

DESCRIPTION

TITLE OF INVENTION: PEPTIDE OR SALT THEREOF, COMPOSITION
CONTAINING SAME, AND COMPOSITION FOR SUPPRESSING DECLINE OF
5 OR IMPROVING COGNITIVE FUNCTIONS

TECHNICAL FIELD

[0001]

The present invention relates to a peptide or a salt thereof and a composition
10 containing the same. The present invention also relates to, for example, a composition
for reducing a decline in cognitive function or improving cognitive function.

BACKGROUND ART

[0002]

15 Improvement of cognitive function such as memory function is a universal
demand across generations. A decline in cognitive function with age has recently
become a problem in countries with a high proportion of elderly individuals in their
population structure, such as Japan, and searches are being conducted for components
that can reduce a decline in cognitive function or improve cognitive function through
20 daily intake of the components as, for example, foods or beverages. For example,
Patent Literature 1 discloses an agent for reducing a decline in cognitive function and/or
improving cognitive function of a subject, the agent containing Cyclo(Gly-Pro).

[0003]

Amyloid β (A β) is a protein produced in the brain. A report shows a strong
25 positive correlation between the amyloid β concentration in the brain and the cognitive
function (Non-Patent Literature 1). Amyloid β that has aggregated into oligomers or
fibers exhibits neurotoxicity. Such aggregated amyloid β is not discharged from the
brain but accumulates therein, which is considered to trigger the onset of Alzheimer's
disease. Thus, reducing the production or accumulation of amyloid β is useful in
30 reducing a decline in cognitive function or improving cognitive function.

[0004]

Amyloid β is generated through the two-stage cleavage of an amyloid β
precursor protein (APP) by β -secretase 1 (BACE1) and γ -secretase. Another report
shows an increase in the activity and expression of BACE1 in the brain of patients with
35 mild cognitive impairment (MCI) and Alzheimer's disease (Non-Patent Literature 2).

Another report shows that inhibition of BACE1 reduces accumulation of amyloid β at an early stage of amyloid β accumulation (Non-Patent Literature 3).

CITATION LIST

5 - Patent Literature

[0005]

Patent Literature 1: JP 2020-196686 A

- Non-Patent Literature

10 [0006]

Non-Patent Literature 1: Naslund et al., JAMA 2000, 283(12), 1571-1577.

Non-Patent Literature 2: Cheng et al., Am. J. Pathol. 2014; 184(1):141-147.

Non-Patent Literature 3: Peters et al., Acta Neuropathol. 2018; 135(5):695-710.

15 SUMMARY OF INVENTION

- Technical Problem

[0007]

20 The present invention aims to provide a peptide or a salt thereof useful in reducing a decline in cognitive function or improving cognitive function. The present invention also aims to provide a composition for reducing a decline in cognitive function or improving cognitive function.

- Solution to Problem

[0008]

25 The present inventors made extensive studies on the above problem to find peptides having an action that reduces at least one of production or accumulation of amyloid β . A peptide or a salt thereof having an action that reduces at least one of production or accumulation of amyloid β is useful in reducing a decline in cognitive function or improving cognitive function.

30 [0009]

Specifically, although not limited thereto, the present invention relates to, for example, a peptide or a salt thereof and a composition described below.

(1) A composition containing: a peptide consisting of an amino acid sequence selected from the group consisting of LYEGY (SEQ ID NO: 7), GGRYY (SEQ ID NO: 1), AHDGGRYY (SEQ ID NO: 2), AEYY (SEQ ID NO: 3), AEYYRA (SEQ ID NO: 4),

35

GVTHNVP (SEQ ID NO: 5), GVTHNVPIYEGY (SEQ ID NO: 6), ISGW (SEQ ID NO: 8), IKGY (SEQ ID NO: 9), pEAPPH (SEQ ID NO: 10), LTEAP (SEQ ID NO: 11), DLTVTN (SEQ ID NO: 12), pEIISAN (SEQ ID NO: 13), ILPVPA (SEQ ID NO: 14), TERGYS (SEQ ID NO: 15), and IGME (SEQ ID NO: 16); or a salt thereof.

5 (2) The composition according to the clause (1), wherein the composition is used to reduce a decline in cognitive function or improve cognitive function.

(3) The composition according to the clause (1) or (2), containing: the peptide consisting of the amino acid sequence LYEGY (SEQ ID NO: 7) or a salt thereof; and the peptide consisting of the amino acid sequence ILPVPA (SEQ ID NO: 14) or a salt thereof, the composition further containing: a peptide consisting of an amino acid
10 sequence FLPM (SEQ ID NO: 21) or a salt thereof; a peptide consisting of an amino acid sequence FQPSF (SEQ ID NO: 22) or a salt thereof; and Cyclo(Trp-Pro) or a salt thereof.

(4) The composition according to any one of the clauses (1) to (3), further
15 containing a peptide consisting of an amino acid sequence selected from the group consisting of VIQY (SEQ ID NO: 17), IWHHT (SEQ ID NO: 18), IVGRPR (SEQ ID NO: 19), VV, and AY, or a salt thereof.

(5) A composition for reducing a decline in cognitive function or improving cognitive function, the composition containing: a peptide consisting of an amino acid
20 sequence selected from the group consisting of LYEGY (SEQ ID NO: 7), GGRIYY (SEQ ID NO: 1), AHDGGRIYY (SEQ ID NO: 2), AEYY (SEQ ID NO: 3), AEYYRA (SEQ ID NO: 4), GVTHNVP (SEQ ID NO: 5), GVTHNVPIYEGY (SEQ ID NO: 6), ISGW (SEQ ID NO: 8), IKGY (SEQ ID NO: 9), pEAPPH (SEQ ID NO: 10), LTEAP (SEQ ID NO: 11), DLTVTN (SEQ ID NO: 12), pEIISAN (SEQ ID NO: 13), ILPVPA
25 (SEQ ID NO: 14), TERGYS (SEQ ID NO: 15), IGME (SEQ ID NO: 16), VIQY (SEQ ID NO: 17), IWHHT (SEQ ID NO: 18), IVGRPR (SEQ ID NO: 19), VV, and AY; or a salt thereof.

(6) The composition according to the clause (5), wherein the composition reduces at least one of production or accumulation of amyloid β .

30 (7) The composition according to the clause (5) or (6), wherein the composition reduces at least one of production or accumulation of amyloid β by inhibiting β -secretase.

(8) The composition according to any one of the clauses (5) to (7), containing: the peptide consisting of the amino acid sequence LYEGY (SEQ ID NO: 7) or a salt
35 thereof; and the peptide consisting of the amino acid sequence ILPVPA (SEQ ID NO:

14) or a salt thereof, the composition further containing a peptide consisting of an amino acid sequence FLPM (SEQ ID NO: 21) or a salt thereof; a peptide consisting of an amino acid sequence FQPSF (SEQ ID NO: 22) or a salt thereof; and Cyclo(Trp-Pro) or a salt thereof.

5 (9) The composition according to any one of the clauses (5) to (8), wherein the composition contains the peptide consisting of the amino acid sequence GVTHNVPIYEGY (SEQ ID NO: 6) or a salt thereof and reduces accumulation of amyloid β by activating neprilysin.

10 (10) The composition according to any one of the clauses (5) to (9), wherein the composition contains the peptide consisting of the amino acid sequence IGME (SEQ ID NO: 16) or a salt thereof and reduces accumulation of amyloid β by promoting microglial phagocytosis.

(11) The composition according to any one of the clauses (1) to (10), wherein the composition is an oral composition.

15 (12) The composition according to any one of the clauses (1) to (11), wherein the composition is a food or beverage or an oral pharmaceutical product.

(13) The composition according to any one of the clauses (1) to (12), wherein the composition is labeled with at least one function claim selected from the group consisting of "enhancing cognitive function", "reducing a decline in cognitive function",
20 "maintaining good cognitive function", "improving memory", "reducing a decline in memory", "maintaining good memory", "improving accuracy of memory", "preventing memory disorder", "ameliorating memory disorder", "maintaining memory, which is a domain of cognitive function", "function suitable for those who are concerned about a decline in memory", "improving accuracy of memory and/or correctness of judgement",
25 "improving memory retention or integration", "maintaining enhancement of cognitive function", "improving executive function", "promoting attention and concentration", "improving learning ability", "maintaining or improving orientation", "delaying an aging-related decline in cognitive function", "enhancing short-term and long-term memory", "promoting verbal and visuospatial
30 memory", and "maintaining or improving logical reasoning".

(14) A peptide consisting of an amino acid sequence selected from the group consisting of LYEGY (SEQ ID NO: 7), GGRYY (SEQ ID NO: 1), AHDGGRYY (SEQ ID NO: 2), AEYY (SEQ ID NO: 3), AEYYRA (SEQ ID NO: 4), GVTHNVP (SEQ ID NO: 5), GVTHNVPIYEGY (SEQ ID NO: 6), ISGW (SEQ ID NO: 8), IKGY (SEQ ID NO: 9), pEAPPH (SEQ ID NO: 10), LTEAP (SEQ ID NO: 11), DLTVTN (SEQ ID NO:
35

12), pEIISAN (SEQ ID NO: 13), ILPVPA (SEQ ID NO: 14), TERGYS (SEQ ID NO: 15), and IGME (SEQ ID NO: 16), or a salt thereof.

- Advantageous Effects of Invention

5 [0010]

The present invention can provide a peptide or a salt thereof useful in reducing a decline in cognitive function or improving cognitive function. The present invention can also provide a composition for reducing a decline in cognitive function or improving cognitive function.

10

BRIEF DESCRIPTION OF DRAWINGS

[0011]

FIG. 1 is a graph showing the evaluation results of the phagocytic activity of microglia (SIM-A9 cells) treated with peptide P16.

15

FIG. 2 is a graph showing the evaluation results of the enzyme activity of neprilysin (NEP) in microglia treated with peptide P6.

FIG. 3A is a graph showing the concentration of amyloid β_{1-38} (A β 38) in the culture supernatant of the H4 glioma cells (H4-hAPP cells) treated with the peptide mixture. FIG. 3B is a graph showing the concentration of amyloid β_{1-42} (A β 42) in the culture supernatant of the H4-hAPP cells treated with the peptide mixture.

20

DESCRIPTION OF EMBODIMENTS

[0012]

The peptide or a salt thereof of the present invention is a peptide consisting of an amino acid sequence selected from the group consisting of GGRYY (SEQ ID NO: 1), AHDGGRYY (SEQ ID NO: 2), AEYY (SEQ ID NO: 3), AEYYRA (SEQ ID NO: 4), GVTHNVP (SEQ ID NO: 5), GVTHNVPIYEGY (SEQ ID NO: 6), LYEGY (SEQ ID NO: 7), ISGW (SEQ ID NO: 8), IKGY (SEQ ID NO: 9), pEAPPH (SEQ ID NO: 10), LTEAP (SEQ ID NO: 11), DLTVTN (SEQ ID NO: 12), pEIISAN (SEQ ID NO: 13), ILPVPA (SEQ ID NO: 14), TERGYS (SEQ ID NO: 15), and IGME (SEQ ID NO: 16) or a salt thereof.

30

[0013]

Herein, the amino acid sequences of the peptides are expressed with conventional one-letter codes described below, unless otherwise specified.

35

A: alanine residue, R: arginine residue, N: asparagine residue, D: aspartic acid

residue, C: cysteine residue, Q: glutamine residue, E: glutamic acid residue, G: glycine residue, H: histidine residue, I: isoleucine residue, L: leucine residue, K: lysine residue, M: methionine residue, F: phenylalanine residue, P: proline residue, S: serine residue, T: threonine residue, W: tryptophan residue, Y: tyrosine residue, V: valine residue

5 Herein, pE represents pyroglutamic acid residue. Pyroglutamic acid (pyro-Glu) is a cyclic amino acid formed by intramolecular dehydration and condensation between the γ -carboxy group and the α -amino group of glutamic acid.
[0014]

10 Herein, the amino acid sequence of a peptide is described in accordance with the conventional notation for peptides such that the N-terminus (amino terminus) is on the left side and the C-terminus (carboxy terminus) is on the right side. The peptides are linear, unless otherwise specified. In the case of an amino acid that may have an optical isomer, the amino acid in the L form is described, unless otherwise specified.
[0015]

15 In the present invention, the salt of a peptide may be any salt that is pharmacologically acceptable or any salt that is acceptable for foods and beverages, and may be either an acidic salt or a basic salt. Examples of the acidic salt include inorganic acidic salts such as hydrochloride, sulfate, nitrate, and phosphate; and organic acidic salts such as acetate, citrate, maleate, malate, oxalate, lactate, succinate, fumarate,
20 and propionate. Examples of the basic salt include alkali metal salts such as sodium salt and potassium salt; and alkaline earth metal salts such as calcium salt and magnesium salt.
[0016]

25 In the present invention, each peptide or a salt thereof may be derived from any origin and may be produced by any method. The peptide or a salt thereof of the present invention can be produced by a known method. Each peptide can be produced by a known peptide synthesis method, for example. In the case of chemical synthesis, the peptide of the present invention can be synthesized by either a solid phase method or a liquid phase method. The peptide obtained by synthesis can be purified by a
30 common purification method using reversed-phase high-performance liquid chromatography, chromatography with an ion exchange resin or a highly porous polymer resin, or affinity chromatography, for example.

 The peptide of the present invention can also be biosynthesized using microorganisms. In the case of biosynthesis using microorganisms, a peptide-
35 encoding gene is first introduced into a vector to produce an expression vector and this

expression vector is introduced into a host microorganism to provide a transformant. This transformant is then cultured to prepare a culture, and the peptide can be purified from this culture. Any host microorganism can be used, such as *Escherichia coli* or yeast. Isolation and purification from the culture can be performed by common
 5 purification methods. The peptide of the present invention can also be produced by enzymatically or chemically hydrolyzing a protein containing the amino acid sequence of the peptide, for example. A salt of the peptide can be easily prepared by a person skilled in the art by any method known in the art.

Peptides or salts thereof other than the peptide or a salt thereof of the present
 10 invention can also be produced, for example, by a known peptide synthesis method or the above biosynthesis method using microorganisms.

[0017]

The peptide or a salt thereof of the present invention has an action that reduces at least one of production or accumulation of amyloid β . The peptide or a salt thereof
 15 of the present invention can be used to reduce at least one of production or accumulation of amyloid β . Reducing at least one of production or accumulation of amyloid β in the brain can reduce a decline in cognitive function or improve cognitive function. The peptide or a salt thereof of the present invention can be used to reduce a decline in cognitive function or improve cognitive function, for example.

20 [0018]

Amyloid β is a peptide also referred to as amyloid β protein, amyloid β peptide, or β -amyloid. Amyloid β is a peptide usually consisting of about 40 amino acids. Examples thereof include amyloid β_{1-42} , amyloid β_{1-40} , and amyloid β_{1-38} . Unassociated amyloid β is also referred to as an amyloid β monomer. Two or more
 25 amyloid β monomers may gather to form an aggregate. For example, two or more (e.g., 2 to 50) amyloid β monomers may gather to form a soluble aggregate (amyloid β oligomer). Example of the amyloid β oligomer include an amyloid β_{1-42} oligomer, an amyloid β_{1-40} oligomer, and an amyloid β_{1-38} oligomer. The amyloid β in the present invention includes unassociated amyloid β and aggregate-forming (e.g., oligomer-
 30 forming) amyloid β .

[0019]

Peptides consisting of the respective amino acid sequences of SEQ ID NOS: 1 to 5 and 7 to 15 or salts thereof have a β -secretase inhibitory action. These peptides or salts thereof can be used to inhibit β -secretase. β -Secretase is a rate-determining
 35 enzyme for production of amyloid β , and its cleavage regulates the amount of total

amyloid β production. Thus, inhibiting β -secretase can reduce production of amyloid β and can reduce accumulation of amyloid β . A peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1 to 5 and 7 to 15 or a salt thereof can be used to reduce at least one of production or accumulation of amyloid β by inhibiting β -secretase, for example.

[0020]

The peptide consisting of the amino acid sequence of SEQ ID NO: 6 (GVTHNVPIYEGY) or a salt thereof has an action that activates neprilysin (NEP). The peptide consisting of the amino acid sequence of SEQ ID NO: 6 or a salt thereof can be used to activate neprilysin. Herein, the activation of neprilysin includes an increase in the enzyme activity of neprilysin and an increase in the neprilysin protein level. Neprilysin is a major enzyme involved in degradation of amyloid β in the brain. Neprilysin can degrade not only amyloid β monomers but also amyloid β oligomers. Activating neprilysin in the brain can reduce accumulation of amyloid β . The peptide consisting of the amino acid sequence of SEQ ID NO: 6 or a salt thereof can be used to reduce accumulation of amyloid β by activating neprilysin, for example.

[0021]

The peptide consisting of the amino acid sequence of SEQ ID NO: 16 (IGME) or a salt thereof has an action that promotes microglial phagocytosis. The peptide consisting of the amino acid sequence of SEQ ID NO: 16 or a salt thereof can be used to promote microglial phagocytosis. Microglia are cells of the central nervous system in the brain. Microglia play an important role in maintaining the brain environment. One of their actions is phagocytosis of waste products in the brain, such as extracellular proteins, e.g., amyloid β , and damaged cells. Promoting the microglial phagocytosis promotes removal of amyloid β . Thus, promoting the microglial phagocytosis is effective in reducing accumulation of amyloid β . The peptide consisting of the amino acid sequence of SEQ ID NO: 16 or a salt thereof can be used to reduce accumulation of amyloid β by promoting the microglial phagocytosis.

[0022]

The peptide or a salt thereof of the present invention can be used as, for example, a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, a cosmetic product, or feed by itself or in the form of a composition containing this peptide or a salt thereof and other components. The food or beverage may be, for example, a general food or beverage, a health food, a health beverage, a food with function claims, a food for specified health uses, a dietary supplement, a food for the

sick, or a raw material thereof.

[0023]

A composition containing the peptide or a salt thereof of the present invention is also encompassed by the present invention.

5 The composition of the present invention contains a peptide consisting of an amino acid sequence selected from the group consisting of GG RYY (SEQ ID NO: 1), AHDGG RYY (SEQ ID NO: 2), AEYY (SEQ ID NO: 3), AEYYRA (SEQ ID NO: 4), GVTHNVP (SEQ ID NO: 5), GVTHNVPIYEGY (SEQ ID NO: 6), LYEGY (SEQ ID NO: 7), ISGW (SEQ ID NO: 8), IKGY (SEQ ID NO: 9), pEAPPH (SEQ ID NO: 10),
10 LTEAP (SEQ ID NO: 11), DLTVTN (SEQ ID NO: 12), pEIISAN (SEQ ID NO: 13), ILPVP A (SEQ ID NO: 14), TERGYS (SEQ ID NO: 15), and IGME (SEQ ID NO: 16), or a salt thereof.

 The composition may contain one of the above peptides or salts thereof or two or more thereof. In an embodiment, the composition preferably contains two or more
15 of the peptides or salts thereof.

 The peptides consisting of the respective amino acid sequences of SEQ ID NOS: 1 to 16 or salts thereof have an action that reduces at least one of production or accumulation of amyloid β as described above. A composition containing at least one of the peptides or salts thereof can be used to reduce at least one of production or
20 accumulation of amyloid β , for example. Preferably, the composition can be used to reduce at least one of production or accumulation of amyloid β in the brain.

[0024]

 The composition may contain the peptide or a salt thereof of the present invention in any amount. For example, the total amount of the peptide or a salt thereof
25 can be 0.001 to 50% by weight. The peptide or a salt thereof can be quantified by a known method, using liquid chromatography-mass spectroscopy (LC-MS/MS), for example. In the case where the composition contains two or more peptides or salts thereof, the total amount of a peptide or a salt thereof refers to the sum of the amounts of these peptides or salts thereof.

30 The LC-MS/MS measurement can be performed under the following conditions.

 The device may be Agilent 1290 binary LC coupled with AB Sciex 5600+ TripleTOF system, for example.

 LC column: CORTECS T3 Column, 120 Å, 1.6 μ m, 2.1 mm $\times$ 100 mm (Waters
35 Corporation)

Amount of sample injected: 5 μ L

Column temperature: 40°C

(Conditions for LC chromatography)

Mobile phase:

5 Solution A: water with 0.1% formic acid

 Solution B: acetonitrile with 0.1% formic acid

Gradient (Concentration of solution B):

0 to 1 min: Solution B, 1%

1 to 8.5 min: Solution B, 1% $\rightarrow$ 40%

10 8.5 to 13 min: Solution B, 40% $\rightarrow$ 90%

13 to 15.5 min: Solution B, 90% $\rightarrow$ 100%

15.5 to 17.8 min: Solution B, 100%

17.8 to 18 min: Solution B, 100% $\rightarrow$ 1%

18 to 20 min: Solution B, 1%

15 [0025]

 Preferably, the composition of the present invention further contains a peptide consisting of an amino acid sequence selected from the group consisting of VIQY (SEQ ID NO: 17), IWHHT (SEQ ID NO: 18), IVGRPR (SEQ ID NO: 19), VV, and AY or a salt thereof. These peptides or salts thereof have a β -secretase inhibitory action.

20 Thus, these peptides or salts thereof have an action that reduces at least one of production or accumulation of amyloid β . These peptides or salts thereof can be used to reduce a decline in cognitive function or improve cognitive function.

[0026]

25 A composition containing a peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1 to 5, 7 to 15, 17 to 19, VV, and AY or a salt thereof can be used as a composition for inhibiting β -secretase, for example. The β -secretase is preferably β -secretase 1. A composition containing the above peptide or a salt thereof can be used to reduce at least one of production or accumulation of amyloid β by inhibiting β -secretase. In an embodiment, the composition of the present invention can be used to

30 reduce at least one of production or accumulation of amyloid β by inhibiting β -secretase.

[0027]

 A composition containing the peptide consisting of the amino acid sequence of SEQ ID NO: 6 (GVTHNVPIYEGY) or a salt thereof can be used as a composition for

35 activating neprilysin. The composition containing the peptide consisting of the amino

acid sequence of SEQ ID NO: 6 or a salt thereof can be used to reduce at least one of production or accumulation of amyloid β by activating neprilysin, for example.

[0028]

5 A composition containing the peptide consisting of the amino acid sequence of SEQ ID NO: 16 (IGME) or a salt thereof can be used as a composition for promoting microglial phagocytosis. A composition containing the peptide consisting of the amino acid sequence of SEQ ID NO: 16 or a salt thereof can be used to reduce accumulation of amyloid β by promoting microglial phagocytosis, for example.

[0029]

10 In an embodiment, the composition of the present invention preferably contains the peptide consisting of the amino acid sequence LYEGY (SEQ ID NO: 7) or a salt thereof. In an embodiment, the composition of the present invention preferably contains the peptide consisting of the amino acid sequence LYEGY (SEQ ID NO: 7) or a salt thereof and the peptide consisting of the amino acid sequence ILPVPA (SEQ ID
15 NO: 14) or a salt thereof, and more preferably further contains a peptide consisting of the amino acid sequence FLPM (SEQ ID NO: 21) or a salt thereof, a peptide consisting of the amino acid sequence FQPSF (SEQ ID NO: 22) or a salt thereof, and Cyclo(Trp-Pro) or a salt thereof.

A composition containing the peptide consisting of the amino acid sequence of
20 SEQ ID NO: 7 or a salt thereof, the peptide consisting of the amino acid sequence of SEQ ID NO: 14 or a salt thereof, the peptide consisting of the amino acid sequence of SEQ ID NO: 21 or a salt thereof, the peptide consisting of the amino acid sequence of SEQ ID NO: 22 or a salt thereof, and Cyclo(Trp-Pro) or a salt thereof can be suitably used to reduce at least one of production or accumulation of amyloid β , for example.
25 Cyclo(Trp-Pro) (cyclotryptophanylproline) is a cyclic dipeptide having a structure in which tryptophan (Trp) and proline (Pro) are bonded by condensation. Herein, the “cyclic dipeptide” refers to a compound characteristically having amino acids as structural units and having a diketopiperazine structure formed by dehydration condensation of the amino group of the N-terminal amino acid and the carboxy group of
30 the C-terminal amino acid. Herein, the amino acids may be described in any order as long as the amino acid structure in the cyclic dipeptide is the same; Cyclo(Trp-Pro) and Cyclo(Pro-Trp) (cycloprolyltryptophan) represent the same cyclic dipeptide.

[0030]

35 In the composition of the present invention, components other than the above peptides or salts thereof can be selected as appropriate according to the form of the

composition, for example. The total amount of a peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1 to 19, VV, and AY or a salt thereof in the composition may be, but is not limited to, 0.001 to 50% by weight, for example. In an embodiment where the composition is a liquid composition such as a beverage, the total amount of the peptide or a salt thereof is preferably 0.01 to 200 mg/mL, more preferably 0.05 to 100 mg/mL.

[0031]

Since a peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1 to 19, VV, and AY or a salt thereof has an action that reduces at least one of production or accumulation of amyloid β , this peptide or a salt thereof and a composition containing the same are useful in reducing a decline in cognitive function or improving cognitive function, for example. In an embodiment, preferably, the composition of the present invention can be used to reduce a decline in cognitive function or improve cognitive function. The composition of the present invention may contain one or two or more of the peptides or salts thereof as active ingredients, for example. The peptide or a salt thereof or a composition containing the same can be used to prevent or ameliorate a condition or disease associated with accumulation of amyloid β , preferably a condition or disease associated with accumulation of amyloid β in the brain. Examples of this condition or disease include a condition or disease involving or resulting from accumulation of amyloid β . Examples of a condition or disease involving or resulting from accumulation of amyloid β in the brain include Alzheimer's disease (including Alzheimer's type dementia), mild cognitive impairment, and aging-related cognitive dysfunction.

Herein, preventing a condition or disease encompasses preventing the onset, delaying the onset, reducing the incidence, reducing the onset risk, and the like. Ameliorating a condition or disease encompasses helping a subject recover from a condition or disease, alleviating a symptom of a condition or disease, changing a symptom of a condition or disease for the better, delaying or preventing the progress of a condition or disease, and the like.

[0032]

The present invention also encompasses a composition for reducing a decline in cognitive function or improving cognitive function. The composition for reducing a decline in cognitive function or improving cognitive function of the present invention contains a peptide consisting of an amino acid sequence selected from the group consisting of GGRYY (SEQ ID NO: 1), AHDGGRYY (SEQ ID NO: 2), AEYY (SEQ

ID NO: 3), AEYYRA (SEQ ID NO: 4), GVTHNVP (SEQ ID NO: 5),
 GVTHNVPIYEGY (SEQ ID NO: 6), LYEGY (SEQ ID NO: 7), ISGW (SEQ ID NO: 8),
 IKGY (SEQ ID NO: 9), pEAPPH (SEQ ID NO: 10), LTEAP (SEQ ID NO: 11),
 DLTVTN (SEQ ID NO: 12), pEIISAN (SEQ ID NO: 13), ILPVPA (SEQ ID NO: 14),
 5 TERGYS (SEQ ID NO: 15), IGME (SEQ ID NO: 16), VIQY (SEQ ID NO: 17),
 IWHHT (SEQ ID NO: 18), IVGRPR (SEQ ID NO: 19), VV, and AY, or a salt thereof.

The composition for reducing a decline in cognitive function or improving
 cognitive function of the present invention usually contains the peptide or a salt thereof
 as an active ingredient. The composition for reducing a decline in cognitive function
 10 or improving cognitive function may contain one of the peptides or salts thereof or two
 or more thereof. In an embodiment, the composition for reducing a decline in
 cognitive function or improving cognitive function preferably contains two or more of
 the peptides or salts thereof because such a composition can provide a higher effect of
 reducing a decline in cognitive function or improving cognitive function.

15 [0033]

In an embodiment, the composition for reducing a decline in cognitive function
 or improving cognitive function of the present invention can be used to reduce a decline
 in cognitive function or improve cognitive function by reducing at least one of
 production or accumulation of amyloid β .

20 [0034]

In an embodiment, the composition for reducing a decline in cognitive function
 or improving cognitive function of the present invention preferably contains a peptide
 consisting of the amino acid sequence of any of SEQ ID NOS: 1 to 5, 7 to 15, 17 to 19,
 VV, and AY, or a salt thereof. A composition containing a peptide consisting of any of
 25 the above amino acid sequences or a salt thereof can reduce at least one of production or
 accumulation of amyloid β by inhibiting β -secretase. In an embodiment, the
 composition preferably contains the peptide consisting of the amino acid sequence
 VIQY (SEQ ID NO: 17) or a salt thereof because such a composition has an excellent β -
 secretase inhibitory action.

30 [0035]

In an embodiment, the composition for reducing a decline in cognitive function
 or improving cognitive function preferably contains the peptide consisting of the amino
 acid sequence of SEQ ID NO: 17. More preferably, the composition further contains a
 peptide consisting of the amino acid sequence of any of SEQ ID NO: 1 to 16, 18, and
 35 19, VV, and AY or a salt thereof.

[0036]

In an embodiment, the composition for reducing a decline in cognitive function or improving cognitive function of the present invention preferably contains the peptide consisting of the amino acid sequence of SEQ ID NO: 6 or a salt thereof. A
5 composition containing this peptide or a salt thereof can reduce at least one of production or accumulation of amyloid β by activating neprilysin.

[0037]

In an embodiment, the composition for improving cognitive function of the present invention preferably contains the peptide consisting of the amino acid sequence
10 of SEQ ID NO: 16 or a salt thereof. A composition containing this peptide or a salt thereof can reduce accumulation of amyloid β by promoting microglial phagocytosis.

[0038]

The composition for reducing a decline in cognitive function or improving cognitive function of the present invention can be used to prevent or ameliorate a
15 disease or condition associated with a decline in cognitive function. The composition for reducing a decline in cognitive function or improving cognitive function can be used to prevent or ameliorate Alzheimer's disease (including Alzheimer's type dementia), mild cognitive impairment, or aging-related cognitive dysfunction. Alzheimer's disease, mild cognitive impairment, and aging-related cognitive dysfunction are known
20 to cause a decline in cognitive function. Examples of symptoms of a decline in cognitive function caused by Alzheimer's disease, mild cognitive impairment, and aging-related cognitive dysfunction include a decline in memory, memory disorder (forgetfulness), aphasia (inability to formulate words), apraxia, agnosia (e.g., getting lost in a familiar place), a decline in orientation (ability to correctly recognize one's
25 situation, such as a place, time, and people's names), a decline in verbal and non-verbal learning abilities, a decline in auditory and visual processing, a decline in executive function, executive dysfunction (an inability to formulate and carry out plans), a decline in concentration, a decline in attention, a decline in judgement, a decline in spatial awareness, a decline in cognitive flexibility, and a decline in information processing
30 speed. The composition for reducing a decline in cognitive function or improving cognitive function of the present invention can be used to prevent or ameliorate symptoms of the decline in cognitive function, for example.

In an embodiment, the composition of the present invention can be used to reduce a decline in cognitive function caused by Alzheimer's disease, mild cognitive
35 impairment, or aging-related cognitive dysfunction. In the present invention, to reduce

a decline in cognitive function encompasses to reduce a further decline (worsening) in cognitive function, i.e. to maintain cognitive function, and to delay a decline (worsening) in cognitive function. To improve cognitive function encompasses to improve cognitive function that has been declined (worsened) once. The “cognitive function” means brain functions such as memory function, learning ability, consolidation of memory, executive function, attention (attention function), and concentration. The cognitive dysfunction means loss of part or all of the cognitive functions described above.

[0039]

The amount of a peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1 to 19, VV, and AY or a salt thereof in the composition for reducing a decline in cognitive function or improving cognitive function is not limited and can be set according to the form of the composition, for example.

In an embodiment, the total amount of the peptide or a salt thereof in the composition for reducing a decline in cognitive function or improving cognitive function may be, for example, 0.001% by weight or more, preferably 0.01% by weight or more and may be 50% by weight or less, preferably 10% by weight or less. In an embodiment, the total amount of the peptide or a salt thereof may be, for example, 0.001 to 50% by weight, preferably 0.01 to 10% by weight, in the composition for reducing a decline in cognitive function or improving cognitive function. In an embodiment where the composition for reducing a decline in cognitive function or improving cognitive function is a liquid composition such as a beverage, the total amount of the peptide or a salt thereof is preferably 0.01 to 200 mg/mL, more preferably 0.1 to 100 mg/mL in the composition.

[0040]

The composition for reducing a decline in cognitive function or improving cognitive function of the present invention is preferably orally ingested (orally administered). The administration amount (also referred to as the intake) of the composition for reducing a decline in cognitive function or improving cognitive function is not limited. The administration amount may be any amount that provides an effect of reducing a decline in cognitive function or improving cognitive function, and may be set as appropriate according to the dosage form, administration method, body weight of a subject, and the like. The administration amount of the composition for reducing a decline in cognitive function or improving cognitive function is preferably an amount that provides at least one effect selected from the group consisting

of an effect of inhibiting β -secretase, an effect of promoting microglial phagocytosis, and an effect of activating neprilysin.

[0041]

In an embodiment where the composition for reducing a decline in cognitive function or improving cognitive function is ingested by or administered to a human (adult) as a subject, the total administration amount of the peptide or a salt thereof in terms of peptide per day is preferably 0.001 mg or more, more preferably 0.01 mg or more, still more preferably 0.1 mg or more, and is preferably 5000 mg or less, more preferably 3000 mg or less, still more preferably 2000 mg or less. In an embodiment where the composition for reducing a decline in cognitive function or improving cognitive function is ingested by or administered to a human (adult), the total administration amount of the peptide or a salt thereof in terms of peptide per day is preferably 0.001 to 5000 mg, more preferably 0.01 to 3000 mg, still more preferably 0.1 to 2000 mg. In the case where the composition contains two or more of the peptides or salts thereof, the total administration amount is the sum of the amounts of these peptides or salts thereof. In the case of a peptide, the amount in terms of peptide refers to the amount of the peptide itself; in the case of a peptide in a salt form, the amount in terms of peptide refers to a value obtained by multiplying the number of moles of the salt by the molecular weight of the peptide.

In an embodiment, for example, the administration amount of the peptide consisting of the amino acid sequence of SEQ ID NO: 1 or a salt thereof in terms of peptide per day may be 0.001 to 5000 mg, preferably 0.01 to 3000 mg.

Regarding the peptides each having any of the amino acid sequences of SEQ ID NOS: 2 to 19, VV, and AY or respective salts thereof, the administration amount in terms of peptide per day may also be 0.001 to 5000 mg, preferably 0.01 to 3000 mg.

Preferably, the above amount is ingested or administered in one or more portions per day, for example, one to several portions (e.g., two or three portions) per day. In an embodiment, preferably, the above amount of the peptide or a salt thereof is orally ingested or administered. In the case of a human (adult), preferably, the above amount of the peptide or a salt thereof is ingested or administered per 60 kg body weight per day. In an embodiment, the composition for reducing a decline in cognitive function or improving cognitive function may be an oral composition that allows the above amount of the peptide or a salt thereof to be ingested by or administered to a person per 60 kg body weight per day.

[0042]

Hereinafter, the composition containing the peptide or a salt thereof of the present invention and the composition for reducing a decline in cognitive function or improving cognitive function are collectively described as "the compositions of the present invention".

5 [0043]

In an embodiment, the compositions of the present invention may each contain a peptide consisting of the amino acid sequence of SEQ ID NO: 1 or a salt thereof. The compositions each preferably further contain a peptide consisting of the amino acid sequence of any of SEQ ID NOS: 2 to 19, VV, and AY or a salt thereof. The
10 compositions may each contain one peptide consisting of the amino acid sequence of any of SEQ ID NOS: 2 to 19, VV, and AY or a salt thereof, or may each contain two or more of such peptides or salts thereof. In an embodiment, the compositions of the present invention may each contain a peptide consisting of the amino acid sequence of SEQ ID NO: 1 or 2 or a salt thereof, and the compositions each preferably further
15 contain a peptide consisting of the amino acid sequence of any of SEQ ID NOS: 3 to 19, VV, and AY or a salt thereof. The compositions may each contain one peptide consisting of the amino acid sequence of any of SEQ ID NOS: 3 to 19, VV, and AY or a salt thereof, or may each contain two or more of such peptides or salts thereof.
[0044]

20 In an embodiment, the compositions of the present invention may each contain a peptide consisting of the amino acid sequence of SEQ ID NO: 3 or a salt thereof. The compositions each preferably further contain a peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1, 2, 4 to 19, VV, and AY or a salt thereof. The compositions may each contain one peptide consisting of the amino acid sequence of
25 any of SEQ ID NOS: 1, 2, 4 to 19, VV, and AY or a salt thereof, or may each contain two or more of such peptides or salts thereof. In an embodiment, the compositions of the present invention may each contain a peptide consisting of the amino acid sequence of SEQ ID NO: 3 or 4 or a salt thereof, and the compositions each preferably further contain a peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1, 2, 5
30 to 19, VV, and AY or a salt thereof. The compositions may each contain one peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1, 2, 5 to 19, VV, and AY or a salt thereof, or may each contain two or more of such peptides or salts thereof.
[0045]

In an embodiment, the compositions of the present invention may each contain
35 a peptide consisting of the amino acid sequence of SEQ ID NO: 5 or a salt thereof.

The compositions each preferably further contain a peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1 to 4, 6 to 19, VV, and AY or a salt thereof. The compositions may each contain one peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1 to 4, 6 to 19, VV, and AY or a salt thereof, or may each contain
5 two or more of such peptides or salts thereof. In an embodiment, the compositions of the present invention may each contain a peptide consisting of the amino acid sequence of SEQ ID NO: 5 or 6 or a salt thereof, and the compositions each preferably further contain a peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1 to 4, 7 to 19, VV, and AY or a salt thereof. The compositions may each contain one peptide
10 consisting of the amino acid sequence of any of SEQ ID NOS: 1 to 4, 7 to 19, VV, and AY or a salt thereof, or may each contain two or more of such peptides or salts thereof.
[0046]

In an embodiment, the compositions of the present invention each preferably further contain at least one selected from the group consisting of a peptide consisting of
15 the amino acid sequence of FLPM (SEQ ID NO: 21) or a salt thereof, a peptide consisting of the amino acid sequence of FQPSF (SEQ ID NO: 22) or a salt thereof, and Cyclo(Trp-Pro) or a salt thereof, and more preferably contain a peptide consisting of the amino acid sequence of FLPM (SEQ ID NO: 21) or a salt thereof, a peptide consisting of the amino acid sequence of FQPSF (SEQ ID NO: 22) or a salt thereof, and
20 Cyclo(Trp-Pro) or a salt thereof.
[0047]

In an embodiment, the compositions of the present invention may each contain a peptide consisting of the amino acid sequence of SEQ ID NO: 7 or a salt thereof. The compositions each preferably further contain a peptide consisting of the amino acid
25 sequence of any of SEQ ID NOS: 1 to 6, 8 to 19, VV, and AY or a salt thereof. The compositions may each contain one peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1 to 6, 8 to 19, VV, and AY or a salt thereof, or may each contain two or more of such peptides or salts thereof. In an embodiment, the compositions of the present invention each preferably contain a peptide consisting of the amino acid
30 sequence of LYEGY (SEQ ID NO: 7) or a salt thereof and a peptide consisting of the amino acid sequence of ILPVPA (SEQ ID NO: 14) or a salt thereof, and more preferably further contain a peptide consisting of the amino acid sequence of FLPM (SEQ ID NO: 21) or a salt thereof, a peptide consisting of the amino acid sequence of FQPSF (SEQ ID NO: 22) or a salt thereof, and Cyclo(Trp-Pro) or a salt thereof.
35 [0048]

In an embodiment, the compositions of the present invention each preferably contain a peptide consisting of the amino acid sequence of SEQ ID NO: 7 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 14 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 21 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 22 or a salt thereof, and Cyclo(Trp-Pro) or a salt thereof, and more preferably further contain a peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1 to 6, 8 to 13, 15 to 19, VV, and AY or a salt thereof. The compositions may each contain one peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1 to 6, 8 to 13, 15 to 19, VV, and AY or a salt thereof, or may each contain two or more of such peptides or salts thereof.

[0049]

In an embodiment, the compositions of the present invention each preferably contain a peptide consisting of the amino acid sequence of SEQ ID NO: 1 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 2 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 3 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 4 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 5 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 6 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 7 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 8 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 9 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 10 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 11 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 12 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 13 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 14 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 15 or a salt thereof, and a peptide consisting of the amino acid sequence of SEQ ID NO: 16 or a salt thereof, and more preferably further contain a peptide consisting of the amino acid sequence of SEQ ID NO: 17 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 18 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 19 or a salt thereof, a peptide consisting of the amino acid sequence of VV or a salt thereof, and a peptide consisting of the amino acid sequence of AY or a salt thereof. The compositions of the present invention each still more

preferably contain a peptide consisting of the amino acid sequence of SEQ ID NO: 21 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 22 or a salt thereof, and Cyclo(Trp-Pro) or a salt thereof.

[0050]

5 The compositions of the present invention are applicable for therapeutic use (medical use) and non-therapeutic use (non-medical use). The non-therapeutic is a concept that does not include medical activities, i.e., a concept that does not include surgery, therapy, or diagnosis of humans.

10 The compositions of the present invention can each be provided in the form of an agent, for example, but it is not limited thereto. The agent can be directly provided as a composition, or can be provided as a composition containing the agent. In an embodiment, the composition for reducing a decline in cognitive function or improving cognitive function can also be referred to as an agent for reducing a decline in cognitive function or improving cognitive function. The composition for inhibiting β -secretase
15 can also be referred to as an agent for inhibiting β -secretase. The composition for promoting microglial phagocytosis can also be referred to as an agent for promoting microglial phagocytosis. The composition for reducing at least one of production or accumulation of amyloid β can also be referred to as an agent for reducing at least one of production or accumulation of amyloid β . The composition for activating neprilysin
20 can also be referred to as an agent for activating neprilysin.

[0051]

 The compositions of the present invention may be for oral administration or parenteral administration. Preferably, the compositions of the present invention are oral compositions. The compositions of the present invention can each be provided in
25 the form of a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like, with a food or beverage or a pharmaceutical product being preferred, and with a food or beverage or an oral pharmaceutical product being more preferred. The compositions of the present invention can also be used in admixture with a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product,
30 feed, or the like. The compositions of the present invention may be in the form of solid (e.g., powder, granules, or tablet), liquid, or paste, for example.

[0052]

 The compositions of the present inventions each contain the peptide or a salt thereof and may further contain a variety of additives accepted as those for food and
35 beverages, pharmaceutical products, or the like, such as diluents, acidulants,

antioxidants, stabilizers, preservatives, flavorings, emulsifiers, colorants, seasonings, pH adjusters, and nutrient enhancers.

[0053]

For example, in the case where the compositions of the present inventions are each provided as a food or beverage, the peptide or a salt thereof used as an active ingredient may be blended with components usable in a food or beverage (e.g., food material and optional food additives), so that a variety of foods and beverages can be provided. Non-limiting examples of the foods and beverages include general foods and beverages, health foods, foods with function claims, foods for specified health uses, dietary supplements, and foods for the sick. The health foods, the foods with function claims, the foods for specified health uses, the dietary supplements, and the like can be used, for example, in various forms of preparations such as liquid, fine granules, tablet, granules, powder, capsule, chewable tablet, dry syrup, and liquid food.

[0054]

In the case where the compositions of the present invention are each provided as a pharmaceutical product or a quasi-pharmaceutical product, the peptide or a salt thereof may be blended with pharmacologically acceptable carriers, optional additives, and the like, so that a variety of dosage forms of pharmaceutical or quasi-pharmaceutical products can be provided. These carriers, additives, and the like are not limited as long as they are pharmacologically acceptable and can be used in pharmaceutical or quasi-pharmaceutical products. For example, one or two or more of excipients, binders, disintegrants, lubricants, antioxidants, colorants, and the like may be used. Examples of the administration (intake) form of pharmaceutical or quasi-pharmaceutical products include oral administration and parenteral administration (e.g., transdermal, transmucosal, enteral, and injection). In the case where the compositions of the present invention are each provided as a pharmaceutical product or a quasi-pharmaceutical product, the composition is preferably prepared in the form of a pharmaceutical product for oral administration or a quasi-pharmaceutical product for oral administration. Examples of dosage forms for oral administration include liquid, tablet, powder, fine granules, granules, sugar-coated tablet, capsule, suspension, emulsion, and chewable tablet. Examples of dosage forms for parenteral administration include injection, intravenous agent, ointment, lotion, adhesive skin patch, suppository, nasal agent, and transpulmonary agent (inhalant). The pharmaceutical product may be pharmaceuticals for non-human animals.

[0055]

In the case where the compositions of the present invention are provided as feed, the peptide or a salt thereof may be simply added to feed. The feed includes feed additives. Examples of the feed include livestock feed for animals such as cows, pigs, chickens, sheep, and horses; feed for small animals such as rabbits, rats, and mice; and
5 pet food for animals such as dogs, cats, and birds.

[0056]

For example, in the case where the compositions of the present invention are each provided as a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like, the compositions can be produced by any common method
10 using the peptide or a salt thereof.

[0057]

In an embodiment, the compositions of the present invention are each preferably a liquid composition, more preferably a beverage. The beverage may be a functional beverage, for example. The beverage may be in any form, and may be a
15 packed beverage. The package of the packed beverage is not limited and may be a package in any form and of any material. For example, any of the following commonly used packages can be used: metal packages such as aluminum cans and steel cans; resin packages such as plastic bottles; paper packages such as drink cartons; glass packages such as glass bottles; and wooden packages such as barrels. Filling a
20 beverage into a package and sealing the package can provide a packed beverage.

[0058]

In an embodiment, the compositions of the present invention may each contain a peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1 to 19, VV, and AY or a corresponding salt thereof in an amount of 0.0001 to 20% by weight,
25 preferably 0.001 to 10% by weight.

[0059]

In the case where the compositions of the present invention each contain a peptide consisting of the amino acid sequence of FLPM (SEQ ID NO: 21) or a salt thereof, a peptide consisting of the amino acid sequence of FQPSF (SEQ ID NO: 22) or
30 a salt thereof, and Cyclo(Trp-Pro) or a salt thereof, the total amount of these peptides or salts thereof may be 0.0001 to 20% by weight, preferably 0.001 to 10% by weight, in the composition. In an embodiment where the compositions of the present invention each contain a peptide consisting of the amino acid sequence of SEQ ID NO: 21 or a salt thereof, the amount thereof may be, for example, 0.0001 to 20% by weight,
35 preferably 0.001 to 10% by weight. In the case where the compositions of the present

invention each contain a peptide consisting of the amino acid sequence of SEQ ID NO: 22 or a salt thereof, the amount thereof may be, for example, 0.0001 to 20% by weight, preferably 0.001 to 10% by weight. In the case where the compositions of the present invention each contain Cyclo(Trp-Pro) or a salt, the amount thereof may be, for example, 0.0001 to 20% by weight, preferably 0.001 to 10% by weight. In an embodiment where the compositions of the present invention are each a liquid composition, such as a beverage, and each contain a peptide consisting of the amino acid sequence of SEQ ID NO: 21 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 22 or a salt thereof, and Cyclo(Trp-Pro) or a salt thereof, the total amount of these peptides or salts thereof is preferably 0.00005 to 20 mg/mL, more preferably 0.0001 to 10 mg/mL. In the case where the compositions of the present invention each contain a peptide consisting of the amino acid sequence of SEQ ID NO: 21 or a salt thereof, a peptide consisting of the amino acid sequence of SEQ ID NO: 22 or a salt thereof, and Cyclo(Trp-Pro) or a salt thereof, the total administration amount of these peptides or salts thereof in terms of peptides per day is preferably 0.0001 to 500 mg, more preferably 0.001 to 300 mg for a human (adult).

[0060]

Preferably, the compositions of the present invention are each ingested or administered continuously. Continuous ingestion or administration of the peptide or a salt thereof is expected to enhance the effect. In an embodiment, preferably, the compositions of the present invention are each continuously ingested or administered preferably for at least one week, more preferably for at least four weeks, still more preferably for at least eight weeks.

The peptide or a salt thereof can be ingested in the form of a food or beverage, for example, so that long-term ingestion thereof, for example, is less likely to cause issues in terms of safety.

[0061]

The compositions of the present invention may each be ingested by or administered to any subject (administration subject). The subject is preferably a human or non-human mammal, more preferably a human. In an embodiment, the administration subject in the present invention may be, for example, a subject in need of or desiring reduction in a decline in cognitive function or improvement of cognitive function. Also, the administration subject in the present invention may be, for example, a subject in need of or desiring prevention or amelioration of a condition or disease associated with a decline in cognitive function, a subject in need of or desiring

reduction in at least one of production or accumulation of amyloid β , and a subject in need of or desiring prevention or amelioration of a condition or disease associated with accumulation of amyloid β . In an embodiment, examples of the administration subject in the present invention include middle-aged and older people. The middle-aged and
5 older people include the elderly. The middle-aged and older people may be those who are 40 years old or older, for example. In an embodiment, the elderly are preferred subjects among the middle-aged and older people. The elderly may be those who are 60 years old or older or 65 years old or older. In an embodiment, the administration subject of the compositions of the present invention may be a healthy person. For
10 example, the compositions may be used on a healthy person for purposes such as reducing a decline in cognitive function or improving cognitive function, reducing at least one of production or accumulation of amyloid β in the brain, preventing accumulation of amyloid β in the brain, and preventing Alzheimer's disease, mild cognitive impairment, or aging-related cognitive dysfunction.

15 [0062]

The compositions of the present invention may each be provided with indications of functions exhibited owing to reduction in a decline in cognitive function or improvement of cognitive function or owing to reduction in at least one of production or accumulation of amyloid β . Such indications are also referred to as function claims.
20 The indications are not limited. Examples of the indications include "enhancing cognitive function", "reducing a decline in cognitive function", "maintaining good cognitive function", "improving memory", "reducing a decline in memory", "maintaining good memory", "improving accuracy of memory", "preventing memory disorder", "ameliorating memory disorder", "maintaining memory, which is a domain of
25 cognitive function", "function suitable for those who are concerned about a decline in memory", "improving accuracy of memory and/or correctness of judgement, which are domains of cognitive function", "improving memory retention or integration", "maintaining enhancement of cognitive function", "improving executive function", "promoting attention and concentration", "improving learning ability", "maintaining or
30 improving orientation", "delaying an aging-related decline in cognitive function", "enhancing short-term and long-term memory", "promoting verbal and visuospatial memory", "maintaining or improving logical reasoning", and labels or function claims considered to be equated thereto.

In an embodiment of the present invention, the compositions of the present
35 invention are each preferably a food or beverage provided with one or two or more of

the indications. The indications may each be an indication of using the composition for achieving the corresponding function. The indication may be directly provided to the compositions or may be attached to containers or packages of the compositions.

[0063]

5 The present invention also encompasses the following methods and uses.

A method for reducing a decline in cognitive function or improving cognitive function, the method including administering a peptide or a salt thereof, the peptide consisting of an amino acid sequence selected from the group consisting of GGRYY (SEQ ID NO: 1), AHDGGRYY (SEQ ID NO: 2), AEYY (SEQ ID NO: 3), AEYYRA (SEQ ID NO: 4), GVTHNVP (SEQ ID NO: 5), GVTHNVPIYEGY (SEQ ID NO: 6),
 10 LYEGY (SEQ ID NO: 7), ISGW (SEQ ID NO: 8), IKGY (SEQ ID NO: 9), pEAPPH (SEQ ID NO: 10), LTEAP (SEQ ID NO: 11), DLTVTN (SEQ ID NO: 12), pEIISAN (SEQ ID NO: 13), ILPVPA (SEQ ID NO: 14), TERGYS (SEQ ID NO: 15), IGME (SEQ ID NO: 16), VIQY (SEQ ID NO: 17), IWHHT (SEQ ID NO: 18), IVGRPR (SEQ ID NO: 19), VV, and AY.

A method for reducing at least one of production or accumulation of amyloid β , the method including administering the peptide or a salt thereof.

Use of a peptide or a salt thereof for reducing a decline in cognitive function or improving cognitive function, the peptide consisting of an amino acid sequence selected from the group consisting of GGRYY (SEQ ID NO: 1), AHDGGRYY (SEQ ID NO: 2),
 20 AEYY (SEQ ID NO: 3), AEYYRA (SEQ ID NO: 4), GVTHNVP (SEQ ID NO: 5), GVTHNVPIYEGY (SEQ ID NO: 6), LYEGY (SEQ ID NO: 7), ISGW (SEQ ID NO: 8), IKGY (SEQ ID NO: 9), pEAPPH (SEQ ID NO: 10), LTEAP (SEQ ID NO: 11), DLTVTN (SEQ ID NO: 12), pEIISAN (SEQ ID NO: 13), ILPVPA (SEQ ID NO: 14),
 25 TERGYS (SEQ ID NO: 15), IGME (SEQ ID NO: 16), VIQY (SEQ ID NO: 17), IWHHT (SEQ ID NO: 18), IVGRPR (SEQ ID NO: 19), VV, and AY.

Use of the peptide or a salt thereof for reducing at least one of production or accumulation of amyloid β .

In the methods and the uses, one of the peptides or salts thereof may be used,
 30 or two or more thereof may be used.

In an embodiment, in the methods and the uses, use of the peptide consisting of the amino acid sequence LYEGY (SEQ ID NO: 7) or a salt thereof and the peptide consisting of the amino acid sequence ILPVPA (SEQ ID NO: 14) or a salt thereof is preferred; additional use of the peptide consisting of the amino acid sequence FLPM (SEQ ID NO: 21) or a salt thereof, the peptide consisting of the amino acid sequence
 35

FQPSF (SEQ ID NO: 22) or a salt thereof, and Cyclo(Trp-Pro) or a salt thereof is more preferred.

[0064]

5 A method for inhibiting β -secretase, the method including administering a peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1 to 5, 7 to 15, 17 to 19, VV, and AY or a salt thereof.

Use of a peptide consisting of the amino acid sequence of any of SEQ ID NOS: 1 to 5, 7 to 15, 17 to 19, VV, and AY or a salt thereof for inhibiting β -secretase.

[0065]

10 A method for activating neprilysin, the method including administering a peptide consisting of the amino acid sequence GVTHNVPIYEGY (SEQ ID NO: 6) or a salt thereof.

Use of a peptide consisting of the amino acid sequence GVTHNVPIYEGY (SEQ ID NO: 6) or a salt thereof for activating neprilysin.

15 [0066]

A method for promoting microglial phagocytosis, the method including administering a peptide consisting of the amino acid sequence IGME (SEQ ID NO: 16) or a salt thereof.

20 Use of a peptide consisting of the amino acid sequence IGME (SEQ ID NO: 16) or a salt thereof for promoting microglial phagocytosis.

[0067]

The above methods may be therapeutic or non-therapeutic. The above uses may be therapeutic or non-therapeutic.

[0068]

25 In the above methods and uses, preferably, the peptide or a salt thereof is ingested by or administered to a subject at least once a day, for example, once to several times (for example, two or three times) per day. The above uses are each preferably for humans or non-human mammals, more preferably for humans. In an embodiment, the peptide or a salt thereof can be used to prevent or ameliorate a condition or disease associated with a decline in cognitive function. In an embodiment, the peptide or a salt thereof can be used to prevent or ameliorate a condition or disease associated with accumulation of amyloid β . The present invention also encompasses a method for preventing or ameliorating a condition or disease associated with accumulation of amyloid β , the method including administering the peptide or a salt thereof.

35 [0069]

In the above methods and uses, the peptide or a salt thereof is used in an amount (effective amount) that provides a desired effect. The effective amount is preferably an amount that can provide an effect of reducing a decline in cognitive function or improving cognitive function, an effect of inhibiting β -secretase, an effect of activating neprilysin, or an effect of promoting microglial phagocytosis. Preferred administration amounts, administration methods, administration subjects, and the like of the peptide or a salt thereof are as described above for the compositions of the present invention. The peptide or a salt thereof may be directly ingested or administered, or may be ingested or administered in the form of a composition containing the same.

For example, the composition of the present invention or the composition for reducing a decline in cognitive function or improving cognitive function may be ingested or administered.

[0070]

The peptide or a salt thereof can be used to produce a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like for use in reducing a decline in cognitive function or improving cognitive function. In an embodiment, the present invention also encompasses use of the peptide or a salt thereof in production of a composition for reducing a decline in cognitive function or improving cognitive function. The peptide or a salt thereof can be used to produce a composition for preventing or ameliorating a condition or disease associated with accumulation of amyloid β (preferably a condition or disease associated with accumulation of amyloid β in the brain).

[0071]

Herein, any numerical range expressed by a lower limit and an upper limit, i.e., "a lower limit to an upper limit" includes the lower limit and the upper limit. For example, a range expressed by "1 to 2" means 1 or more and 2 or less, with 1 and 2 being inclusive. Herein, the range may be any combination of any upper limit and any lower limit.

All the academic literature and patent literature described herein are incorporated herein by reference.

EXAMPLES

[0072]

The present invention is described in more detail below with reference to examples, but these examples are not intended to limit the scope of the present

invention.

[0073]

<Peptides>

Peptides P1 to P21 were used in Examples 1 and 2. Table 1 shows the amino acid sequences of the peptides P1 to P21. The peptides P1 to P21 are linear peptides. The peptides P1 to P21 were synthesized by GenScript Biotech. The amino acid sequences of the peptides were expressed with one-letter codes. pE represents pyroglutamic acid residue. All amino acids constituting the peptides are in the L form.

[0074]

10 [Table 1]

Peptide	Amino acid sequence	SEQ ID NO
P1	GGRYY	1
P2	AHDGGRYY	2
P3	AEYY	3
P4	AEYYRA	4
P5	GVTHNVP	5
P6	GVTHNVPIYEGY	6
P7	LYEGY	7
P8	ISGW	8
P9	IKGY	9
P10	pEAPPH	10
P11	LTEAP	11
P12	DLVTN	12
P13	pEIISAN	13
P14	ILPVPA	14
P15	TERGYS	15
P16	IGME	16
P17	VIQY	17
P18	IWHHT	18
P19	IVGRPR	19
P20	VV	-
P21	AY	-

[0075]

<Example 1>

(Materials and methods)

A. Reagents

BACE1 Inhibitor Screening Kit (Fluorometric) was obtained from Abcam. The other reagents were purchased from Sigma-Aldrich.

[0076]

5 B. BACE1 inhibitory assay

The peptides P1 to P5, P7 to P15, and P17 to P21 shown in Table 1 were used as candidate compounds. BACE1 Inhibitor Screening Kit (Fluorometric) was used to evaluate the BACE1 inhibitory activity of each candidate compound.

[0077]

10 BACE1 inhibition assay was performed according to the kit manufacturer's protocol. The candidate compounds were each individually dissolved in dimethyl sulfoxide (DMSO) at a high, 100-fold (100×) stock concentration and then diluted with assay buffer to twice (2×) the assay concentration. The resulting dilutions of the respective candidate compounds were used in the following experiment.

15 A BACE1 enzyme assay mixture was prepared by diluting stock enzyme (25×) with assay buffer. A 50-μL portion of the resulting enzyme dilution was mixed with 50 μL of each of the dilutions of the respective candidate compounds. The resulting mixtures were transferred into wells of a flat bottom 96-well black plate, whereby reaction mixtures were prepared. A Tecan Spark® multimode microplate reader was
20 used to monitor the fluorescence ($\lambda_{Ex}/\lambda_{Em} = 345/500$ nm) readout of the assay plate for 30 minutes at 37°C. The relative inhibition (%) of each sample was calculated from the fluorescence reading according to the manufacturer's protocol.

[0078]

(Statistical analysis)

25 Statistical analysis was performed using GraphPad Prism version 7.0 (San Diego, California, USA). The best-fit IC₅₀ value (hereinafter, referred to “IC₅₀”) of each peptide was determined by fitting the corresponding concentration and relative inhibition into log (peptide) vs. normalized inhibition equation.

[0079]

30 (Results)

Each of the peptides P1 to P5, P7 to P15, and P17 to P21 had BACE1 inhibitory action. Table 2 shows IC₅₀ (μg/mL) of each of the peptides P1 to P5, P7 to P15, and P17 to P21.

[0080]

35 [Table 2]

Peptide	Amino acid sequence	IC50 (ug/mL)
P1	GGRYY	141.6
P2	AHDGGRYY	401.7
P3	AEYY	672.0
P4	AEYYRA	257.7
P5	GVTHNVP	799.0
P7	LYEGY	89.2
P8	ISGW	93.4
P9	IKGY	94.3
P10	pEAPPH	124.9
P11	LTEAP	162.1
P12	DLTVTN	218.3
P13	pEIISAN	516.5
P14	ILPVPA	801.0
P15	TERGYS	702.5
P17	VIQY	39.3
P18	IWHHT	70.7
P19	IVGRPR	82.7
P20	VV	407.5
P21	AY	826.5

[0081]

Tables 3 to 5 show the percent relative inhibition of each peptide tested at various concentrations. Tables 3 to 5 show the average (%) of the BACE1 inhibition values (N = 2). The concentration and the relative inhibition value (%) of each peptide were fitted into a log (peptide) vs. normalized inhibition equation to determine the experimental IC50 values (table 2).

[0082]

[Table 3]

Concentration (mg/mL)	BACE1 inhibition (%)					
4.5	98.2	89.5	90.3	94		105
1.5	93.6	76.4	70.3	84.3	71.9	75.5
0.5	78.2	54.6	43.4	67.3	30.2	57.4
0.166665	53.6	30.5	17	38.8		46.7
0.15					17.2	
0.0555555	27.3	17.5	0.0618	15.3		28.1
0.05					1.55	
0.015	10.6	3.3	1.51	11.6	5.39	12.5
0.005					11.6	
Peptide	GGRYY	AHDGGRYY	AEYY	AEYYRA	GVTHNVP	LYEGY
	P1	P2	P3	P4	P5	P7

[0083]

[Table 4]

Concentration (mg/mL)	BACE1 inhibition (%)					
4.5				94.7	94.1	88.5
1.5	70.1	90.7	89.1	87.8	84.9	68.8
0.5	55.6	79.4	73.8	72.3	71.5	48.4
0.166665				55	44.7	28.1
0.15	39.4	63	54.1			
0.0555555				24.1	19.5	15.4
0.05	58.4	34.2	57			
0.015	33.7	18.1	29.7	9.54	0.975	7.3
0.005	33.3		22.6			
Peptide	ISGW	IKGY	pEAPPH	LTEAP	DLTVTN	pEISAN
	P8	P9	P10	P11	P12	P13

5 [0084]

[Table 5]

Concentration (mg/mL)	BACE1 inhibition (%)						
4.5	93.5					99.1	90.6
1.5	66.9	70.2	90.8	80.3	87.7	88.7	63.6
0.5	27.7	30.6	80.9	56.8	78	50.1	36
0.166665	26.6					27.8	14.7
0.15		30.2	51.4	49.4	54.1		
0.0555555	0.893					8.34	7.01
0.05		28.3	46.9	40.2	33		
0.015	14.2	1.61	38.1	25.6	25.4	5.33	4.3
0.005		8.52	39.9	38.5	33.1		
Peptide	ILPVPA	TERGYS	VIQY	IWHHT	IVGRPR	VV	AY
	P14	P15	P17	P18	P19	P20	P21

[0085]

BACE1 is an aspartyl protease that plays a major role in generation of amyloid-beta ($A\beta$) peptides from amyloid precursor protein. A strongly positive correlation is present between the $A\beta$ peptide concentration and cognitive function (Non-Patent Literature 1 mentioned above); this strongly positive correlation, coupled with the evidence of increased BACE1 activity in Alzheimer's disease models (Reference 1), suggests that BACE1 inhibition is a promising strategy for delaying a decline in cognitive function (Reference 2). The results of the BACE1 inhibition assay show that the peptides P1 to P5, P7 to P15, and P17 to P21 successfully inhibited BACE1 with IC50 ranging from 39.3 to 826.5 $\mu\text{g/mL}$ (Table 2).

Reference 1: Fukumoto et al., Arch. Neurol. 2002, 59(9), 1381-1389.

Reference 2: McDade et al., Nature Reviews Neurology 2021, 17(11), 703-714.

[0086]

<Example 2>

15 Materials and methods

A. Reagents

Gibco Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 Ham 1:1 (DMEM/F12), fetal bovine serum (FBS), horse serum (HS), penicillin/streptomycin (P/S), poly-D-lysine (PDL)-coated 24-well plate, Hanks' balanced salt solution (HBSS), Dulbecco's phosphate-buffered saline (DPBS), sodium pyruvate, D-glucose, BlockAid™ Blocking Solution, IBA1 polyclonal antibody (IBA1), and Hoechst 33342, trihydrochloride, trihydrate were obtained from Thermo Fischer Scientific.

Amyloid $\beta(1-42)$, HiLyte™ Fluor 488($A\beta_{1-42}$), and a 1% NH_4OH solution were purchased from Anaspec. Triton® X-100 and paraformaldehyde (PFA) were obtained from Sigma-Aldrich.

[0087]

B. Preparation of $A\beta_{1-42}$

Soluble species of $A\beta_{1-42}$ peptide ($A\beta_{1-42}$ monomer) was prepared according to the manufacturer's (Anaspec's) recommendations. Lyophilized $A\beta_{1-42}$ peptide was dissolved in a 1% NH_4OH solution to a concentration of 2 mg/mL. The peptide solution was further diluted with DPBS to be a stock solution with a final concentration of 1 mg/mL. Reconstituted peptide was aliquoted into several freezer vials and stored at -20°C .

[0088]

35 C. Cell culture

SIM-A9 murine microglial cells (ATCC CRL-3265) were cultured in DMEM/F12 supplemented with 10% FBS, 5% HS, and 1% P/S in a humidified gas chamber containing 5% CO₂ at 37°C.

[0089]

5 D. Phagocytosis assay

Peptide P16 (IGME, SEQ ID NO: 16) shown in Table 1 was used as a test compound.

SIM-A9 cells (2×10^5 cells/well) were seeded into a PDL-coated 24-well plate in a DMEM/F12 medium and pre-treated with 8.8 µg/mL peptide P16 for 24 hours. Following the pre-treatment, the cells were washed with DMEM/F12 and incubated with 4.51 µg/mL Aβ₁₋₄₂ for further six hours. At the end of the incubation period, the Aβ₁₋₄₂-containing medium was removed and the cells were washed with DPBS, whereby extracellular Aβ₁₋₄₂ was removed. Next, the cells were fixed with 4% PFA, permeabilized with 0.2% Triton[®] X-100, and blocked with BlockAid[™] Blocking Solution at room temperature for one hour. IBA1 antibody was used to visualize the cell shapes, and Hoechst 33342 was used to stain the nuclei. Five sets of images were acquired per well for analysis, and the cells containing intracellular Aβ₁₋₄₂ were determined by co-localization of Aβ₁₋₄₂/IBA1/nuclei signals. The phagocytic activity was evaluated based on the number of cells containing intracellular Aβ₁₋₄₂. The values were each represented as the average number of microglial cells normalized to Control group (cells untreated with peptide P16).

[0090]

E. Neprilysin (NEP) activity assay

Peptide P6 (GVTHNVPIYEGY, SEQ ID NO: 6) shown in Table 1 was used as a test compound.

qf-Aβ(12-16) AAC (a peptide having a sequence VHHQKAAC (SEQ ID NO: 20) with methylcoumarinamide (MCA) linked to the Cys side chain and 2,4-dinitrophenol (DNP) as a quencher linked to the N-terminus of the peptide) was synthesized. The synthesized qf-Aβ(12-16) AAC was dissolved in DMSO as a 2 mM stock solution. SIM-A9 cells (4×10^4 cells/well) were seeded into a PDL-coated 96-well plate in a DMEM/F12 medium as described above and pre-treated with 2.2 µg/mL peptide P6 for 24 hours. Next, the medium was replaced with 200 µL assay buffer (DPBS containing 5.5 mM D-glucose, 0.3 mM sodium pyruvate, 25 mM sodium bicarbonate, and 1.5 µM zinc sulfate) containing 4 µM qf-Aβ(12-16) AAC, and the cells were incubated for one hour. To determine the NEP activity, 150 µL of the assay

buffer was taken for fluorescence measurement with TECAN Spark® (TECAN, USA) at an excitation wavelength of 328 nm and a fluorescence wavelength of 393 nm.

[0091]

F. Statistical analysis

5 Statistical analysis was performed using GraphPad Prism version 7.0 (San Diego, California, USA). All results are expressed as mean $\pm$ standard deviation (SD). The statistical analysis was performed using Student's t-test. Data was defined as significant when the P-value satisfied $p < 0.05$.

[0092]

10 (Results)

A. Effect of peptide on A β ₁₋₄₂ phagocytosis by SIM-A9 cells

Microglia are brain-resident immune cells and perform various functions including surveying the brain microenvironment and removing cellular debris. Microglial cells are known to phagocytose various forms of A β ; enhancing this
15 mechanism of clearance may be a potential therapeutic strategy for Alzheimer's disease (AD).

FIG. 1 shows the evaluation results of the phagocytic activity of microglia (SIM-A9 cells) treated with peptide P16. The vertical axis represents the relative value of the number of microglial cells containing intracellular A β ₁₋₄₂. The results are
20 expressed as relative values to the Control. The values represent the mean $\pm$ SD ($n = 3$). The symbol * denotes significant difference with $p < 0.05$ against Control. The results show that the pre-treatment with peptide P16 significantly promotes the phagocytic activity of the SIM-A9 cells incubated with A β ₁₋₄₂ monomers (FIG. 1).

[0093]

25 B. Effect of peptide on NEP enzyme activity

A β is also degraded by endopeptidases such as NEP. Enhancing the activity of A β -degrading enzyme is another clearance mechanism under investigation for treatment of AD.

FIG. 2 is a graph showing the evaluation results of the enzyme activity of NEP
30 in microglia treated with peptide P6. The values represent the mean $\pm$ SD ($n = 3$). The results are expressed as relative values to the Control. The symbol ** denotes significant difference with $p < 0.01$ against Control. The results show that the treatment with peptide P6 significantly enhances the enzyme activity of NEP (FIG. 2).

[0094]

35 <Example 3>

(Materials and methods)

A. Reagents and peptides

Gibco Opti-MEM™ I Reduced Serum Medium (Opti-MEM), Fetal calf serum (FCS), Penicillin/Streptomycin (P/S), Hygromycin B and Blasticidin S HCl were
 5 obtained from Thermo Fischer Scientific. MSD® 96-well MULTISPOT® 6E10 Abeta Triplex Assay kit (amyloid β detection kit) was purchased from Meso Scale Discovery. N-[N-(3,5-difluorophenacetyl-L-alanyl)]-S-phenylglycine t-butyl ester (DAPT, γ -secretase inhibitor) was obtained from Calbiochem.

[0095]

10 The test peptides used in Example 3 are shown below. All test peptides were synthesized by GenScript Biotech.

LYEGY: peptide consisting of the amino acid sequence LYEGY (SEQ ID NO: 7)

15 14) ILPVPVA: peptide consisting of the amino acid sequence ILPVPVA (SEQ ID NO: 14)

FLPM: peptide consisting of the amino acid sequence FLPM (SEQ ID NO: 21)

FQPSF: peptide consisting of the amino acid sequence FQPSF (SEQ ID NO: 22)

Cyclo(Trp-Pro): cyclic dipeptide having a structure in which tryptophan and proline are bonded by condensation

20 The peptides LYEGY, ILPVPVA, FLPM, and FQPSF are linear peptides.

[0096]

B. Cell culture and Treatments

The human H4 glioma cell line was stably transfected with pAG3 vector containing human amyloid precursor protein isoform 695 (APP695) with the Swedish
 25 double mutation K595N/M596L, whereby H4-hAPP cells were obtained. The pAG3 vector is a modified pcDNA3 plasmid containing a hygromycin-B resistance cassette with the transgene under the control of a fused CMV and chicken β -actin promoter.

The H4-hAPP cells were cultured in Opti-MEM (cell culture medium) supplemented with 10% FCS, 1% P/S, 200 μ g/mL hygromycin B, and 2.5 μ g/mL
 30 blasticidin S HCl in a humidified incubator. The cells were seeded into a 96-well plate at a density of 25000 cells/well and incubated overnight at 37°C in a humidified gas chamber containing 5% CO₂.

The test peptides were reconstituted in the cell culture medium, whereby a peptide mixture containing the five type peptides was prepared. Table 6 shows the
 35 concentrations of the peptides in the peptide mixture.

[0097]

[Table 6]

Peptide	Concentration (mg/mL)
ILPVPA	0.00196
FLPM	0.001602
FQPSF	0.004196
LYEGY	0.014052
Cyclo(Trp-Pro)	0.001303

[0098]

5 The H4-hAPP cells were treated with the peptide mixture or a cell culture medium supplemented with DAPT as positive control (DAPT concentration: 400 nM). H4-hAPP cells not treated with the peptide mixture or DAPT were used as control. After treatment at 37°C for 24 h, cell culture supernatants were collected for amyloid beta (A β) analysis. Two independent experiments were performed.

10 [0099]

C. Amyloid β ELISA assay

 The cell culture supernatants were diluted 1:10 and analyzed for human amyloid β_{1-38} (A β 38) and human amyloid β_{1-42} (A β 42) with 6E10 Abeta Triplex Assay kit. Multiplex assay was performed according to the manufacturer's instructions and
15 the plates were read with Sector Imager 2400 (Meso Scale Discovery). The Amyloid β concentrations were calculated by reference to the standard curve. The sensitivity of the multiplex kit was <5 pg/mL.

[0100]

D. Statistical analysis

20 Statistical analysis was performed using GraphPad Prism version 5.0 (San Diego, California, USA). All results are expressed as mean $\pm$ standard deviation (SD). The statistical analysis was performed by analysis of variance (ANOVA), followed by posthoc Dunnett's multiple comparison test. Data was defined as significant when the P value satisfied $P < 0.05$.

25 [0101]

(Results)

 FIG. 3A and FIG. 3B show the results (**: $P < 0.01$, ***: $P < 0.001$ vs. Control). FIG. 3A is a graph showing the concentration of amyloid β_{1-38} (A β 38) in the culture supernatant of the H4-hAPP cells treated with the peptide mixture. FIG. 3B is

a graph showing the concentration of amyloid β_{1-42} (A β 42) in the culture supernatant of the H4-hAPP cells treated with the peptide mixture. The results are expressed as mean $\pm$ SD (N = 2). The results show that the peptide mixture reduced production of amyloid β_{1-38} and amyloid β_{1-42} by the H4-hAPP cells.

- 5 [Sequence Listing]
SP2024-0024.xml

CLAIMS

[Claim 1] A composition comprising:

a peptide consisting of an amino acid sequence selected from the group

5 consisting of

LYEGY (SEQ ID NO: 7),

GGRYY (SEQ ID NO: 1),

AHDGGRYY (SEQ ID NO: 2),

AEYY (SEQ ID NO: 3),

10 AEYYRA (SEQ ID NO: 4),

GVTHNVP (SEQ ID NO: 5),

GVTHNVPIYEGY (SEQ ID NO: 6),

ISGW (SEQ ID NO: 8),

IKGY (SEQ ID NO: 9),

15 pEAPPH (SEQ ID NO: 10),

LTEAP (SEQ ID NO: 11),

DLTVTN (SEQ ID NO: 12),

pEIISAN (SEQ ID NO: 13),

ILPVPA (SEQ ID NO: 14), TERGYS (SEQ ID NO: 15), and

20 IGME (SEQ ID NO: 16); or

a salt thereof.

[Claim 2] The composition according to claim 1,

wherein the composition is used to reduce a decline in cognitive function or

25 improve cognitive function.

[Claim 3] The composition according to claim 1 or 2, comprising:

the peptide consisting of the amino acid sequence LYEGY (SEQ ID NO: 7) or

a salt thereof; and

30 the peptide consisting of the amino acid sequence ILPVPA (SEQ ID NO: 14) or

a salt thereof,

the composition further comprising:

a peptide consisting of an amino acid sequence FLPM (SEQ ID NO: 21) or a

salt thereof;

35 a peptide consisting of an amino acid sequence FQPSF (SEQ ID NO: 22) or a

salt thereof; and

Cyclo(Trp-Pro) or a salt thereof.

[Claim 4] The composition according to claim 1 or 2, further comprising a
 5 peptide consisting of an amino acid sequence selected from the group consisting of
 VIQY (SEQ ID NO: 17), IWHHT (SEQ ID NO: 18), IVGRPR (SEQ ID NO: 19), VV,
 and AY, or a salt thereof.

[Claim 5] A composition for reducing a decline in cognitive function or
 10 improving cognitive function, the composition comprising:
 a peptide consisting of an amino acid sequence selected from the group
 consisting of LYEGY (SEQ ID NO: 7), GGRYY (SEQ ID NO: 1), AHDGGRYY (SEQ
 ID NO: 2), AEYY (SEQ ID NO: 3), AEYYRA (SEQ ID NO: 4), GVTHNVP (SEQ ID
 NO: 5), GVTHNVPIYEGY (SEQ ID NO: 6), ISGW (SEQ ID NO: 8), IKGY (SEQ ID
 15 NO: 9), pEAPPH (SEQ ID NO: 10), LTEAP (SEQ ID NO: 11), DLTVTN (SEQ ID NO:
 12), pEIISAN (SEQ ID NO: 13), ILPVPA (SEQ ID NO: 14), TERGYS (SEQ ID NO:
 15), IGME (SEQ ID NO: 16), VIQY (SEQ ID NO: 17), IWHHT (SEQ ID NO: 18),
 IVGRPR (SEQ ID NO: 19), VV, and AY; or
 a salt thereof.

20

[Claim 6] The composition according to claim 5,
 wherein the composition reduces at least one of production or accumulation of
 amyloid β .

[Claim 7] The composition according to claim 5 or 6,
 wherein the composition reduces at least one of production or accumulation of
 amyloid β by inhibiting β -secretase.

[Claim 8] The composition according to claim 5 or 6, comprising:
 30 the peptide consisting of the amino acid sequence LYEGY (SEQ ID NO: 7) or
 a salt thereof; and
 the peptide consisting of the amino acid sequence ILPVPA (SEQ ID NO: 14) or
 a salt thereof,
 the composition further comprising:
 35 a peptide consisting of an amino acid sequence FLPM (SEQ ID NO: 21) or a

salt thereof;

a peptide consisting of an amino acid sequence FQPSF (SEQ ID NO: 22) or a salt thereof; and

Cyclo(Trp-Pro) or a salt thereof.

5

[Claim 9] The composition according to claim 5 or 6,

wherein the composition comprises the peptide consisting of the amino acid sequence GVTHNVPIYEGY (SEQ ID NO: 6) or a salt thereof and reduces accumulation of amyloid β by activating neprilysin.

10

[Claim 10] The composition according to claim 5 or 6,

wherein the composition comprises the peptide consisting of the amino acid sequence IGME (SEQ ID NO: 16) or a salt thereof and reduces accumulation of amyloid β by promoting microglial phagocytosis.

15

[Claim 11] The composition according to claim 1 or 5,

wherein the composition is an oral composition.

[Claim 12] The composition according to claim 1 or 5,

20

wherein the composition is a food or beverage or an oral pharmaceutical product.

[Claim 13] The composition according to claim 1 or 5,

25

wherein the composition is labeled with at least one function claim selected from the group consisting of "enhancing cognitive function", "reducing a decline in cognitive function", "maintaining good cognitive function", "improving memory", "reducing a decline in memory", "maintaining good memory", "improving accuracy of memory", "preventing memory disorder", "ameliorating memory disorder", "maintaining memory, which is a domain of cognitive function", "function suitable for those who are concerned about a decline in memory", "improving accuracy of memory and/or correctness of judgement, which are domains of cognitive function", "improving memory retention or integration", "maintaining enhancement of cognitive function", "improving executive function", "promoting attention and concentration", "improving learning ability", "maintaining or improving orientation", "delaying an aging-related decline in cognitive function", "enhancing short-term and long-term memory",

35

"promoting verbal and visuospatial memory", and "maintaining or improving logical reasoning".

[Claim 14] A peptide consisting of an amino acid sequence selected from the
5 group consisting of LYEGY (SEQ ID NO: 7), GGRYY (SEQ ID NO: 1), AHDGGRYY
(SEQ ID NO: 2), AEYY (SEQ ID NO: 3), AEYYRA (SEQ ID NO: 4), GVTHNVP
(SEQ ID NO: 5), GVTHNVPIYEGY (SEQ ID NO: 6), ISGW (SEQ ID NO: 8), IKGY
(SEQ ID NO: 9), pEAPPH (SEQ ID NO: 10), LTEAP (SEQ ID NO: 11), DLTVTN
(SEQ ID NO: 12), pEIISAN (SEQ ID NO: 13), ILPVPA (SEQ ID NO: 14), TERGYS
10 (SEQ ID NO: 15), and IGME (SEQ ID NO: 16), or a salt thereof.