# Quantification of presenilin-1 mRNA in Alzheimer's disease brains

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**Abstract The presenilin-1 (PS-1) gene on chromosome 14 carries mutations which cosegregate with early-onset familial Alzheimer's disease. We quantified PS-1 mRNA in post-mortem mid-temporal and superior frontal cortices from 14 Alzheimer's disease subjects, 9 non-demented controls and 5 subjects with other neurological diseases using solution hybridisation-RNase protection assay. APP and APLP2 mRNAs had previously been quantified in these samples (Johnston et al. (1996) Mol. Brain Res., in press) and subjects were apolipoprotein E (APOE) genotyped. There were no significant differences between PS-1 mRNA levels per pg total RNA in mid-temporal or superior frontal cortices of the Alzheimer's disease subjects, compared to controls. PS-1 mRNA levels corresponded to 10% of total APP and 30% of APLP2 mRNA levels, and were not significantly affected by age, post-mortem delay, tissue pH, or APOE genotype. PS-1 mRNA showed significant positive correlations with APP and APLP2 mRNA levels in mid-temporal cortex and with APP mRNA in superior frontal cortex. This may reflect a co-regulation of the expression of these genes, or the fact that they are expressed in similar neuronal populations.**

*Key words:* Presenilin-1 mRNA; Alzheimer's disease; Amyloid precursor protein; Amyloid precursor-like protein 2; Solution hybridization-RNase protection; Post-mortem human brain

## 1. Introduction

Several molecules which are likely to be involved in the aetiology of Alzheimer's disease have now been identified. The genetic heterogeneity of this disease suggests that a number of mechanisms may be involved, which eventually converge on a final common pathological cascade. In a sub-group of familial Alzheimer's disease, pathogenic mutations are present in the amyloid precursor protein (APP) gene. These mutations alter the production of a metabolite of APP, $A\beta_{42}$, a peptide component of the amyloid plaques which characterise Alzheimer's disease neuropathology [2–4]. Recently, a gene on chromosome 14, S182 or presenilin-1 (PS-1), was identified and shown to carry mutations which cosegregate with early-onset familial Alzheimer's disease [5]. A homologous gene on chromosome 1, presenilin-2, also carries mutations which are pathogenic for the disease [6,7]. To date, over 30 pathogenic mutations have been identified on the PS-1 gene [8]. The normal function of PS-1, or its role in the aetiology of Alzheimer's disease, have not yet been determined, although there are indications that the PS-1 mutations, like the APP mutations, increase $A\beta_{42}$ production and deposition [9,10]. The protein sequence of PS-1 contains seven hydrophobic, potentially transmembrane regions, suggestive of an integral membrane protein such as a receptor, channel protein or structural membrane protein [5]. Full length PS-1 is 467 amino acids in size and PS-1 RNA is alternatively spliced to produce at least two other isoforms which would encode proteins with 463 [11] and 374 [12] amino acids. A range of shorter mRNA transcripts have also been detected in post-mortem human brain using RT-PCR techniques, although full length PS-1 was the most abundant isoform found in this tissue [13]. PS-1 expression has been detected in several regions of human brain [5,11–15] and in situ hybridisation studies indicate that this expression is largely neuronal [14,15]. An immunohistochemical study of PS-1 in mouse brain confirmed this finding, revealing mainly neuronal, intracellular and granular PS-1 staining, suggestive of a vesicular location [16].

The aim of the present study was to determine whether altered PS-1 mRNA expression is a feature of Alzheimer's disease pathology. We established a quantitative solution hybridisation-RNase protection assay to determine PS-1 mRNA levels in mid-temporal and superior frontal cortices from a series of Alzheimer's disease subjects and matched controls. We had previously quantified total amyloid precursor protein (APP), APP containing the Kunitz-type protease inhibitor insert (APP KPI) and amyloid precursor-like protein 2 (APLP2) mRNA levels in these subjects [1]. In addition, since inheritance of the apolipoprotein E (APOE) ε4 allele increases the risk of developing Alzheimer's disease [17,18], we genotyped the subjects in order to establish whether any relationship existed between APOE genotype and PS-1 mRNA levels.

## 2. Materials and methods

### 2.1. Post-mortem brain tissue

Clinical information about the subjects included in this study has been presented in detail elsewhere [1]. Brain tissue was obtained via the rapid autopsy program of The Netherlands Brain Bank. Regions of mid-temporal cortex (gyrus temporalis medialis) and superior frontal cortex (gyrus frontalis superior) were dissected from 14 Alzheimer's disease subjects, 9 non-demented control subjects with no history of neurological or psychiatric disorders, and a positive disease control group of 5 individuals with other neurological diseases (Parkinson's disease, Lewy body disease and progressive supranuclear palsy). The diagnosis of 'probable AD', based on NINCDS-ADRDA criteria [19], was confirmed by extensive post-mortem neuropathological examination using conventional histopathological staining techniques on formalin-fixed tissue. Diagnosis was based on the presence and distribution of plaques and tangles, and the extent of disruption of the fibre pattern (dystrophic neurites) in the hippocampus, superior frontal area (area 10), orbito-frontal cortex (area 11), superior temporal cortex (area 22), cortex of the temporal pole (area 38), the superior parietal (area 7), inferior parietal (area 40) and the cortex of the occipital lobe (area 17 and 18). Brain pH values were determined on ventricular CSF at autopsy to provide an indication of agonal status [20–22]. Mean tissue pH ± S.D. was 6.51 ± 0.35 for the Alzheimer's disease subjects; 6.82 ± 0.66 for the non-demented controls; and 6.48 ± 0.22 for the positive disease controls. The groups were also matched for age (age ± S.D. was 77.7 ± 9.7 years for the Alzheimer's disease subjects; 76.0 ± 14.7 years for the non-demented controls; and 81.4 ± 12.3 years for the positive disease controls). Tissue was dissected fresh and the meninges and white matter removed

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before snap freezing under liquid nitrogen and storage at −70°C. The period between time of death and freezing of the tissue (post-mortem delay) was kept as short as possible, but was significantly longer in the non-demented control group (374 ± 102 min) than in the Alzheimer's disease group (266 ± 68 min; $p \leq 0.01$, Student's $t$-test). The post-mortem delay for the positive disease control group was 271 ± 64 min (no significant difference from non-demented controls).

*2.2. Total nucleic acid extraction*

Total nucleic acid (TNA) was prepared by digestion of homogenised brain tissue (100–200 mg) with proteinase K (IBI, 200 μg/ml) and subsequent extraction with phenol/chloroform, as described [23]. TNA was dissolved in 0.2×SET (0.2% SDS, 2 mM EDTA and 4 mM Tris, pH 7.6). TNA and DNA concentrations were determined by spectrophotometry and using Hoechst 33258 fluorimetry [24], respectively. RNA concentrations were calculated by subtracting the DNA concentrations from the TNA concentrations.

*2.3. Probe synthesis*

Radiolabelled antisense RNA probe, designed to hybridise to positions 384–554 of PS-1 (S182 coding sequence, accession no. L42110), was transcribed in vitro from a DNA template generated by polymerase chain reaction (PCR) as described below. 1 μg total RNA extracted from a lymphoblastoid cell line was reverse transcribed using M-MLV reverse transcriptase (Gibco, BRL) in a final volume of 50 μl, according to the manufacturer's instructions. 1 μl of this reaction product was then used as template for PCR amplification of positions 203–618 of PS-1 with primers 5′-AAGAGGCTTTGTTTTCTGTG and 5′-CTCGGTATCTTCTGTGAATG in a final volume of 25 μl for 30 cycles (annealing temperature 52°C). This PCR product was diluted 100-fold, and 1 μl used as template for a second PCR (100 μl) where each primer encoded an RNA polymerase promoter (either SP6 or T7), in addition to a 20 nucleotide complementary region. The primers used were 5′-TAATACGACTCACTATA GGGGATC CACCCTGAGCCATTATCTAA (T7 promoter underlined) and 5′ (biotinylated)-ATTTAGGTGACACTATA GGATCCC TGACTTAATGGTAGCC ACGA (SP6 promoter underlined) for 30 cycles (annealing temperature 53°C), producing a 218 base pair product. Single-stranded DNA was prepared using streptavidin-coated magnetic Dynabeads (Dynal, Oslo) and the PCR product verified by sequencing using the Sequenase kit, version 2.0 (USB) according to the manufacturer's instructions.

The PCR product was purified using a Wizard PCR Preps DNA purification system (Promega) before use as template DNA for in vitro transcription of sense and antisense RNA. Radiolabelled antisense RNA probe was transcribed from approx. 4 ng PCR template in the presence of [$^{35}$S]CTP (Du Pont Scandinavia) using SP6 RNA polymerase, according to the manufacturer's instructions (Riboprobe Gemini II system, Promega). Template DNA was degraded by incubation with 1 unit RQ1 ribonuclease-free DNase (Promega) for 30 min at 37°C, and unincorporated nucleotides removed by fractionation through a disposable Nick column packed with Sephadex G-50 (Pharmacia, Sweden). Intact transcripts were ethanol precipitated and dissolved in 500 μl 0.2×SET (see Section 2.2). Probe concentration was determined by trichloroacetic acid precipitation, capture on glass fibre filters (GF/C, Whatman), and scintillation counting. Probe integrity was also checked by polyacrylamide gel electrophoresis (see Section 2.5).

Sense standard RNA was transcribed from approx. 30 ng purified PCR product using T7 RNA polymerase, and template DNA degraded using 10 units RQ1 ribonuclease-free DNase (Promega) as described above. Intact transcripts, identified by their absorbance at 260 nm, were purified by two consecutive fractionations through Sephadex G-50 columns. The final concentration of intact sense RNA transcripts was determined spectrophotometrically. Antisense and sense RNA transcripts were aliquoted and stored at −80°C until use.

*2.4. Solution hybridisation-RNase protection assay*

This assay was carried out as described in detail elsewhere for APP and APLP2 mRNA quantification [23]. Briefly, $^{35}$S-labelled antisense RNA probe was hybridised in solution with either increasing amounts of unlabelled sense in vitro transcribed RNA, enabling construction of a standard curve; or with TNA sample. Hybridisations were run for 18 h at 75°C in the presence of 25% formamide, 0.75 mM DTT, 0.6 M NaCl, 20 mM Tris and 4 mM EDTA in a reaction volume of 40 μl, covered with paraffin oil. Non-hybridised RNA was then degraded by incubation with 1 ml RNase T1 (2 μg/ml), RNase A (40 μg/ml) and salmon sperm DNA (100 μg/ml) (Sigma) at 37°C for 45 min. Ribonuclease-resistant hybrids were precipitated with 10% trichloroacetic acid, captured on Whatman GF/C filters, and quantified by scintillation counting.

The amount of specific mRNA present was calculated by comparison with the standard curve, using a correction factor of 1.08 to account for the difference in length of probe/standard RNA (185 bp) and probe/endogenous mRNA (171 bp) hybrids. Hybridisation signal was thus converted to the equivalent number of moles of standard RNA, indicating the number of copies of PS-1 mRNA detected, and the results expressed as a function of either the DNA or total RNA content of the sample.

*2.5. Polyacrylamide gel electrophoresis of probes and nuclease-resistant hybrids*

Probe (50 000 cpm) was hybridised with either standard RNA (5 or 10 pg) or 68 μg TNA (containing 40 μg total RNA) extracted from control superior frontal cortex and treated with ribonuclease as described in Section 2.4. SDS (0.6%) and Proteinase K (300 μg/ml) were added and incubated at 37°C for 30 min. The hybrids were then phenol/chloroform extracted, precipitated with 99.5% ethanol, dissolved in loading buffer (95% formamide, 20 mM EDTA) and run on a 5% denaturing polyacrylamide gel. The gel was soaked in Amplify (Amersham, UK) fluorographic reagent for 30 min prior to visualisation of the bands by exposure of Biomax MR film (Kodak) to the dried gel.

*2.6. APOE genotyping*

DNA was extracted from 25 mg frozen brain tissue using the QIAamp Tissue kit (Qiagen). APOE genotype was determined by minisequencing reactions on solid-phase bound DNA using the Affigene APOE kit (Sangtec Medical, Sweden).

## 3. Results

*3.1. Methodological experiments*

A representative standard curve, generated by hybridising increasing amounts (1 to 10 pg) of standard RNA with a fixed amount (15 000 cpm) of radiolabelled antisense riboprobe, is shown in Fig. 1. A linear increase in hybridisation signal was seen using up to 50 pg standard RNA under these hybridisation conditions. The addition of increasing amounts of TNA sample also produced the predicted linear increase in hybridisation signal. The reproducibility of the assay was examined by running one TNA sample in duplicate on five different occasions and calculating the coefficient of variation (S.D. expressed as a percentage of the mean value). The coefficient of variation for this assay was 3.2%.

The integrity and size of the PS-1 probe, standard and protected probe following hybridisation with either standard RNA or TNA sample were established by polyacrylamide gel electrophoresis as described in Section 2.5. Probes and hybrids were of the expected sizes and no extra bands or smears indicating unspecific hybridisation or contamination with ribonuclease were seen (Fig. 2).

*3.2. PS-1 mRNA in Alzheimer's disease and control cortex*

The levels of PS-1 mRNA detected in the mid-temporal and superior frontal cortices of the Alzheimer's disease and control subjects are presented in Table 1. The results are expressed as copies of PS-1 mRNA detected per pg total RNA or per pg DNA present in the TNA sample. There were no significant differences between the number of PS-1 mRNA copies detected per pg total RNA in the mid-temporal and superior frontal cortices of the Alzheimer's disease subjects, as compared to either the non-demented or positive

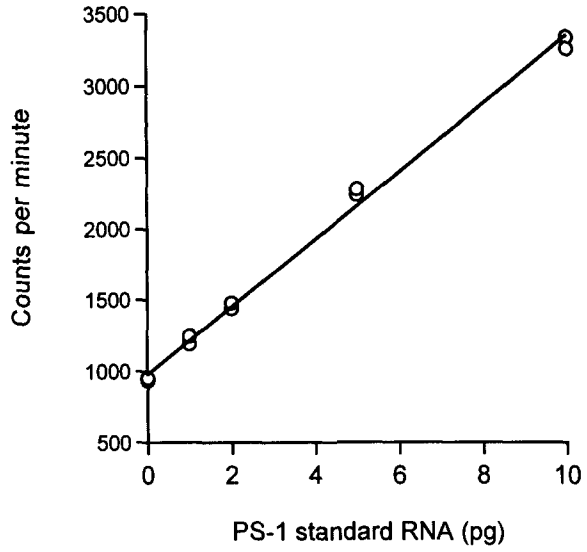


Fig. 1. A typical standard curve for the PS-1 solution hybridisation-RNase protection assay. Radiolabelled PS-1 riboprobe was hybridised with increasing amounts of PS-1 sense standard RNA. The equation of the line generated in each assay was used to convert the sample hybridisation signals to pg PS-1 mRNA.

disease control groups. Expression of the results as a function of tissue DNA content, however, revealed a significantly reduced number of PS-1 mRNA copies in the Alzheimer's disease subjects, compared to non-demented controls, in both mid-temporal and superior frontal cortices ($p \leq 0.01$, Student's $t$-test). The differences seen when results were expressed per pg total RNA or DNA in the sample appeared to reflect differences between the study groups in the total RNA and DNA yields per mg wet weight tissue (Table 1), as discussed in detail elsewhere [1]. Briefly, the DNA yield per mg wet weight tissue was significantly higher in the Alzheimer's disease mid-temporal and superior frontal cortices than in the non-demented controls ($p \leq 0.05$, Student's $t$-test), while the total RNA yield was unaffected. In contrast, the total RNA yield per mg wet weight tissue was significantly lower in the positive disease controls than in the non-demented controls in both cortical regions ($p \leq 0.05$, Student's $t$-test) while the DNA yield was unaltered.

### 3.3. Comparison of PS-1, APP and APLP2 mRNA levels

We compared the PS-1 mRNA levels with our recently reported APP/APLP2 mRNA data in these samples [1] to determine the relative levels of expression of these genes. Two probes were used to detect APP, one which hybridised to all the reported isoforms, and one which only detected the isoforms encoding the kunitz-type protease inhibitor (KPI) domain. PS-1 mRNA was present at lower levels than both APP and APLP2 in cerebral cortex, at approx. 10% of total APP, 14% of APP KPI and 30% of APLP2 mRNA in both mid-temporal and superior frontal cortices. These ratios were not altered in the Alzheimer's disease group as compared to non-demented or positive disease controls.

We also analysed the data for potential correlations between the expression of PS-1 and APP or APLP2 in both the Alzheimer's disease and control groups, as shown in Fig. 3. Interestingly, we found significant positive correlations between PS-1 mRNA levels and total APP, APP KPI and APLP2 mRNA levels (per pg total RNA) in mid-temporal cortex of both the Alzheimer's disease and control subjects. Correlation coefficients ($r$) ranged from 0.58 to 0.86 ($p \leq 0.05$, Fisher's $r$ to $z$ test). Similarly, in the superior frontal cortex, the levels of PS-1 mRNA correlated significantly with total APP and APP KPI mRNAs ($r$ values between 0.61 and 0.75; $p \leq 0.02$, Fisher's $r$ to $z$ test).

Significant correlations were also found when the specific mRNA levels were expressed as a function of sample DNA content. PS-1 mRNA levels showed positive correlations to total APP, APP KPI and APLP2 mRNA (per pg DNA) levels in both cortical regions of the Alzheimer's disease and control subjects ($r$ values between 0.56 and 0.85; $p \leq 0.05$, Fisher's $r$ to $z$ test,).

### 3.4. Effect of APOE genotype

The PS-1 mRNA levels detected were grouped according to

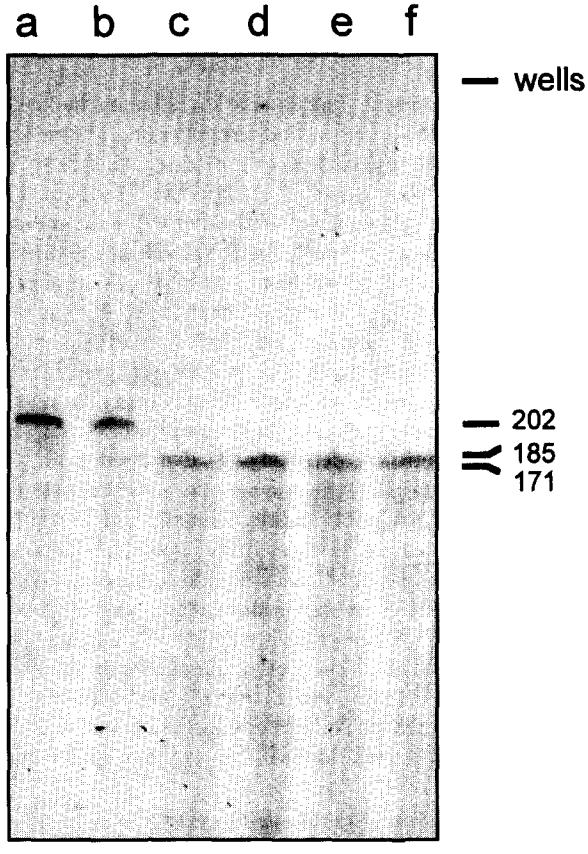


Fig. 2. Polyacrylamide gel electrophoresis of radiolabelled riboprobe and nuclease resistant hybrids. Experiments were performed as described in Section 2.5. Lane a, PS-1 standard RNA, transcribed in the presence of [$^{35}$S]CTP in order to allow visualisation (202 bases). The standard RNA used in all other experiments was transcribed in the absence of [$^{35}$S]CTP as described in Section 2.3. Lane b, PS-1 probe alone (202 bases). Both probe and standard RNA were intact and of the theoretically predicted size. Ribonuclease-protected probe following hybridisation with PS-1 standard RNA was run in lanes c (5 pg standard) and d (10 pg standard). The length of the probe protected by standard RNA was 185 bases. Ribonuclease-protected probe following duplicate hybridisations with 68 μg TNA extracted from control superior frontal cortex was run in lanes e,f. The length of the probe protected by sample mRNA was 171 bases. The band sizes indicated were confirmed by comparison with radiolabelled RNA transcribed from the Riboprobe Gemini positive control plasmid and other previously characterised riboprobes.

APOE genotype for Alzheimer's disease and control subjects separately. Eight of the 14 Alzheimer's disease subjects carried at least one APOE ε4 allele, compared with 4 of the 14 control subjects. The Alzheimer's disease subjects with at least one APOE ε4 allele tended to have lower levels of PS-1 mRNA in mid-temporal cortex than subjects with the APOE ε3/3 genotype (0.92 ± 0.21 versus 1.40 ± 0.60), although this difference failed to reach statistical significance ($p = 0.06$, unpaired $t$-test). This trend was absent when the results were expressed per pg DNA in the sample and was not seen for the control groups or in superior frontal cortex.

### 3.5. *Effects of age, post-mortem delay and tissue pH*

Data for the Alzheimer's disease and control groups were analysed separately to determine potential effects of post-mortem delay, age, tissue pH and brain weight on the levels of PS-1 mRNA. There were no significant correlations between PS-1 mRNA levels in mid-temporal and superior frontal cortices and either age, tissue pH, brain weight or post-mortem delay.

## 4. Discussion

The aim of the present study was to investigate whether PS-1 mRNA levels are altered in mid-temporal or superior frontal cortices affected by Alzheimer's disease. This is to our knowledge the first systematic quantitative study of PS-1 mRNA expression in a series of Alzheimer's disease and control post-mortem brains. The RNA-RNA solution hybridisation-RNase protection assay described provides a sensitive and reproducible method for quantification of PS-1 mRNA in total nucleic acid samples. The inclusion of a standard curve enables absolute rather than relative quantification and simultaneously corrects for inter-assay variability, making the method ideal for analysing large sample series [23]. The amount of PS-1 mRNA detected was expressed both as a function of the total RNA in the sample, indicating its contribution to the RNA pool, and as a function of the sample DNA content, indicating how much PS-1 mRNA was present per cell sampled, as each cell has a standard amount of DNA.

We did not identify any significant differences between the levels of PS-1 mRNA per pg total sample RNA in mid-temporal or superior frontal cortices of the Alzheimer's disease and control groups. This indicates that PS-1 mRNA levels are not invariably altered in Alzheimer's disease cerebral cortex, although further investigation will be required to determine whether PS-1 RNA splicing is altered in the disease, as suggested recently [13]. It is also possible that PS-1 expression is affected by the presence of pathogenic PS-1 mutations [25].

When the results were expressed as a function of sample DNA content, the Alzheimer's disease subjects had lower levels of PS-1 mRNA than the control subjects in both mid-temporal and superior frontal cortices, indicating reduced levels of PS-1 mRNA per cell in the Alzheimer's disease tissue. It should be noted, however, that higher DNA yields were obtained per mg Alzheimer's disease tissue than control tissue, which we have speculated may be due to increased cell density resulting from atrophic changes, combined with compromised neuronal activity as a result of the neurodegenerative process [1]. These results, taken together, indicate that the PS-1 mRNA contribution to the cortical mRNA pool (copies per pg total RNA), is not affected in Alzheimer's disease, while the level of detectable PS-1 per cell sampled (copies per pg total DNA), is reduced.

We have previously used solution hybridisation-RNase protection assays to quantify the levels of APP and APLP2 mRNA in the same series of samples used in the present study [1]. Two probes were used to detect APP, one which hybridised to all the reported isoforms, and one which only detected the isoforms encoding the KPI region. Briefly, total APP and APLP2 mRNA levels were significantly reduced in Alzheimer's disease mid-temporal, but not superior frontal cortex, suggesting that regional reductions in these mRNAs correlate with severity of disease pathology. In addition, a small significant increase in the proportion of APP KPI mRNA was seen in both cortical regions in Alzheimer's disease, although it is unclear whether this occurs early in the disease process, predisposing to Aβ production and aggregation, or reflects later events such as gliosis and neuronal cell death. The present study showed that PS-1 mRNA was present at lower levels than both APP and APLP2 in cerebral cortex, corresponding to 10% of total APP and 30% of APLP2 mRNA. The relationship between APP/APLP2 and PS-1 mRNA levels was not significantly altered in the Alzheimer's disease group as compared to either of the control groups.

We also identified significant positive correlations between PS-1 mRNA levels and total APP, APP KPI and APLP2 mRNA levels in mid-temporal cortex. Similarly, in superior frontal cortex, the levels of PS-1 mRNA correlated with total APP and APP KPI mRNAs. These observations may indicate

Table 1
Levels of presenilin-1 mRNA in Alzheimer's disease and control cortices

| | Copies/pg RNA | Copies/pg DNA | μg RNA/mg tissue | μg DNA/mg tissue |
|---|---|---|---|---|
| Mid-temporal cortex | | | | |
| Alzheimer's disease ($n = 14$) | 1.13 ± 0.47 | 1.28 ± 0.34[b] | 0.83 ± 0.15 | 0.71 ± 0.17[a] |
| Non-demented controls ($n = 8$) | 1.28 ± 0.35 | 1.75 ± 0.35 | 0.79 ± 0.06 | 0.58 ± 0.12 |
| Positive disease controls ($n = 4$) | 1.25 ± 0.39 | 1.37 ± 0.24 | 0.59 ± 0.06[b] | 0.51 ± 0.08 |
| Superior frontal cortex | | | | |
| Alzheimer's disease ($n = 14$) | 1.38 ± 0.47 | 1.41 ± 0.38b | 0.69 ± 0.08 | 0.69 ± 0.13[b] |
| Non-demented controls ($n = 9$) | 1.40 ± 0.42 | 1.98 ± 0.49 | 0.75 ± 0.11 | 0.52 ± 0.09 |
| Positive disease controls ($n = 5$) | 1.42 ± 0.31 | 1.46 ± 0.32* | 0.63 ± 0.05[a] | 0.63 ± 0.09 |

Results represent the mean (± S.D.) levels of presenilin-1 mRNA in Alzheimer's disease, non-demented control and positive disease control subjects (number of subjects indicated in parentheses). Each sample was assayed in duplicate as described in Section 2 and the mean value used in subsequent calculations. Significance levels are shown for the comparison of the group indicated with the non-demented controls ([a]$p \leq 0.05$, [b]$p \leq 0.01$), Student's $t$-test.

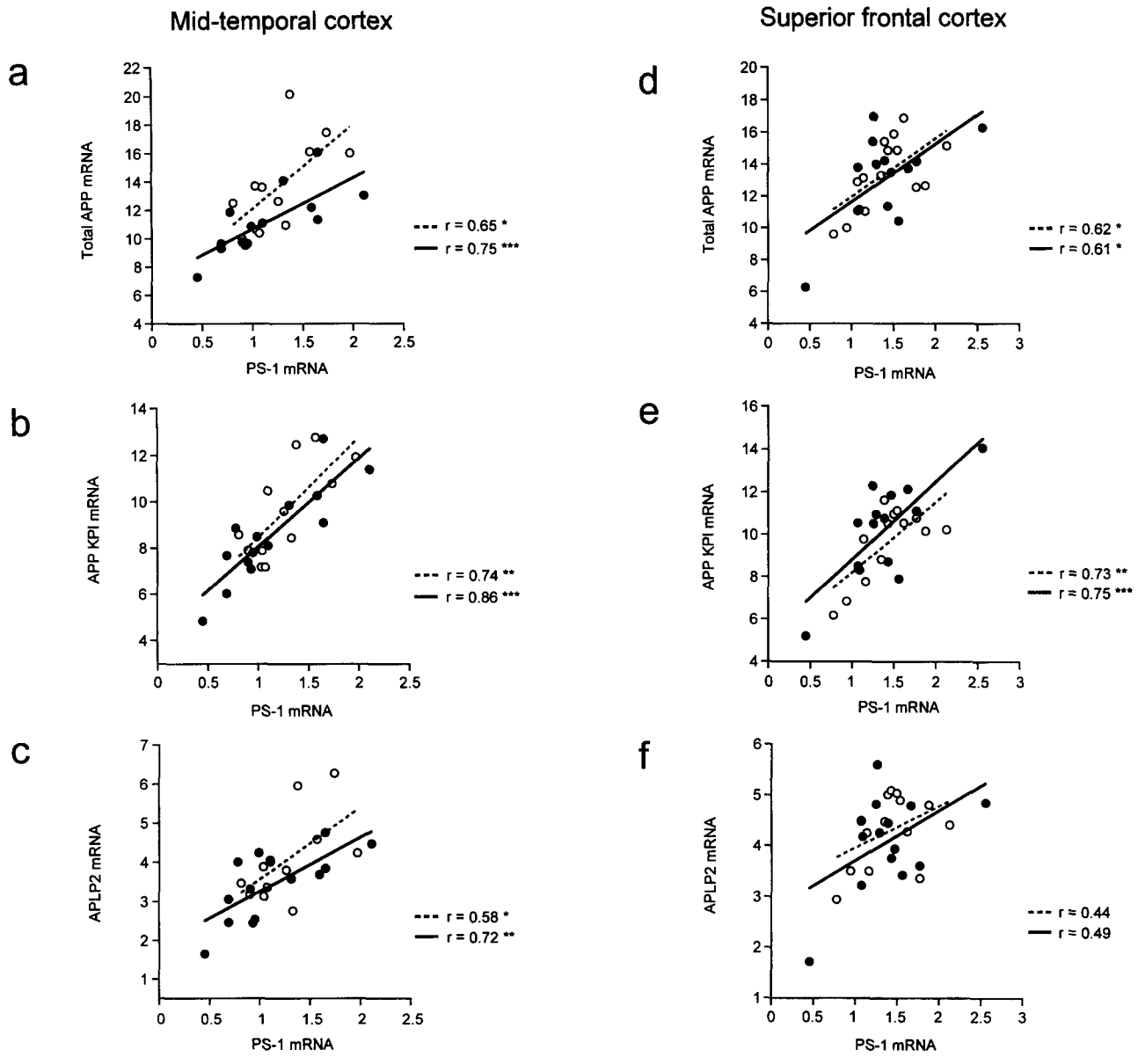


Fig. 3. Correlations between PS-1 and APP/APLP2 mRNA levels in cerebral cortex PS-1 mRNA levels, expressed as copies per pg total RNA, are shown plotted against total APP mRNA, APP KPI mRNA and APLP2 mRNA levels for the Alzheimer's disease (●) and control subjects (○). The regression plot is represented by a solid line for the Alzheimer's disease subjects, and by a dashed line for the control subjects. Correlation coefficients ($r$) are shown for each group and the significance levels for Fisher's $r$ to $z$ test are indicated where $^{*}p \leq 0.05$, $^{**}p \leq 0.01$ and $^{***}p \leq 0.001$.

a co-regulation of the expression of these genes. Some of the speculation around the potential function of PS-1 has focused on its homology to *C. elegans* genes, including *spe-4*, involved in spermatogenesis [5], and a stronger homology to *sel-12*, a gene involved in intercellular signalling and important in vulval development [26]. Sel-12, a nine-transmembrane protein, interacts with a single membrane spanning protein, lin-12. This has led to the proposal that PS-1 interacts with APP in a similar fashion [27], in which case a co-regulation of their expression may be important. Alternatively, the correlations between PS-1, APP and APLP2 mRNA levels in cerebral cortex may reflect the fact that these genes seem to be expressed in similar neuronal populations [16].

We investigated whether APOE genotype had any influence on the levels of PS-1 mRNA present in mid-temporal and superior frontal cortices, since the mechanism by which inheritance of the APOE ε4 allele increases susceptibility to Alzheimer's disease is unclear [17,18]. APOE genotype did not affect PS-1 mRNA levels detected in this study, suggesting that its role in the aetiology of Alzheimer's disease is not exerted at the level of PS-1 mRNA expression.

In summary, the present study shows that PS-1 mRNA levels are not grossly altered in Alzheimer's disease mid-temporal and superior frontal cortices. The PS-1 mRNA levels detected in these brain regions corresponded to 10% of total APP and 30% of APLP2 mRNA levels. The positive correlations observed between PS-1 mRNA, APP and APLP2 mRNA levels in mid-temporal and superior frontal cortices are interesting, although further work will be required to determine whether this reflects a true co-regulation of the expression of these genes, or the fact that they are expressed in similar neuronal populations.

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## References

[1] Johnston, J.A., Norgren, S., Ravid, R., Wasco, W., Winblad, B., Lannfelt, L. and Cowburn, R.F. (1996) Mol. Brain Res., in press.
[2] Suzuki, N., Cheung, T.T., Cai, X.-D., Odaka, A., Otvos, L., Jr., Eckman, C., Golde, T.E. and Younkin, S.G. (1994) Science 264, 1336–1340.
[3] Johnston, J.A., Cowburn, R.F., Norgren, S., Wiehager, B., Venizelos, N., Winblad, B., Vigo-Pelfrey, C., Schenk, D., Lannfelt, L. and O'Neill, C. (1994) FEBS Lett. 354, 274–278.
[4] Citron, M., Vigo-Pelfrey, C., Teplow, D.B., Miller, C., Schenk, D., Johnston, J., Winblad, B., Venizelos, N., Lannfelt, L. and Selkoe, D.J. (1994) Proc. Natl. Acad. Sci. USA 91, 11993–11997.
[5] Sherrington, R., Rogaev, E.I., Liang, Y., Rogaeva, E.A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K., Tsuda, T., Mar, L., Foncin, J.-F., Bruni, A.C., Montesi, M.P., Sorbi, S., Rainero, I., Pinessi, L., Nee, L., Chumakov, I., Pollen, D., Brookes, A., Sanseau, P., Polinsky, R.J., Wasco, W., Da Silva, H.A.R., Haines, J.L., Pericak-Vance, M.A., Tanzi, R.E., Roses, A.D., Fraser, P.E., Rommens, J.M. and St George-Hyslop, P.H. (1995) Nature 375, 754–760.
[6] Levy-Lahad, E., Wasco, W., Poorkaj, P., Romano, D.M., Oshima, J., Pettingell, W.H., Yu, C.-e., Jondro, P.D., Schmidt, S.D., Wang, K., Crowley, A.C., Fu, Y.-H., Guenette, S., Galas, D., Nemens, E., Wijsman, E.M., Bird, T.D., Schellenberg, G.D. and Tanzi, R.T. (1995) Science 269, 973–977.
[7] Rogaev, E.I., Sherrington, R., Rogaeva, E.A., Levesque, G., Ikeda, M., Llang, Y., Chi, H., Lin, C., Holman, K., Tsuda, T., Mar, L., Sorbi, S., Nacmias, B., Placentini, S., Amaducci, L., Chumakov, I., Cohen, D., Lannfelt, L., Fraser, P.E., Rommens, J.M. and St George-Hyslop, P.H. (1995) Nature 376, 775–778.
[8] Van Broeckhoven, C. (1995) Nat. Genet. 11, 230–232.
[9] Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T.D., Hardy, J., Hutton, M., Kukull, W., Larson, E., Levy-Lahad, E., Viitanen, M., Peskind, E., Poorkaj, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D. and Younkin, S. (1996) Nat. Med., in press.
[10] Lemere, C.A., Lopera, F., Kosik, K.S., Ossa, J., Saido, T.C., Ruiz, A., Martinez, A., Madrigol, L., Hincapie, L., Arango, L., Selkoe, D.J. and Arango V, J.C. (1996) Neurobiol. Aging (Suppl.) 17, S17.
[11] Alzheimer's Disease Collaborative Group (1995) Nat. Genet. 11, 219–222.
[12] Sahara, N., Yahagi, H., Takagi, H., Kondo, T., Okochi, M., Usami, M., Shirasawa, T. and Mori, H. (1996) FEBS Lett. 381, 7–11.
[13] Anwar, R., Moynihan, T.P., Ardley, H., Brindle, N., Coletta, P.L., Cairns, N., Markham, A.F. and Robinson, P.A. (1996) J. Neurochem. 66, 1774–1777.
[14] Kovacs, D.M., Fausett, H.J., Page, K.J., Kim, T.-W., Moir, R.D., Merriam, D.E., Hollister, R.D., Hallmark, O.G., Mancini, R., Felsenstein, K.M., Hyman, B.T., Tanzi, R.E. and Wasco, W. (1996) Nat. Med. 2, 224–229.
[15] Suzuki, T., Nishiyama, K., Murayama, S., Yamamoto, A., Sato, S., Kanazawa, I. and Sakaki, Y. (1996) Biochem. Biophys. Res. CommUN. 219, 708–713.
[16] Moussaoui, S., Czech, C., Pradier, L., Blanchard, V., Bonici, B., Gohin, M., Imperato, A. and Revah, F. (1996) FEBS Lett. 383, 219–222.
[17] Corder, E.H., Saunders, A.H., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L. and Pericak-Vance, M.A. (1993) Science 261, 921–923.
[18] Saunders, A.M., Strittmatter, W.J., Schmechel, D., St George-Hyslop, P.H., Pericak-Vance, M.A., Joo, S.H., Rosi, B.L., Gusella, J.F., Crapper-MacLachlan, D.R., Alberts, M.J., Hulette, C., Crain, B., Goldgaber, D. and Roses, A.D. (1993) Neurology 43, 1467–1472.
[19] McKhann, G., Drachmann, D., Folstein, M., Katzman, R., Price, D. and Stadlan, E.M. (1984) Neurology 34, 939–944.
[20] Hardy, J.A., Wester, P., Winblad, B., Gezelius, C., Bring, G. and Eriksson, A. (1985) J. Neural Transm. 61, 253–264.
[21] Ravid, R. and Swaab, D.F. (1993) J. Neural Trans. (Suppl.) 39, 143–153.
[22] Kingsbury, A.E., Foster, O.J.F., Nisbet, A.P., Cairns, N., Bray, L., Eve, D.J., Lees, A.J. and Marsden, C.D. (1995) Mol. Brain Res. 28, 311–318.
[23] Johnston, J.A., Norgren, S., Anneren, G., Cowburn, R.F. and Lannfelt, L. (1996) Mol. Brain Res., in press.
[24] Labarca, C. and Paigen, K. (1980) Ann. Biochem. 102, 344–352.
[25] Barton, A.J.L., Crook, B.W., Karran, E.H., Pearson, R.C.A., Dewar, D., Mann, D.M.A., Roberts, G.W. and Group, t.A.s.D.C. (1996) Neurobiol. Aging (Suppl.) 17, S17.
[26] Levitan, D. and Greenwald, I. (1995) Nature 377, 351–354.
[27] Dewji, N.N. and Singer, S.J. (1996) Science 271, 159–160.