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Upregulation of Presenilin 1 in Brains of Sporadic, Late-Onset Alzheimer's Disease

Roberta Borghi[a], Alessandra Piccini[a], Erica Barini[a,b], Gabriella Cirmena[a], Michela Guglielmotto[c], Elena Tamagno[c], Michele Fornaro[d], George Perry[e,f], Mark A. Smith[f], Anna Garuti[a] and Massimo Tabaton[a,*]
[a]*Department of Internal Medicine, University of Genova, Genova, Italy*
[b]*Department of Neuroscience and Brain Technologies, The Italian Institute of Technology, Genova, Italy*
[c]*Department of Experimental Medicine and Oncology, University of Torino, Torino, Italy*
[d]*Department of Neurosciences, Ophthalmology and Genetics, University of Genova, Genova, Italy*
[e]*College of Sciences, University of Texas at San Antonio, San Antonio, TX, USA*
[f]*Department of Pathology, Case Western Reserve University, Cleveland, OH, USA*

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Abstract. The activity of the β-secretase involved in the cleavage of amyloid-β (Aβ) is increased in sporadic late-onset Alzheimer's disease (AD). Whether the corresponding γ-secretase activity is altered is still uncertain. We evaluated mRNA expression and protein levels of presenilin 1 (PS1) and γ-secretase activity in the frontal cortex of 32 cases with late-onset sporadic AD and those of 29 control subjects. We found a significant increase in PS1 mRNA, protein levels and γ-secretase activity in AD cases. These findings suggest that upregulation of PS1 leads to Aβ overproduction and accumulation in sporadic AD.

Keywords: Alzheimer's disease, amyloid-β, γ-secretase, presenilin 1

INTRODUCTION

Alzheimer's disease (AD), the most common neurodegenerative disorder, is characterized by cerebral deposition of amyloid plaques, neurofibrillary pathology, and synaptic loss [1]. Amyloid-β (Aβ), the major component of amyloid plaques, is a mixture of heterogeneous peptides [2] derived from two sequential endoproteolytic cleavages of the amyloid-β protein precursor (AβPP). The enzymes that orchestrate this cleavage are referred to as β- and γ-secretase [3,4]. The β-secretase cleavage site is the result of cleavage by a single protein, BACE1. γ-secretase, in contrast, is a multi-subunit proteolytic complex; presenilin 1 (PS1) and presenilin 2 are its catalytic subunits [5,6]. Full-length PS1 protein is endoproteolytically cleaved into amino- and carboxy-terminal fragments that interact with each other to form the catalytic component of γ-secretase [7]. Mutant PS1, the major genetic defect in early-onset familial AD (FAD), alters the activity of the γ-secretase, leading to an increase in the relative production of the longer Aβ species, Aβ_{42} [8]. The mechanism underlying altered AβPP processing and Aβ_{42} accumulation in sporadic AD is still unclear. The expression and the activity of BACE1 are increased in AD brains [9], and several factors, such as oxida-

*Correspondence to: Dr. Massimo Tabaton, Unit of Geriatric Medicine, Department of Internal Medicine, University of Genova, 16132 Genova, Italy. Tel.: +39 010 3537064; Fax: +39 010 506938; E-mail: mtabaton@neurologia.unige.it.

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tive stress [10], hypoxia [11], and ischemia [11] were shown to determine this event. In contrast, there are conflicting reports on the activity of γ-secretase claiming reduced [12,13], unchanged [14,15], or increased levels of PS1 mRNA [16,17] in the cerebral cortex of animal models and sporadic AD cases. To resolve this issue we analyzed the expression and protein levels of PS1 and γ-secretase activity in brain tissue from large groups of AD and neurologically normal cases (CTR).

MATERIALS AND METHODS

Tissues

We used frozen cerebral cortex (superior frontal gyrus) from 29 cognitively normal CTR cases (mean age at death was 69.72 $\pm$ 12.95; mean postmortem delay 9.2 h $\pm$ 3), with few amyloid plaques without neurofibrillary pathology (provided by the brain bank of the Case Western Reserve University, Cleveland, OH, USA; Dr. Giaccone, Carlo Besta National Neurological Institute, Milano, Italy and Dr. Zanusso, University of Verona, Italy) and from 32 late-onset sporadic AD cases (mean age at death was 73.31 $\pm$ 9.53; mean postmortem delay 10.2 hours $\pm$ 4) with a clinical history of disease and pathological diagnosis according to the CERAD criteria (provided by the Case Western Reserve University brain bank, Cleveland, OH).

q-RT-PCR analysis

Total RNA was extracted from 100 mg of frozen tissue, using the TRIZOL method (Invitrogen, Carlsbad, CA, USA). Three μg of total RNA were reverse-transcribed using random primers. Primers and probes for PS1 and β-actin were obtained from a pre-developed assay-on-demand (Applied Biosystems, Foster City, CA, USA). Five μl of the resulting cDNA dilution were used for quantitative PCR amplification performed in duplicate using the Prism 7900HT (Applied Biosystems) with the fluorescent Taqman method. PS1 mRNA quantities were normalized to β-actin as the internal control mRNA and were expressed relative to a calibrator sample. The calibrator was provided by serial dilution (10^6 to 10 gene's copies: 1 000 000 -100 000-10 000-1000-100-10 gene's copies.) of control plasmids containing cloned sequences of ABL obtained from Ipsogen (Marseille, France) used as a standard curve. The levels of PS1 and β-actin transcripts in each sample were determined using the standard curve.

Evaluation of the γ-secretase activity and PS1 protein levels

To evaluate γ-secretase activity, brains were homogenized in a hypotonic buffer containing 10 mM Tris-HCl (pH 7.4), 1 mM EGTA and 1 mM EDTA. To extract the dissolved proteins, samples were centrifuged 12000xg for 20 min and supernatants were recovered. To measure the enzyme activity, 20 μg proteins were incubated with 20 μM of a fluorescent conjugated peptide substrate [NMA-GGVVIATVK (DNP)-DRDRDR-NH2, Calbiochem, Merck, Darmstadt, Germany] at 37°C for 2 h. The degree of the substrate cleavage was measured by the emitted fluorescence using a reader (Perkin-Elmer nLS-55) with an excitation wavelength of 355 nm and an emission of 440 nm. To confirm the specificity of γ-secretase cleavage the inhibitor L685-458 (Bachem) was added at the final concentration of 1 μM together with the fluorescent substrate.

For quantification of PS1 protein levels, 50 μg of supernatant were loaded on 13% SDS-PAGE gels and transferred onto PVDF membranes (Amersham Biosciences, Buckingamshire, UK). Immunoblots were probed both with an anti-PS1 monoclonal antibody, recognizing the N-terminal fragment of the protein (1:200; R&D Systems, Inc., Minneapolis, MN, USA), and with an anti-PS1 polyclonal antibody recognizing the C-terminal fragment of the protein (1:100; Cell Signaling INC, Beverly, MA, USA); the reactive bands were detected using ECL plus (Amersham Biosciences). To normalize protein levels, membranes were stripped with Restore Western blot stripping reagent (Pierce, Rockford, IL, USA) and re-probed with a monoclonal antibody against actin (1:6000; Sigma, St. Louis, USA). The density of the specific bands was quantified using Quantity One software system (Biorad, Hercules, CA, USA).

The data were statistically analyzed using t-student tests, results were expressed as mean $\pm$ standard error. Values are expressed relative to the average of control cases (100%).

Apolipoprotein E genotype determination

Apolipoprotein E (ApoE) genotype was determined by restriction isotyping of amplified ApoE sequences. Briefly, DNA was extracted from brain using QIAamp DNA mini kit (Qiagen GmbH, Hilden, Germany) and ApoE sequences were amplified using following primers: Fw 5'TCG GCC GCA GGG CGC TGA TGG

3'; Rev 5' CTC GCG GGC CCC GGC CTG GTA 3'. PCR products were digested using Hin6I (Fermentas, Burlington, Canada) and then separated on a metaphor-agarose gel.

Evaluation of Aβ levels

The amounts of total $A\beta_{1-40}$ and $A\beta_{1-42}$ were evaluated by sandwich ELISA (Wako Pure Chemical Industries, Osaka, Japan). Total brain homogenates in Tris buffer of CTR and AD were diluted 1:20 for the determination of $A\beta_{1-42}$ and 1:100 for the determination of $A\beta_{1-40}$ with the standard diluent buffer and processed according to manufacturer's protocol. The correlation of Aβ with γ-secretase activity was statistically analyzed using Kruskal-Wallis test with Dunn's Multiple comparison test as post-test and the study of linear correlation with its correlation coefficient. A p-value lower than 5% was considered significant.

RESULTS

We observed a significant increase in PS1 mRNA levels (+151%) in AD cases compared to controls (AD 251.2 ± 54.04; CTR 100 ± 18.04; $p = 0.016$; Fig. 1A). The PS1 N-terminal fragment (27 KDa), analyzed by immunoblotting, showed a significant 64% increase in the AD sample as compared to CTR (AD 163.9 ± 19.33; CTR 100 ± 13.77; $p = 0.011$; Fig. 1B); a correspondent increase (62%) of the PS1 C-terminal fragment (20 KDa) was detected in the AD samples (AD 162.5 ± 15.79; CTR 100 ± 15.74; $p = 0.045$; Fig. 1B). A fluorimetric test was used to measure the enzymatic activity of γ-secretase in the brain samples. We found a significant increase in the γ-secretase activity (+30%) in the AD tissue as compared to the CTR (AD 129.7 ± 3.4; CTR 100 ± 2.88; $p < 0.0001$; Fig. 1C). As predicted, the inhibitor prevented the γ-secretase activity increase (AD 58.13 ± 3.057; CTR 57.06 ± 5.08; $p > 0.05$; Fig. 1C). In presence of the γ-secretase inhibitor the CTR activity decreased 43% and the AD activity decreased 55.2%. We did not find significant correlation between age of controls and PS1 protein levels ($n = 29$, slope 0.011 ± 0.001; $p = 0.33$; $R = 0.189$), PS1 mRNA levels ($n = 29$, slope −0.0030 ± 0.002; $p = 0.154$; $R = 0.277$), and γ-secretase activity ($n = 29$, slope −0.05 ± 0.108; $p = 0.623$; $R = 0.095$). As expected the ApoE ε4 is more frequent in AD compared to controls (AD 32.5%; CTR 15.3%). There were not significant differences of PS1 parameters between ApoE ε4 allele carriers and not in AD cases, see results in Table 1. The levels of $A\beta_{1-42}$ were significantly higher in AD compared to CTR (AD 58.64 ± 2.79, CTR 30.6 ± 2.53, $p < 0.001$; pg/mg of protein); also $A\beta_{1-40}$ levels were significantly higher in AD (AD 319.4 ± 38.06, CTR 176.76 ± 13.64, $p = 0.0012$; pg/mg of protein).We found a significant posi-

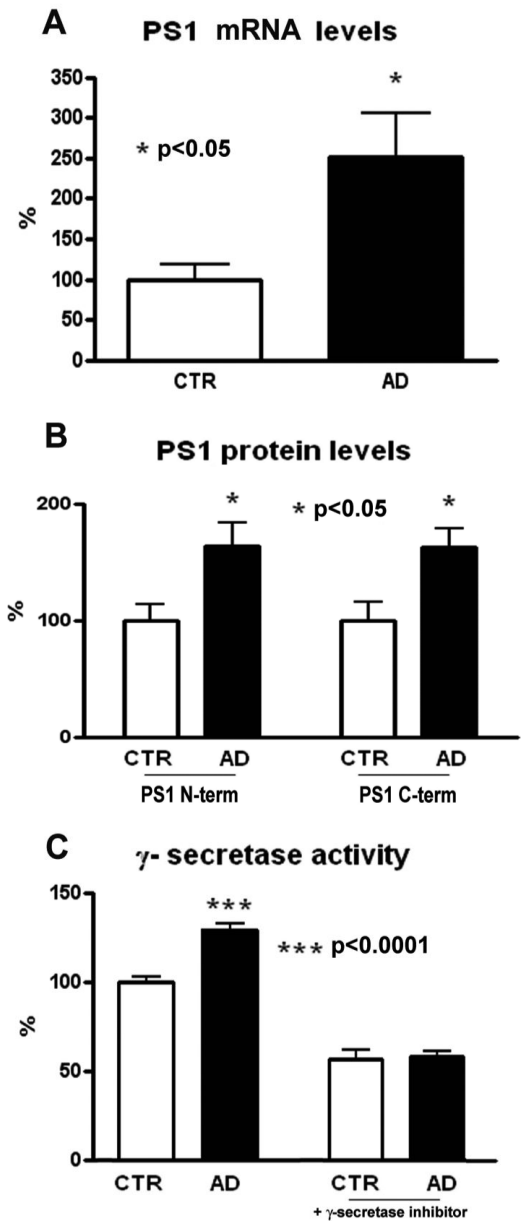

Fig. 1. Evaluation of PS1 expression and γ-secretase activity in AD and normal brain. A) PS1 mRNA, B) protein levels, and C) γ-secretase activity are significantly increased in AD cases as compared to controls.

Table 1
ε4+ and ε4- results for PS1 parameters in AD and CTR

Cases	PS1 mRNA levels (AU mean ± SE)	PS1 protein levels (OD mean ± SE)	γ-secretase activity (AUF mean ± SE)
AD ε4+	0.47 ± 0.14	1.76 ± 0.42	128.1 ± 4.31
AD ε4-	0.36 ± 0.16	1.85 ± 0.33	116.9 ± 1.88
p	$p = 0.63$	$p = 0.88$	$p = 0.23$
CTR ε4+	0.26 ± 0.22	1.57 ± 0.64	108 ± 0.82
CTR ε4-	0.2 ± 0.043	1.25 ± 0.37	102.7 ± 5.78
P	$p = 0.65$	$p = 0.68$	$p = 0.48$

AU, arbitrary unit; SE, standard error; OD, optical density; AUF, fluorescence arbitrary unit.

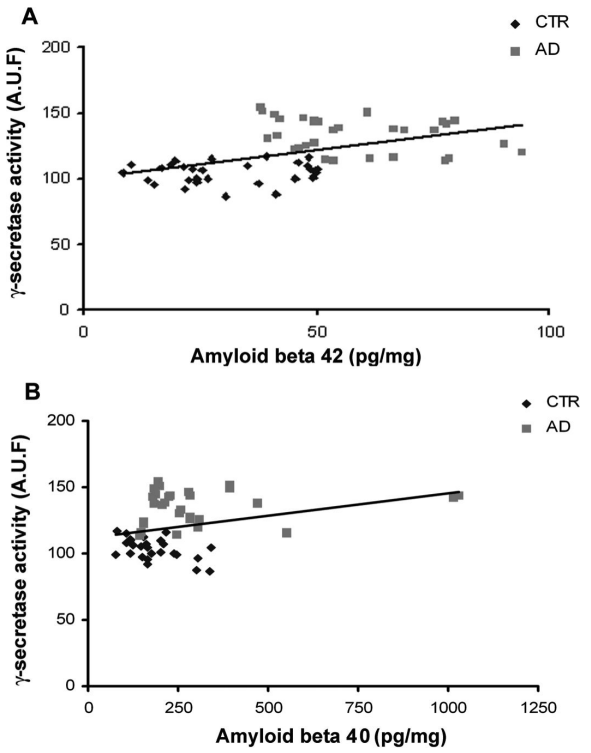

Fig. 2. Linear correlation between γ-secretase activity and Aβ_{1-42} (A) and Aβ_{1-40} (B). We found a significant positive linear correlation between γ-secretase activity, Aβ_{1-42} and Aβ_{1-40}.

tive linear correlation between γ-secretase activity and Aβ_{1-42} ($n = 61$, slope 0.4457 ± 0.102; $p < 0.001$, $R = 0.49$, Fig. 2A) and Aβ_{1-40} ($n = 61$, slope 0.034 ± 0.012; $p = 0.0098$, $R = 0.328$, Fig. 2B).

DISCUSSION

The mechanisms underlying Aβ accumulation, in late-onset sporadic AD are not precisely defined, in part because several different factors are likely to contribute to the pathology. Increased activity of the β- and the γ-secretases is suspected to cause the Aβ overproduction. Indeed, BACE1 expression and activity are increased in AD brain [9]. Hypoxia [11], ischemia [11], oxidative stress [10], each produces (through different mechanisms) the over-expression of BACE1 with a consequent increase in Aβ production. We previously demonstrated that γ-secretase influences the expression and activity of BACE1 through the increased relative production of Aβ_{42} [18,19], suggesting the existence of a positive feedback loop between the γ-secretase and the β-secretase cleavages of AβPP. We have also shown *in vitro*, as well as *in vivo*, that oxidative stress triggers this loop through the over-expression of PS1 and the over-activity of γ-secretase [19,20]. The same effect, i.e., activation of BACE1, is obtained with PS1 mutations linked to familial AD, through the alteration of the γ-secretase that leads to a relative overproduction of Aβ_{42}. Thus, either the augmentation or alteration of γ-secretase is able to activate a cyclic mechanism that fosters increased production of Aβ peptides.

Our study confirms the data from the experimental models, showing that PS1, the catalytic subunit of γ-secretase, is augmented in sporadic AD. The increase of PS1 is proportional to the amount of Aβ in the brain, as expected. The PS1 parameters are not correlated with the ApoE ε4 frequency that affects Aβ aggregation and clearance, but not Aβ production. Finally, our results indicate that the overactivity of both β- and γ-secretase is a major pathologic cause of Aβ accumulation in sporadic AD.

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Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=528).

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