant
difference between the control subjects and the Alzheimer pa-
tients. The value of the pH of CSF is an indication of the agonal
state of the patients (22, 23). A significant difference between the
groups was found in the post-mortem delay; however, no
correlation was found between post-mortem delay and APP 1
levels or protein levels. Furthermore, a significant decrease in
brain weight of AD patients, which is an inevitable character-
istic of the disorder, as well as a significant decrease in CSF
total protein content were observed (Table II).

| CON | AD | Mann-Whitney p value |
|-----|----|---------------------|
| Total number of patients | 4 | 8 | |
| Male | 3 | 4 | |
| Female | 1 | 4 | |
| Age (years) | 61.5 | 75.5 | 0.306 |
| pH | 6.59 | 6.56 | 0.349 |
| Brain weight (g) | 1332.5 | 1100 | 0.089 |
| APP 1 (ng/mg protein) | 0.75 | 2.55 | 0.042* |

* Significant statistical difference between non-demented controls and AD patients.

Western Analysis of APP 1 in CSF—The nature of the APP 1
immunoreactivity in CSF was determined by a Western blot.
First, we determined whether the AMY6 antibody, which is
directed against the AMY6 peptide, will stain purified His6-
APP 1 on a Western blot. This protein is produced in a pro-
karyotic expression system, and, therefore, no glycosylation of
APP 1 takes place. Furthermore, six histidines are added to
the N terminus, and the signal peptide sequence is not cleaved
off from His6-APP 1. Consequently, His6-APP 1 (Fig 4A, lane
3) will run at a slightly higher molecular mass than the se-
creted endogenous APP 1 (−50–60 kDa band indicated with an
asterisk) in Fig. 4B, lane 2). The same blot probed with a
buffer without the first antibody or with pre-adsorbed AMY6
showed no signal at all (Fig. 4A, lanes 1 and 2). In human CSF
of a non-demented control (NB92-030, female, 78 year-old,
Apoe 33), a banding pattern was observed after staining with
the AMY6 antibody (Fig. 4B, lane 2). The band of −50–60 kDa
(asterisk) is most likely the APP 1 band, because this band has
been significantly reduced in the blot probed with pre-adsorbed
antibody (Fig. 4B, lane 1). In agreement with the findings in
the culture medium of the APP 1 transfected SH-SY5Y cells,
only the fully processed form of APP 1 protein is secreted. Fig.
4C provides a direct comparison between His6-APP 1, APP 1
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the left lane (Fig. 4C) with the Multimark® multi-colored
standard of Invitrogen and in the right lane (Fig. 4C) with the
Rainbow marker of Amersham Biosciences. It is clear from this

![Fig. 2. APP and APP 1 in neuronal cells. Western blot of lysates (lys) and cell culture medium (med) of human neuronal SH-SY5Y cells transfected with pcDNA3-CMV-APP 1 (APP) or pcDNA3-CMV-APP 1 (APP 1). The blots were probed with the 22C11 antibody (A) or the AMY6 antibody (B). APP (#, panel A) and APP 1 (*, panel A) are stained with 22C11 in cell lysate and culture medium. A specific staining for APP 1 is observed with the AMY6 antibody (*, panel B). Endogenous APP and sAPP are only detected by the 22C11 staining (arrowheads).]
28 male non-demented controls (median age 78) and 122 CSF samples from 90 female and 32 male AD patients (median age 79.5) were analyzed;

on-line version of this article. Data are depicted as median values. CON, control; PMD, post-mortem delay.

| Table II Determination of APP\(^{1}\) in CSF of non-demented controls and AD patients |
|---------------------------------|-----------------|-----------------|-----------------|
| Total number of patients        | CON 50          | AD 122          |
| Male                            | 28              | 32              |
| Female                          | 22              | 90              |
| Age (years)                     | 78.0            | 79.5            | 0.057           |
| PMD (min)                       | 385             | 255             | <0.001*         |
| pH                              | 6.70            | 6.62            | 0.325           |
| Brain weight (g)                | 1302            | 1105            | <0.001*         |
| Protein (\(\mu g/ml\))         | 439             | 272             | <0.001*         |
| APP\(^{1}\) (ng/ml)             | 1.75            | 0.51            | <0.001*         |
| APP\(^{1}\) (ng/mg protein)     | 3.34            | 1.95            | 0.003*          |

\(\ast\) Significant statistical difference between non-demented controls and AD patients.

Fig. 3. Quantification of APP\(^{1}\). Presence of APP\(^{1}\) in brain homogenate (A) and CSF (B) of non-demented controls (CON, open boxes) and AD patients (gray boxes) as measured with a RIA. The antibody used in the RIA was directed against the AMY6 epitope (Fig. 1). The boxes depict the 25th and 75th percentile values, with the median value given by the horizontal line in each box. The whiskers range from the 10th to the 90th percentiles. Total protein was measured with a Bradford assay (19). The amount of APP\(^{1}\) is corrected for protein content. In panel A, CON n = 4 and AD n = 8; significance tested with a Mann-Whitney (20) nonparametric test, #, \(p = 0.042\). In panel B, 50 CSF samples from 22 female and 28 male non-demented controls (median age 78) and 122 CSF samples from 90 female and 32 male AD patients (median age 79.5) were analyzed; significance tested with a Mann-Whitney (20) nonparametric test, ** \(p < 0.001\).
Thus far we had only observed the presence of APP mRNA in the hippocampus and cortex of AD and Down syndrome patients and the APP protein in the neuritic plaques and neurofibrillary tangles of these brain areas in AD and Down syndrome patients (4, 6).

The failure to detect a GA deletion in the mRNA of APP in non-demented controls can be explained by the relatively low sensitivity of the immunoscreening assay we used in our earlier study (4). In the cDNAs from APP mRNA isolated from the cortex and hippocampus of AD brains, we found between 2 and 12 APP immunopositive clones of 20,000. It is therefore conceivable that we missed the mutation in the two non-demented control patients we screened in that study. Recent extensive studies on the frequency of molecular misreading of APP in the cell lines and temporal cortex of non-demented control, AD, and Down syndrome patients (4, 6).

The analysis of the CSF samples of non-demented controls and AD patients showed clearly that the APP level was significantly decreased in neuropathologically confirmed AD patients (Braak score 4–6). The RIA technique to measure APP is highly specific, because there is direct competition between endogenous APP and iodinated-peptide for the same antibody. Fig. 4B shows that the APP antibody used in this study, AMY6, recognized several different proteins on Western blots.

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Thus far we had only observed the presence of APP mRNA in the hippocampus and cortex of AD and Down syndrome patients and the APP protein in the neuritic plaques and neurofibrillary tangles of these brain areas in AD and Down syndrome patients (4, 6). The failure to detect a GA deletion in the mRNA of APP in non-demented controls can be explained by the relatively low sensitivity of the immunoscreening assay we used in our earlier study (4). In the cDNAs from APP mRNA isolated from the cortex and hippocampus of AD brains, we found between 2 and 12 APP mRNA, cell lysate of APP, transfected SH-SY5Y cells, and the culture medium of these cells were put on one gel to compare directly the differences in molecular mass between these different APP proteins. The blot is stained with the AMY6 antibody. The molecular mass marker on the left is the MultiMark® multi-colored standard of Invitrogen, and the molecular mass marker on the right is the Rainbow marker of Amersham Biosciences.
and sAPP (12), are decreased in CSF of AD patients. Another explanation for the drop in the concentration of APP*1 is that the enlargement of the ventricles may cause a subsequent dilution of CSF proteins, because the volume of ventricular CSF in AD patients has been shown to be twice as much as in non-demented controls (29). Furthermore, ventricular dilatation and reduced CSF production will presumably result in a progressive reduction in CSF turnover during aging (30). An alternative reason for the reduced levels of APP*1 in the CSF of AD patients could, therefore, be the proteolytic breakdown of APP*1 in CSF. From the patients we analyzed, there is no information available on the degree of ventricular dilatation. Therefore, we decided to measure the total protein content of the CSF, which will provide information on the dilution of CSF and, therefore, these data might reflect the degree of ventricular dilatation. As can be observed in Fig. 5B, the protein content of CSF obtained from AD patients with Braak stage 4–6 is significantly less compared with that from the patients with Braak 0–2. It is also clear from Fig. 5 that dilution of the CSF is not the cause of the decrease in APP*1 levels. A strong argument in favor of a specific reduction of APP*1 secretion in patients with early AD changes comes from the Braak stage 1 group. In those controls less than half of the APP*1 levels of the Braak stage 0 group has been found (Fig. 5A), but no decline in total protein was observed (Fig. 5B). It is highly unlikely that a ventricular dilatation by more than a factor two would already have occurred in these non-demented controls with only very mild AD changes.

The clinical diagnosis of probable and possible AD is largely based on neuropsychological examinations. Although the accuracy of the clinical diagnosis has improved, a definite diagnosis still can only be made after autopsy (1). A biomarker that can aid the clinical diagnosis and even detect early neuropathological changes would be extremely valuable (31–34). In this respect, CSF markers are expected to be useful, because the biochemical processes in the brain are likely to be reflected by the proteins that are present in the CSF. Aβ42 and sAPP levels have often been reported to be decreased in CSF of AD patients, whereas Aβ40 levels were unchanged. The former finding also indicates that there is an initial problem with protein secretion in neurons. Another protein that plays an important role in the pathogenesis of AD is the microtubule-associated protein tau (35). The level of tau protein is increased in CSF of AD patients (36) and patients with mild cognitive impairments (37), probably reflecting neuronal death. Recently, it has been shown that altered tau and Aβ42 concentration can help to diagnose AD patients in subjects with mild cognitive impairments (38). In post-mortem ventricular CSF it has been shown previously that the melatonin concentration is closely correlated to the Braak stage. In patients with more severe neuropathology a lower concentration of melatonin was measured (28), which is in agreement with our findings. In conclusion, the measurement of AD-related proteins in CSF, including APP*1, can be of great use in improving the clinical diagnostic accuracy of AD (24), which is, at present, only definite after autopsy.

In this manuscript we show that APP*1 is a 50–60 kDa secretory protein. Furthermore, we provide evidence that APP*1 is already retained in neurons in the brains of non-demented controls with initial AD pathology. This retention probably reflects an impaired capacity of protein secretion and other early pathological changes in affected neurons or dying neurons. Measuring levels of secretory proteins, like APP*1, in CSF can help to reveal these early deficits in the function of neurons. Above all, the strong correlation between APP*1 levels in CSF and observed pathological changes could help in diagnosing AD at an early stage.
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REFERENCES

1. Cummings, J. L., and Cole, G. (2002) J. Am. Med. Assoc. 287, 2335–2338
2. Hardy, J., and Selkoe, D. J. (2002) Science 297, 353–356
3. Selkoe, D. J. (2001) Physiol. Rev. 81, 741–766
4. Van Leeuwen, F. W., De Kleijn, D. P. V., Van den Hurk, H. H., Neubauer, A., Sonnemans, M. A. F., Slujs, J. A., Koycu, S., Ramdjelal, R. D. J., Saleci, A., Martens, G. J. M., Grosveld, F. G., Burbach, J. P. H., and Hol, E. M. (1998) Science 279, 242–247
5. van Den Hurk, W. H., Willems, H. J., Bloemen, M., and Martens, G. J. (2001) J. Biol. Chem. 276, 11496–11498
6. Hol, R. M., Neubauer, A., De Kleijn, D. P. V., Slujs, J. A., Ramdjelal, R. D. J., Sonnemans, M. A. F., and Van Leeuwen, F. W. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 1779–1788
7. Vassar, R., Bennett, B. D., Babu-Khan, S., Kahn, S., Mendiaz, E. A., Denis, P., Toplow, D. B., Ross, S., Amarante, P., Loeloff, R., Luo, Y., Fisher, S., Fuller, J., Edenson, S., J., Schenk, D., Seubert, P., Suomensaari, S. M., Wang, S., Walker, D., John, Keim, P., Knops, J., Lieberburg, I., Power, M., Tan, H., Tatsuno, G., Tung, J., Sjogren, M., Davidsson, P., Tullberg, M., Minthon, L., Wikkelso, C., Reisine, T., Poirier, J., and Davies, P. (2002) Neuron 22, 383–394
8. Sinha, S., Anderson, J. P., Barbour, R., Basi, G. S., Cassavello, R., Davis, D., Dean, M., Doyle, H. F., Frigon, N., Hong, J., Jacobson-Croak, K., Jewett, N., Keim, P., Knops, J., Lieberburg, I., Power, M., Tan, H., Tatsuno, G., Tung, J., Schenk, D., Steubert, P., Sonnemans, S. M., Wang, S., Walker, D., John, V., et al. (1999) Nature 392, 537–540
9. Selkoe, D. J. (1998) Trends Cell Biol. 8, 447–453
10. Sinha, S., and Lieberburg, I. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 11049–11053
11. Van Nostrand, W. E., Wagner, S. L., Shankle, W. R., Farrow, J. S., Dick, M., Rozemuller, J. M., Kuiper, M. A., Wolters, E. C., Zimmerman, J., Cowman, C. W., and Cunningham, D. D. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 2551–2555
12. Sennvik, K., Fastbom, J., Blennow, K., Wahlin, L. O., Winblad, B., and Bengtsson, O. (2000) Neurosci. Lett. 287, 169–172
13. Lannfelt, L., Basun, H., Wahlin, L. O., Rowe, B. A., and Wagner, S. L. (1995) Nat. Med. 1, 829–832
14. Andreasen, N., and Blennow, K. (2002) Peptides 23, 1205–1214
15. Palmert, M. R., Podlisny, M. B., Witker, D. S., Oltersdorf, T., Younkin, L. H., Selkoe, D. J., and Younkin, S. G. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 6338–6342
16. Hersberger, M., Santiago-Garcia, J., Paterrotes-White, S., Yan, J., and Xu, X. (2001) J. Neurochem. 76, 1308–1314
17. Hilbich, C., Menning, U., Grund, C., Masters, C. L., and Beyreuther, K. (1993) J. Biol. Chem. 268, 26571–26577
18. Buedel, J. L., Roffler-Tarlton, S., Schachmiller, M., and Freedman, L. S. (1978) Cancer Res. 38, 3751–3757
19. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
20. Conover, W. J. (1980) Practical Nonparametric Statistics, 2d Ed., 229–237
21. John Wiley & Sons, Inc., New York
22. Biedler, J. L., Roffler-Tarlov, S., Schachmiller, M., and Freedman, L. S. (1978) Cancer Res. 38, 3751–3757
23. Ravid, R., Van Zwieten, E. J., and Swaab, D. F. (1992) Proc. Brain Res. 53, 83–88
24. Braak, H., and Braak, E. (1991) Acta Neuropathol. 82, 239–259
25. Peraas, G. C., Masters, C. L., and Beyreuther, K. (1997) J. Neurosci. 17, 7714–7724
26. Hock, V. Y., Toneff, T., Aaron, W., Yasothornriksit, S., Bundey, R., and Resine, T. (2002) J. Neurochem. 81, 257–256
27. Weidemann, A., Konig, G., Bunke, D., Fischer, P., Salbaum, J. M., Masters, C. L., and Beyreuther, K. (1989) Cell 57, 115–126
28. Zhou, J.-N., Liu, R.-Y., Kamphorst, W., Hofman, M. A., and Swaab, D. F. (2003) J. Neuropathol. Exp. Neurol. 62, 767–779
29. May, M., Raz, J., and Lieberburg, I. (2002) Neuron 35, 485–508
30. Tanna, N. K., Kohn, M. I., Horwich, D. N., Jelks, P. R., Zimmerman, R. A., Alves, W. M., and Alavi, A. (1991) Radiology 178, 123–130
31. Teunissen, C. E., de Vente, J., Steinbusch, H. W., and De Bruijn, C. (2002) Neurobiol. Aging 23, 485–508
32. Klunk, W. E. (2002) Neurobiol. Aging 23, 517–519
33. Khachaturian, Z. S. (2002) Neurobiol. Aging 23, 509–511
34. Papassotiropoulos, A., and Hock, C. (2002) Neurobiol. Aging 23, 513–514
35. Spillantini, M. G., and Goedert, M. (1998) Trends Neurosci. 21, 428–433
36. Sjogren, M., Davidsonson, P., Tullberg, M., Minthon, L., Wallin, A., Wikkelso, C., Granerus, A. K., Vandersteichele, H., Vanmichael, E., and Blennow, K. (2001) J. Neurol. Neurosurg. Psychiatry 70, 624–630
37. De Leon, M. J., Segal, S., Tarshish, C. Y., DeSanti, S., Zinkowski, R., Ma, A., Convit, A., Caraos, C., Rusinek, H., Tsui, W., Saint Louis, L. A., DeBernardis, J., Kerkman, D., Qadri, F., Gary, A., Lesbre, P., Wasiunski, T., Fuerj, J., and Davies, P. (2002) Neurosci. Lett. 333, 183–186
38. Riemenschneider, M., Lautenschlager, N., Wagenfuehl, S., Diehl, J., Drzezga, A., and Kurz, A. (2002) Arch. Neurol. 59, 1729–1734