SUPPLEMENTARY INFORMATION

The 28-amino acid form of an APLP1-derived Aβ-like peptide is a surrogate marker for Aβ42 production in the central nervous system

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Running title: Novel APLP1-derived Aβ-like peptides in human CSF

Supplementary table 1. List of APLP1 peptides detected in human CSF

APLP1 derivative	M_r (observed)	M_r (calculated)	Amino acid sequence
APL1β25	2329	2327.2	DELAPAGTGVSREAVSGLLIMGAGG
APL1β27	2473	2471.3	DELAPAGTGVSREAVSGLLIMGAGGGS
APL1β28	2586	2584.3	DELAPAGTGVSREAVSGLLIMGAGGGSL

All amino acid sequences were confirmed by LC/MS/MS analysis (See Supplementary figure 1).

Supplementary table 2. Clinical information for the sporadic AD, non-AD, and non-demented patients in the study

No.	sex	diagnosis		Age
A1	M	syphilis, cancer (pancreas)	clinically	n.r.
A2	M	familial ataxia, schizophrenia	clinically	n.r.
A3	F	secondary parkinsonism, dementia (atypical)	clinically	n.r.
A6	M	dementia (atypical), subdural hematoma	clinically	n.r.
A7	F	viral encephalomyelitis by cytomegalovirus	clinically	n.r.
A8	F	chronic alcoholism, dementia	clinically	n.r.
A12	F	encepharomyelitis	clinically	n.r.
A14	M	bacterial meningoencephalitis, schizophrenia	clinically	n.r.
A16	M	acute subdural hematoma, NPH	clinically	n.r.
A17	M	local brain injury	clinically	n.r.
A18	M	sequela of intracerebral hemorrhage	clinically	n.r.
A19	M	NPH, chronic renal insufficiency	clinically	n.r.
A21	F	amnesia	clinically	n.r.
A22	M	communicating hydrocephalus, cerebral aneurysm	clinically	n.r.
A23	F	NPH, chronic renal insufficiency	clinically	n.r.
A25	M	cerebral thrombosis	clinically	n.r.
A28	M	polyneuropathy, sepsis, schizophrenia	clinically	n.r.
A29	M	syphilis, cirrhosis	clinically	n.r.
A32	M	encepharomyelitis	clinically	n.r.
A33	M	Behcet's disease	clinically	n.r.
A34	M	schizophrenia	clinically	n.r.
A35	M	trauma (body injury)	clinically	n.r.
A37	F	sporadic AD	clinically	n.r.
A38	M	syphilis, dementia	clinically	n.r.
C60	M	sporadic AD, MMSE 22/30 (74y), MMSE 10/30 (77y)	clinically	75
C63	M	sporadic AD	clinically	58
C67	M	dementia (atypical, unspecified), MMSE 0/30 (51y)	clinically	51
C71	F	dementia (atypical)	clinically	46
C75	M	progressive aphasia, MMSE29/30 (70y)	clinically	76
C76	M	sporadic AD, MMSE 7/30 (68y)	clinically	68
C77	F	Pick disease, psychosis (54y)	clinically	55
C78	F	sporadic AD, MMSE20/30 (74y), MMSE16 (79y)	clinically	74
C84	M	FTLD, MMSE 8/30 (60y)	clinically	60
C85	F	sporadic AD, MMSE 7/30 (51y)	clinically	52
C98	F	sporadic AD, MMSE 25 (68y)	clinically	68
C99	F	non-demented, MMSE 28/30 (78y)	clinically	79
C100	M	dementia (atypical), MMSE 14/30 (74y), ADAS 26.7/	clinically	73
C101	M	sporadic AD, MMSE 11/30 (72y)	clinically	72
C103	F	sporadic AD, MMSE 22/30 (76y)	clinically	76
C106	F	NPH, MMSE 23/30 (79y)	clinically	79
C108	M	NPH, MMSE 12/30 (81y)	clinically	82
C111	M	NPH, MMSE 23/30 (70y), ADAS9.6/70 (71y)	clinically	70
C112	F	sporadic AD, MMSE 19/30 (79y)	clinically	79
C113	M	familial dementia (atypical), MMSE 1/30 (78y)	clinically	79
C114	F	sporadic AD, MMSE 18/30 (80y)	clinically	80
D001	F	sporadic AD, Age at onset 76y	clinically	84
D002	F	sporadic AD, Age at onset 63y	clinically	83
D003	F	sporadic AD, Age at onset 76y	clinically	86
D046	F	sporadic AD, Age at onset 49y, ApoE 3/3	clinically	54
D065	F	sporadic AD	clinically	55
D092	M	sporadic AD, Age at onset 60y	clinically	70
D143	F	sporadic AD, Age at onset 64y	clinically	66
D174	M	sporadic AD, Age at onset 69y, ApoE 4/4	clinically	78
D175	F	sporadic AD, Age at onset 71y	clinically	83
D241	F	sporadic AD	clinically	68
D383	F	sporadic AD, Age at onset 78y	clinically	78
D415	M	sporadic AD, Age at onset 59y, ApoE 3/4	clinically	63
D444	F	sporadic AD	clinically	73
D452	F	sporadic AD, Age at onset 51y, ApoE 3/3	clinically	54
D538	M	sporadic AD, Age at onset 74y, ApoE 4/4	clinically	82
D595	F	sporadic AD	clinically	65
D682	M	sporadic AD, Age at onset 60y, ApoE 3/3	clinically	69
D731	M	sporadic AD, Age at onset 74y	clinically	76
D734	F	sporadic AD, Age at onset 72y, ApoE 3/4	clinically	74
D887	F	sporadic AD	clinically	n.r.
D909	F	sporadic AD, Age at onset 52y	clinically	56
D930	F	sporadic AD, Age at onset 76y	clinically	80
D937	F	sporadic AD, Age at onset 55y, ApoE 3/3	clinically	71
E001	M	MCI, MMSE 28/30, converted to sporadic AD	clinically	70
E002	M	MCI, MMSE 28/30, converted to sporadic AD	clinically	61
E003	M	MCI, MMSE 25/30, converted to sporadic AD	clinically	74
E004	F	MCI, MMSE 27/30, converted to sporadic AD	clinically	72
E006	F	MCI, MMSE 27/30, converted to sporadic AD	clinically	78
E007	M	MCI, MMSE 27/30, converted to sporadic AD	clinically	77
E008	M	MCI, MMSE 24/30, converted to sporadic AD	clinically	76
E009	M	MCI, MMSE 24/30, converted to sporadic AD	clinically	72
E010	F	MCI, MMSE 26/30, converted to sporadic AD	clinically	78
E103	F	sporadic AD	clinically	68
E104	M	sporadic AD	clinically	72
E105	F	sporadic AD, MMSE 18/30	clinically	74
E106	M	sporadic AD, MMSE 18/30	clinically	79

n.r., not recorded.

Supplementary figure 1

Determination of the amino acid sequences of the APLP1 peptides in human CSF by

LC/MS/MS analysis.

Left panels; MS/MS spectra of APL1β25 (A), APL1β27 (B), and APL1β28 (C) in human CSF. LC/MS/MS analysis was performed for CSF peptides with molecular weights (A, 2327 Da [m/z 1164.6]; B, 2471 Da [m/z 1236.6]; C, 2584 Da [m/z 1293.2]) and elution times (A, ~21.1 min; B, ~20.6 min; C, ~21.8 min) identical to those of synthetic APL1β species. Enhanced product-ion analysis was performed at the time of elution, and MS/MS spectra were obtained. **Upper right panels; MS/MS spectra of APL1β species with peaks labeled by the Mascot MS/MS Ion Search database.** MS/MS data were submitted to the Mascot MS/MS Ion Search database (A, B, and C; left panels), which determines the amino acid sequences of the parent ions by comparing them with all the protein sequences registered in the SWISS-PROT data base. The database returns the MS/MS spectra with labeled peaks in which calculated and measured molecular weights are identical (A, B and C; upper right panels). **Lower right panels; The probability-based mowse scores of APL1β species in CSF.** The probability-based mowse scores (*P=109 [A], 112 [B], and 53 [C]) indicated that the only possible parent ions that could be generated from human APLP1 for the 2327, 2741, and 2585 Da peptides were the amino acid sequences DELAPAGTGVSREAVSGLLIMGAGG, DELAPAGTGVSREAVSGLLIMGAGGGS, and DELAPAGTGVSREAVSGLLIMGAGGGSL, respectively. Note that the probability score is –10*Log(*P*), where *P* is the probability that the observed match is a random event.

Supplementary figure 2

Juxtamembrane sequence of APLP1 and βAPP are cleaved by recombinant BACE1 and 2 *in vitro*.

Based on the *in vitro* BACE cleavage assay of βAPP (Koike et al., 1999), we established an *in vitro* BACE cleavage assay for the APLP1 juxtamembrane domain. The APLP1 juxtamembrane domain (Nma-EIQRDELAK(Dnp)-RR-NH2) was mixed with recombinant ectodomain of BACE1/2. Following incubation for 3 h at 30ºC, the fluorescence was measured at an excitation wavelength of 320 nm and an emission wavelength of 405 nm. The recombinant BACE1/2 resulted in an increase in the fluorescence. The increase could be inhibited by addition of inhibitor IV (10 μM). These results show that APLP1 as well as the βAPP peptide (Farzan et al., 2000; Hussain et al., 2000) were cleaved by BACE1/2. Note that activities of the recombinant BACE1 and BACE2 enzymes were normalized by their proteolytic activities for the βAPP peptide. The experiments were performed three times and values represent means ± SD.

Supplementary figure 3

Selection of optimal daughter ions and conditions for quantification by LC/MS/MS analysis.

Parental ions for APL1β25 (A), APL1β27 (B), and APL1β28 (C) were degraded into

several daughter ions by MS/MS (collision energy, 50–80 eV). The daughter ions for which the peak heights were relatively high were selected for quantification. For APL1β25/28, the b2, y20, and y21 ions were used, whereas for APL1β27, the b2, y21, and y22 ions were used.

Supplementary figure 4

FA fractions from AD brain tissues contain almost no APL1β.

(A) FA fractions from sporadic AD brain tissues (65 mg) contained very high amounts of Aβ but almost no detectable APL1β. (B, C) Additional presentations of the data of Figure 4A and B. Note that FA fractions extracted from the same amount of sporadic AD (AD1 and AD2) or non-AD (N1 and N2) brain samples (65 mg) were used in the experiment shown in (A) and (C).

Supplementary figure 5

Immunohistochemical detection of senile plaques in serial AD sections.

Both with (E,F, and G) and without (B, C, and D) FA treatment (brain sections were dipped in 100% FA solution for 10 s), paraffin sections of brain tissues were stained with the anti-APL1β antibodies OA601 (C and F) and OA663 (D and G). Senile plaques were not detected with the antibodies, although under the experimental conditions, a considerable number of senile plaques were detected using anti-Aβ antibody 4G8 even in the absence of FA treatment (B). Panels A and H show methenamine silver staining.

Supplementary figure 6

APL1β forms almost no protofibril *in vitro*.

To further characterize the non-aggregative nature of APL1β, we incubated the APL1β species (B, C, and D) *in vitro* under conditions where Aβ40 (A) forms amyloid fibrils (Hartley et al., 1999). To observe potential protofibril formation by APL1β, we increased the incubation period 12-fold compared to that necessary for the formation of Aβ40 protofibrils. Following the incubation, we examined amyloid protofibril formation by size exclusion chromatography. We could not detect any APL1β-derived protofibrils (B, C, and D), although we confirmed that Aβ (A) forms protofibrils under these conditions.

Supplementary figure 7

Sulindac sulfide and compound-W, which lower the relative Aβ42 level, do not decrease the relative APL1β28 level.

(A) In addition to S2474, fenofibrate, another PS/γ-secretase modifier, also induces an increase in the relative secretion of APL1β28 to total APL1β in naive SH-SY5Y cells. Blue, red, and yellow bars indicate the ratios of APL1β25, APL1β27, and APL1β28 to total APL1β,

respectively. In the conditioned media, the ratio of Aβ42 to total Aβ was elevated (data not shown). However, sulindac sulfide and Compound-W (CW) decreased the relative generation of Aβ42 to total Aβ in naive SH-SY5Y cells (B), but they did not decrease the relative generation of APL1β28 to total APL1β (C). (D) The relationship between the relative levels of APL1β28 to Aβ42 in the presence of the indicated γ-secretase modulators.

Supplementary figure 8

Displacement of endogenous PS proteins in PS/γ-secretase complex by the exogenous mutant form in stable cell lines.

We prepared K293 cell lines stably expressing both βAPP sw and wt APLP1. From these cell lines, we chose stable clones that expressed exogenous PS1 mutants at levels high enough so that endogenous PS1/2 in the PS/γ-secretase complex was displaced by the exogenous PS1 mutants. To do this, we first chose stable cell lines that overexpress PS1 holoprotein, which is degraded via the ubiquitin-proteasome pathway immediately after translation (Steiner et al., 1998), such that the positive signal is barely detectable in untransfected cells. Subsequently, from these cell lines, we further selected clones in which endogenous PS2 is considerably displaced by the exogenous PS1 mutant proteins. The PS1 holoprotein band (~45 kDa) was clearly observed, whereas the PS2 CTF band (~18 kDa) was barely detectable. Relative levels of APL1β and Aβ species in conditioned media from these PS1 mutant-expressing cells were determined and are shown in Figure 5D.

Supplementary figure 9

Each APL1β species in CSF is degraded in a similar rate.

We investigated whether there is a difference in the rate of degradation for the various APL1β species in CSF between sporadic AD patients (B) and non-demented controls (A). The experiments were performed three times, and representative data are shown. We incubated each APL1β species (2.5 nmol) in the CSF (100 μl) for the indicated time. After boiling for 5 min in SDS-sample buffer, the samples were separated in Tris-Tricine gels and then analyzed by Western blotting with the OA601 antibody. Note that the rate of degradation of each APL1β species did not differ.

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