

2-1-2008

# Abeta42 mutants with different aggregation profiles induce distinct pathologies in *Drosophila*.

Koichi Iijima

*Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; Laboratory of Neurodegenerative Diseases and Gene Discovery, Farber Institute for Neurosciences, Thomas Jefferson University, Philadelphia, PA; Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA, Koichi.Iijima@jefferson.edu*

Hsueh-Cheng Chiang

*Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; Department of Neurobiology and Behavior, State University of New York, Stony Brook, NY*

Stephen A Hearn

*Cold Spring Harbor Laboratory, Cold Spring Harbor, NY*

Inessa Hakker

*Cold Spring Harbor Laboratory, Cold Spring Harbor, NY*

Anthony Gatt

*Laboratory of Neurodegenerative Diseases and Gene Discovery, Farber Institute for Neurosciences, Thomas Jefferson University, Philadelphia, PA*

**Let us know how access to this document benefits you**

Follow us and additional works at: <http://jdc.jefferson.edu/bmpfp>

 Part of the [Medical Biochemistry Commons](#), [Medical Genetics Commons](#), and the [Medical Neurobiology Commons](#)

---

## Recommended Citation

Iijima, Koichi; Chiang, Hsueh-Cheng; Hearn, Stephen A; Hakker, Inessa; Gatt, Anthony; Shenton, Christopher; Granger, Linda; Leung, Amy; Iijima-Ando, Kanae; and Zhong, Yi, "Abeta42 mutants with different aggregation profiles induce distinct pathologies in *Drosophila*." (2008). *Department of Biochemistry and Molecular Biology Faculty Papers*. Paper 33.

<http://jdc.jefferson.edu/bmpfp/33>

---

**Authors**

Koichi Iijima, Hsueh-Cheng Chiang, Stephen A Hearn, Inessa Hakker, Anthony Gatt, Christopher Shenton, Linda Granger, Amy Leung, Kanae Iijima-Ando, and Yi Zhong

# A $\beta$ 42 Mutants with Different Aggregation Profiles Induce Distinct Pathologies in *Drosophila*

Koichi Iijima<sup>1,2,4\*</sup>, Hsueh-Cheng Chiang<sup>1,5</sup>, Stephen A. Hearn<sup>1</sup>, Inessa Hakker<sup>1</sup>, Anthony Gatt<sup>2</sup>, Christopher Shenton<sup>3</sup>, Linda Granger<sup>2,3</sup>, Amy Leung<sup>1</sup>, Kanae Iijima-Ando<sup>1,3,4</sup>, Yi Zhong<sup>1\*</sup>

**1** Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, United States of America, **2** Laboratory of Neurodegenerative Diseases and Gene Discovery, Farber Institute for Neurosciences, Thomas Jefferson University, Philadelphia, Pennsylvania, United States of America, **3** Laboratory of Neurogenetics and Protein Misfolding Diseases, Farber Institute for Neurosciences, Thomas Jefferson University, Philadelphia, Pennsylvania, United States of America, **4** Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, United States of America, **5** Department of Neurobiology and Behavior, State University of New York, Stony Brook, New York, United States of America

## Abstract

Aggregation of the amyloid- $\beta$ -42 (A $\beta$ 42) peptide in the brain parenchyma is a pathological hallmark of Alzheimer's disease (AD), and the prevention of A $\beta$  aggregation has been proposed as a therapeutic intervention in AD. However, recent reports indicate that A $\beta$  can form several different prefibrillar and fibrillar aggregates and that each aggregate may confer different pathogenic effects, suggesting that manipulation of A $\beta$ 42 aggregation may not only quantitatively but also qualitatively modify brain pathology. Here, we compare the pathogenicity of human A $\beta$ 42 mutants with differing tendencies to aggregate. We examined the aggregation-prone, EOFAD-related Arctic mutation (A $\beta$ 42Arc) and an artificial mutation (A $\beta$ 42art) that is known to suppress aggregation and toxicity of A $\beta$ 42 *in vitro*. In the *Drosophila* brain, A $\beta$ 42Arc formed more oligomers and deposits than did wild type A $\beta$ 42, while A $\beta$ 42art formed fewer oligomers and deposits. The severity of locomotor dysfunction and premature death positively correlated with the aggregation tendencies of A $\beta$  peptides. Surprisingly, however, A $\beta$ 42art caused earlier onset of memory defects than A $\beta$ 42. More remarkably, each A $\beta$  induced qualitatively different pathologies. A $\beta$ 42Arc caused greater neuron loss than did A $\beta$ 42, while A $\beta$ 42art flies showed the strongest neurite degeneration. This pattern of degeneration coincides with the distribution of Thioflavin S-stained A $\beta$  aggregates: A $\beta$ 42Arc formed large deposits in the cell body, A $\beta$ 42art accumulated preferentially in the neurites, while A $\beta$ 42 accumulated in both locations. Our results demonstrate that manipulation of the aggregation propensity of A $\beta$ 42 does not simply change the level of toxicity, but can also result in qualitative shifts in the pathology induced *in vivo*.

**Citation:** Iijima K, Chiang H-C, Hearn SA, Hakker I, Gatt A, et al (2008) A $\beta$ 42 Mutants with Different Aggregation Profiles Induce Distinct Pathologies in *Drosophila*. PLoS ONE 3(2): e1703. doi:10.1371/journal.pone.0001703

**Editor:** Hilal Lashuel, Swiss Federal Institute of Technology Lausanne, Switzerland

**Received:** October 3, 2007; **Accepted:** January 29, 2008; **Published:** February 27, 2008

**Copyright:** © 2008 Iijima et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** This work was supported by grants from the Alzheimer's Association (to K.I.), Pilot Research Award from Thomas Jefferson University (to K.I.), the Hereditary Disease Foundation (to K.I.-A.), the start-up funds from Farber Institute for Neurosciences (to K.I. and K. I.-A.), and the National Institutes of Health (to Y.Z.).

**Competing Interests:** The authors have declared that no competing interests exist.

\* E-mail: Koichi.Iijima@jefferson.edu (KI); zhongyi@cshl.edu (YZ)

## Introduction

The amyloid- $\beta$ -42 (A $\beta$ 42) peptide has been suggested to play a central role in the pathogenesis of Alzheimer's disease (AD), a devastating, and currently incurable, neurodegenerative disorder [1]. Aggregation of A $\beta$ 42 peptide in the brain parenchyma is a pathological hallmark of AD [2]. Genetic studies of early-onset familial AD (EOFAD) provide a strong causative link between A $\beta$ 42 and AD [3], and some mutations in the A $\beta$  peptide promote amyloid fibril formation [4,5]. These data suggest that A $\beta$ 42 aggregation might be involved in AD pathogenesis [6], and A $\beta$ 42 aggregation is therefore an attractive target for therapeutic intervention in AD [7].

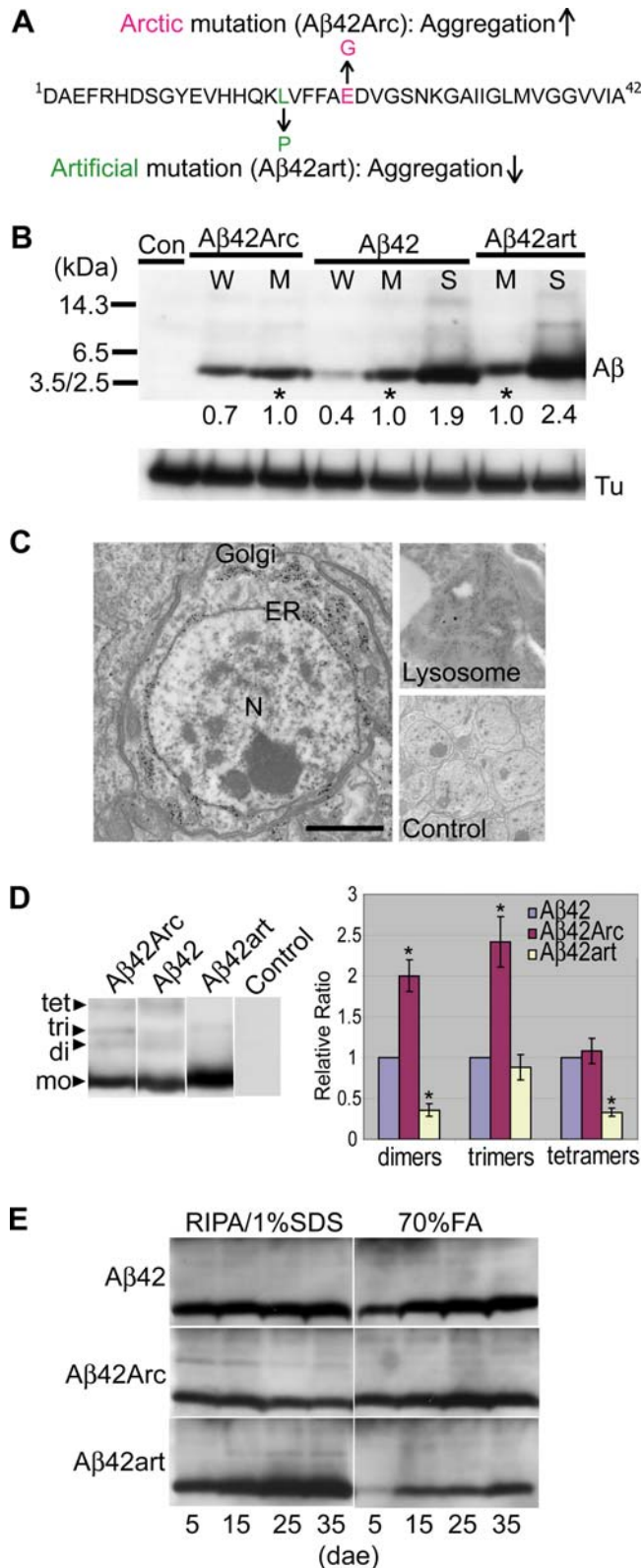
*In vitro*, the neurotoxicity of A $\beta$ 42 has been often correlated with the tendency of A $\beta$ 42 to aggregate [8,9]. However, recent evidence indicates that A $\beta$ 42 can form a variety of misfolded structures, including multiple monomer conformers, different types of prefibrillar assemblies, and structurally distinct amyloid fibrils, and that such structural polymorphisms may mediate the diverse toxic effects of A $\beta$ 42 [10–13]. These results suggest that

manipulation of A $\beta$ 42 aggregation *in vivo* may not simply change the magnitude of toxicity, but also qualitatively modify its pathogenic effects.

We have previously shown that expression of the human A $\beta$ 42 peptide in *Drosophila* brains induces age-dependent memory defects, locomotor dysfunction, and neurodegeneration accompanied by A $\beta$ 42 deposits [14]. Using this model system, we investigated the correlation between the aggregation tendencies of A $\beta$ 42 and memory defects, as well as neurodegeneration, through genetic manipulation of A $\beta$ 42 aggregation. We demonstrated that manipulation of the aggregation propensity of A $\beta$ 42 qualitatively as well as quantitatively modified the pathogenicity of A $\beta$ 42 *in vivo*.

## Results and Discussion

The human A $\beta$ 42 with the Arctic mutation (E22G substitution, A $\beta$ 42Arc) (Figure 1A), which causes early onset familial AD (EOFAD) [4], is more aggregation-prone and toxic *in vitro* [5,15] and accelerates the formation of amyloid deposits in the brains of AD model mice [16,17]. In contrast, an artificial mutation, (L17P



**Figure 1. Expression, distribution, aggregation, and accumulation profiles of mutant A $\beta$ 42 peptides in fly brains.** **A**, Sequences of A $\beta$ 42, A $\beta$ 42Arc, and A $\beta$ 42art. **B**, Expression levels of A $\beta$  in independent transgenic lines (W; weak, M; moderate, S: strong expression) at 1-2dae (top panel, A $\beta$ 42), when accumulation of each A $\beta$  in the insoluble fraction was minimum, were compared, and relative ratios were shown below each lane and in Table S1 (n=3). Asterisks

←

indicate the fly lines primarily used in this study. *elav-Gal4<sup>155</sup>* flies were used as control. Tubulin was used as a loading control (bottom panel: Tu). **C**, ImmunoEM detection of A $\beta$ 42 in the endoplasmic reticulum (ER) and Golgi, as well as a lysosome. Gold particles are absent in the control (Control). N: nucleus, Scale bar: 1  $\mu$ m. Neurons in Kenyon cell region of A $\beta$ 42 fly brains at 25dae were analyzed. **D**, Detection of dimers (di), trimers (tri) and tetramers (tet) in fly brains. The level of each oligomer was shown as a ratio relative to that of A $\beta$ 42. Asterisks indicate significant differences from A $\beta$ 42 (n=3, P<0.05, Student's t-test). **E**, Age-dependent accumulation of A $\beta$  peptides in detergent-soluble and insoluble fractions. The ages of the flies are indicated at the bottom. doi:10.1371/journal.pone.0001703.g001

substitution, A $\beta$ 42art) (Figure 1A), suppresses amyloid fibril formation and toxicity *in vitro* [9,18] and prevents the formation of amyloid deposits in *C. elegans* muscle [19].

A signal sequence was fused to the N-terminus of each A $\beta$  [14], to target the peptide to the secretory pathway. Multiple transgenic lines carrying a *UAS-A $\beta$ 42*, *UAS-A $\beta$ 42Arc*, or *UAS-A $\beta$ 42art* transgene were established. Expression of each A $\beta$  in the brain was driven by the pan-neuronal *elav-Gal4<sup>155</sup>* driver [20]. Since *elav-Gal4* is on the X chromosome, male progeny expressed more A $\beta$  peptide and developed stronger phenotypes than female progeny due to dosage compensation (data not shown). The results presented in this study are from male flies, unless otherwise indicated.

Western blot analysis detected monomeric forms of A $\beta$ 42, A $\beta$ 42Arc, and A $\beta$ 42art as 4 kDa signals (Figure 1B). Monomeric and oligomeric forms of A $\beta$ 42art migrated slower than those of A $\beta$ 42 due to an amino acid substitution (Figure 1B and D). Immunoprecipitation followed by mass spectrometry analysis confirmed that the fused signal peptide was correctly cleaved, and intact A $\beta$ 42, A $\beta$ 42Arc, and A $\beta$ 42art peptides were produced (Figure S1A–C). Immuno-electron microscopy (Immuno-EM) detected A $\beta$ 42 signals in the secretory pathway, including ER, Golgi, and lysosomes (Figure 1C), with minimal signals in the mitochondria and cytoplasm of neurons in the Kenyon cell region of A $\beta$ 42 fly brains. A $\beta$ 42Arc and A $\beta$ 42art peptides were also detected in the secretory pathway (data not shown). Secretion of A $\beta$  peptides occurred in *Drosophila* cultured cells (Figure S2), and, in *Drosophila* brains, immuno-EM analysis occasionally detected A $\beta$ 42 accumulation in glial cells suggesting that A $\beta$ 42 peptides were secreted from neurons and then taken up by glial cells (Figure S3).

All A $\beta$  peptides caused late-onset locomotor defects and premature death when expressed in neurons. Since the severity of these phenotypes positively correlated with the expression level of the peptides (Figure S4 and Table S1), we selected transgenic lines with similar expression levels (Figure 1B, asterisks) to characterize the accumulation profiles and the pathogenic effects of each A $\beta$  peptide in the *Drosophila* brain.

To compare the ability of each A $\beta$  peptide to form small oligomers, we quantified the levels of dimers (8 kDa), trimers (12 kDa), and tetramers (16 kDa), as detected by Western blotting (Figure 1D). This analysis revealed that A $\beta$ 42Arc formed 2-fold more dimers and trimers than did A $\beta$ 42, while A $\beta$ 42art formed 50% fewer dimers and tetramers. We also tested whether higher molecular weight A $\beta$  oligomers were formed in fly brains with dot blot analysis using A11 antibody [21]. We did not observe A11-positive oligomers in any of A $\beta$ 42Arc, A $\beta$ 42 and A $\beta$ 42art brain lysates (Figure S5). During aging, A $\beta$ 42 and A $\beta$ 42Arc accumulated in the insoluble fraction of brain lysates (Figure 1E; extracted by 70% formic acid), with no significant accumulation in the soluble fraction (Figure 1E; extracted by RIPA/1%SDS). Accumulation of A $\beta$ 42Arc in the insoluble fraction was more aggressive than that of

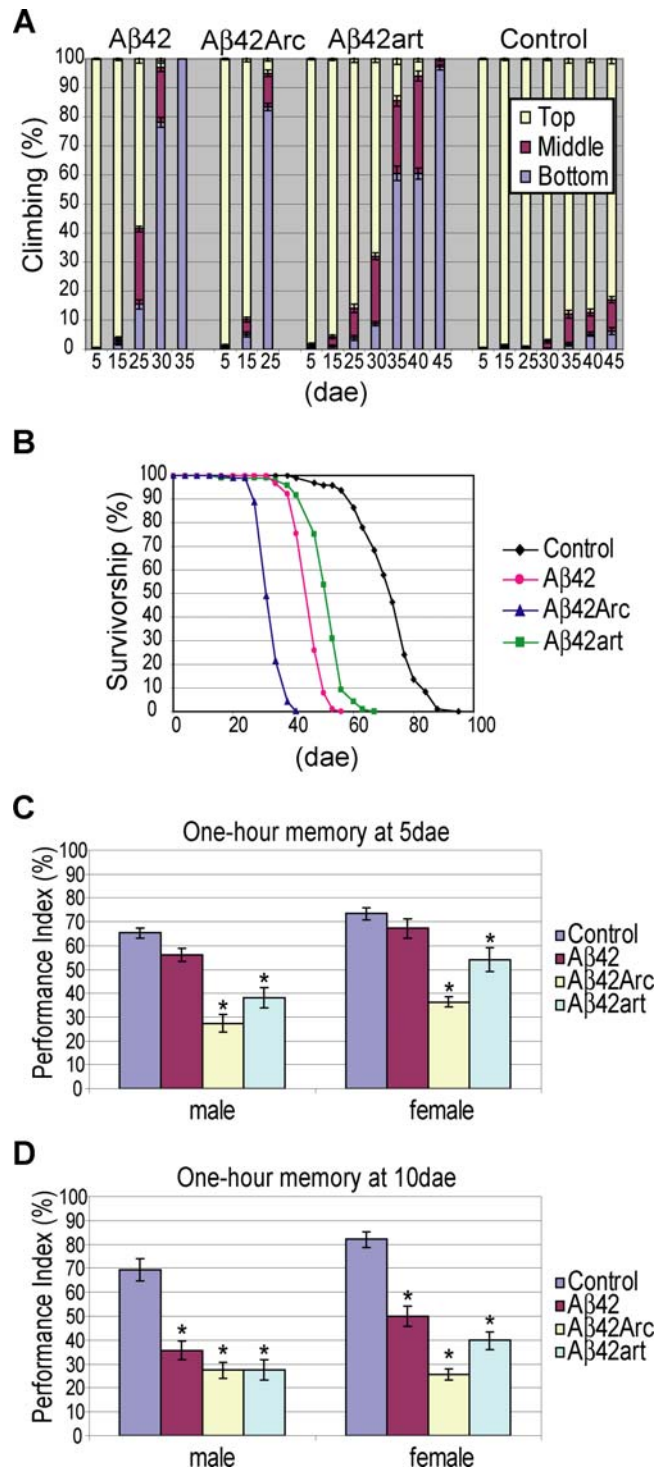


A $\beta$ 42 (Figure 1E, compare A $\beta$ 42 and A $\beta$ 42Arc in 5 days-after-eclosion (dae) flies). In contrast, A $\beta$ 42art strongly accumulated in the soluble fraction with greatly reduced accumulation in the insoluble fraction (Figure 1E). It should be noted that although age-dependent accumulation of A $\beta$ 42Arc in FA fraction from 5 to 25dae was clearly observed, the level of A $\beta$ 42Arc at 35dae was less than that at 25dae, presumably due to a progressive cell loss in A $\beta$ 42Arc fly brains (See below). These results demonstrate that A $\beta$ 42Arc is consistently more, and A $\beta$ 42art is significantly less, prone to aggregate *in vivo*.

The severity of locomotor dysfunction and premature death phenotypes of the transgenic flies correlated well with the aggregation proneness of the A $\beta$  peptides. Climbing ability was used to quantify locomotor activity [22]. Climbing disability in 80% of the flies occurred by 25, 35, and 45 dae in A $\beta$ 42Arc, A $\beta$ 42, and A $\beta$ 42art flies, respectively (Figure 2A). A similar tendency was observed for the premature death phenotype. The average lifespan of A $\beta$ 42Arc flies (32.9 dae) was shorter than that of A $\beta$ 42 flies (46.2 dae), while A $\beta$ 42art flies (51.4 dae) lived longer than A $\beta$ 42 flies (Figure 2B).

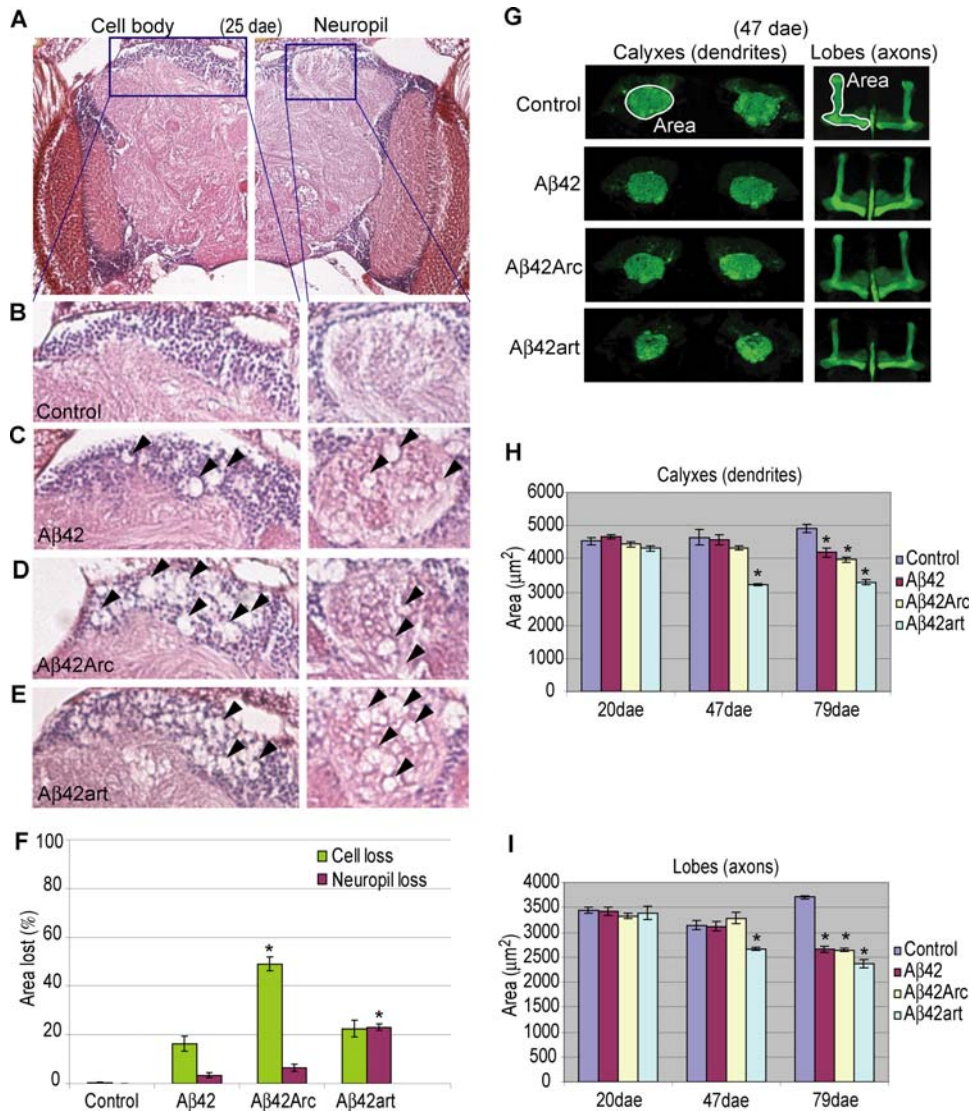
However, the onset of memory defects measured by Pavlovian olfactory classical conditioning [23] did not follow this simple trend. This assay was conducted with younger flies (5 or 10 dae), before the flies developed locomotor defects (Figure 2A). Data obtained from male and female flies are presented separately, since expression of A $\beta$  is higher in males than in females, as a result of dosage compensation. For 5 dae flies in both male and female groups, A $\beta$ 42Arc flies showed the most severe 1 hour memory defects (memory scores were measured 1 hour after the training session), and A $\beta$ 42art flies were also defective (Figure 2C). In contrast, memory in A $\beta$ 42 flies was indistinguishable from control flies (Figure 2C). For 10 dae flies, all A $\beta$  flies reached a similar level of memory defects in the male group (left panel in Figure 2D). In the female group, memory scores remained the lowest in A $\beta$ 42Arc flies, and both A $\beta$ 42art and A $\beta$ 42 flies showed similar defects (Figure 2D). A similar tendency was observed with the learning assay (learning scores were obtained immediately after training) (Figure S6). Of note, learning scores were normal in both 10 dae A $\beta$ 42 and A $\beta$ 42art female flies, indicating that these flies were specifically defective in short-term memory, the major clinical manifestation observed in patients in the early stages of AD [24]. The sensory motor activity of the flies, including sensing odors and electric shock, was indistinguishable from controls at 10 dae (Table S2), indicating that the observed defects can be interpreted as learning and memory defects.

Remarkably, A $\beta$ 42, A $\beta$ 42Arc, and A $\beta$ 42art each induced distinct pathologies. Neurodegeneration in the A $\beta$  fly brains was observed as a vacuolar appearance both in the cell body and neuropil regions. To quantify the area lost in these regions, we focused on the mushroom body structure, in which the cell bodies (Kenyon cell body), dendrites (Calyxes), and axon bundles (Lobes) were easily identified [25]. Our analysis revealed that, at 25 dae, A $\beta$ 42Arc fly brains showed more extensive cell loss than that in A $\beta$ 42 or A $\beta$ 42art brains (Figures 3A–F). However, the level of neuropil degeneration was greatest in A $\beta$ 42art flies (Figures 3A–F). The enhanced neuropil degeneration observed in A $\beta$ 42art flies was further confirmed by confocal analysis. In this assay, each A $\beta$ 42 peptide was preferentially expressed in mushroom body neurons using the *OK107-gal4* driver, and the structure of dendrites (Calyxes) and axons (Lobes) was visualized by co-expressed CD8-GFP[26] (Figure 3G). Quantification of the size of these structures revealed that A $\beta$ 42art induced earlier onset and more severe atrophy in both the dendrites (Calyxes) and axons (Lobes) among all A $\beta$  flies (Figure 3G–I). The severity of the



**Figure 2. Behavioral defects in A $\beta$ 42, A $\beta$ 42Arc, and A $\beta$ 42art flies.** **A**, Locomotor dysfunction. The percent of flies at the top (yellow), middle (magenta) or bottom (blue) of the vial at 10 seconds after knocking flies to the bottom are shown (average  $\pm$  SEM (n=10)). **B**, Premature death. The percent survivorship was plotted against the age (dae). **C** and **D**, Memory defects. One hour memory was assessed by Pavlovian olfactory conditioning at 5 dae (C) and 10 dae (D). Asterisks indicate a significant difference from control (n=6 or 8,  $\alpha < 0.05$ , Tukey-Kramer significant difference). Average memory scores  $\pm$  SEM are shown.

doi:10.1371/journal.pone.0001703.g002



**Figure 3. Cell body and neuropil degeneration in A $\beta$ 42, A $\beta$ 42Arc, and A $\beta$ 42art flies.** **A–E**, Neurodegeneration in A $\beta$  flies at 25 dae. The cell body and neuropil region in the mushroom body are enlarged. Arrowheads indicate neurodegeneration (C to E). **F**, Percentage of the area lost in the cell body (green) and neuropil (magenta) regions are shown as averages  $\pm$  SEM ( $n = 7–9$  hemispheres). Asterisks indicate significant differences from A $\beta$ 42 ( $P < 0.05$ , Student's *t*-test). **G**, Atrophy of Calyxes (dendrites) and Lobes (axons) in A $\beta$  flies. **H** and **I**, Areas of Calyxes and Lobes were measured as indicated in (G) and presented as averages  $\pm$  SEM ( $n = 6$  hemispheres). Asterisks indicate significant differences from control ( $P < 0.05$ , Student's *t*-test). The ages of the flies are indicated at the bottom. doi:10.1371/journal.pone.0001703.g003

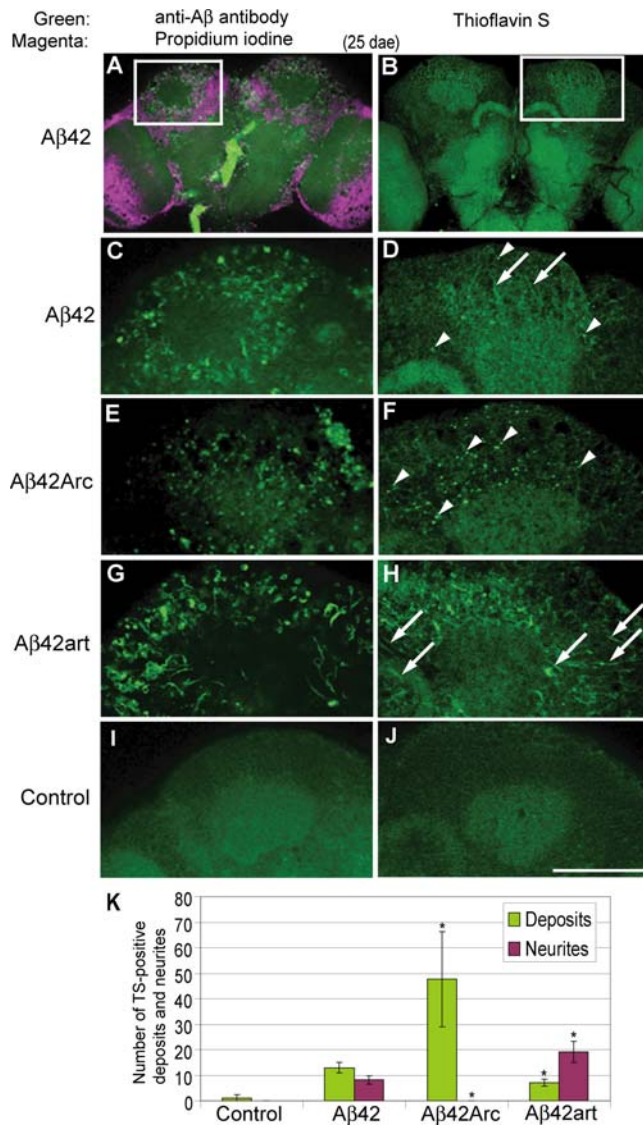
atrophy in A $\beta$ 42 and A $\beta$ 42Arc was similar. Observed differences were not due to differences in brain size (Figure S7).

Immunostaining and Thioflavin S (TS) staining, which labels aggregated A $\beta$ 42, revealed that the degenerated structures in A $\beta$ 42, A $\beta$ 42Arc, and A $\beta$ 42art flies were closely correlated with the intraneuronal accumulation sites of each A $\beta$  peptide. A $\beta$ 42Arc accumulated primarily in the cell soma as large deposits (Figure 4E, F, arrowheads in F), while A $\beta$ 42art was distributed primarily in the neurites (Figure 4G, H, arrows in H). A $\beta$ 42 was detected both in the cell body and in the neurites, but to a lesser extent than the mutants (Figure 4C, D, arrowheads and arrows in D). Quantification of TS-positive deposits and neurites in A $\beta$  fly brains is shown in Figure 4K. These fly brains were negative for Congo-Red staining (Figure S8), and no intraneuronal amyloid fibrils were observed by electron microscopy, suggesting that the majority of TS-positive aggregates did not contain amyloid fibril

structures. These distinct patterns of neurodegeneration and A $\beta$  accumulation were confirmed in several independent transgenic lines (data not shown).

This study highlights that the complex toxicities of A $\beta$ 42 are associated with different aggregation propensities *in vivo*. First, the increase in A $\beta$ 42 aggregation proneness associated with the pathogenic Arctic mutation (E22G) correlated with more severe detrimental effects on memory, locomotor ability, and lifespan than those caused by A $\beta$ 42 (Figure 2). These data are consistent with the fact that A $\beta$ 42Arc causes EOFAD [4], and indicates that aggregation proneness contributes to A $\beta$ 42 toxicity *in vivo*. Second, an artificial mutation (L17P) that decreased A $\beta$ 42 aggregation proneness suppressed the toxicities toward locomotor function and lifespan, but caused earlier onset of memory defects (Figure 2), showing that not all pathogenic effects of A $\beta$ 42 correlate directly with aggregation proneness. Third, the differences in aggregation





**Figure 4. Distribution and aggregation of A $\beta$ 42, A $\beta$ 42Arc, and A $\beta$ 42art peptides in fly brains.** A,C,E,G and I, Immunostaining of brains of 25 dae flies with anti-A $\beta$  antibody (green). In (A), nuclei were stained with propidium iodide (magenta). B,D,F,H and J, Thioflavin S (TS) staining of brains of 25 dae flies. Arrowheads and arrows indicate TS-positive deposits and neurites, respectively. No signal was detected in the control (I, J). Scale bar in J: 50  $\mu$ m. (C) and (D) are enlarged images of the boxed regions in (A) and (B), respectively. K, Numbers of TS-positive deposits and neurites were presented as averages  $\pm$  SD (n = 6 hemispheres). Asterisks indicate significant differences from A $\beta$ 42 (P < 0.05, Student's t-test). doi:10.1371/journal.pone.0001703.g004

tendencies of A $\beta$ 42 and derivatives correlated with qualitative shifts in pathology in the fly brain, exemplified by distinct neurodegeneration patterns accompanying the different accumulation profiles of A $\beta$ 42 peptides (Figure 3 and 4). Importantly, these differences are not due to a difference in genetic background [27], since these A $\beta$  flies were generated in the same genetic background.

Under physiological conditions, the A $\beta$ 42 peptides are generated from amyloid precursor protein (APP) by  $\beta$ - and  $\gamma$ -secretases in the secretory pathway including, the transgolgi-network, endosome-system, and plasma membrane [28]. Because *Drosophila* has no or very low endogenous  $\beta$ -secretase activity [29], we used the artificial expression system to achieve high expression levels of

A $\beta$ 42 in fly brains. In our transgenic flies, A $\beta$ 42 peptides were expressed in the ER and distributed to the late secretory pathway compartments, axons, dendrites, and presynaptic terminals, as well as secreted from neurons (Figure 1C, S2 and S3). Although the ER is not a major cellular site for A $\beta$  generation under physiological conditions [28], several lines of evidence suggest that, under abnormal conditions, A $\beta$  may be generated, retained, or recycled back to the ER and may induce ER stress [30–35]. Our fly models may recapitulate neuronal dysfunction and degeneration induced by such abnormal intracellular metabolisms of A $\beta$ 42. It would be also important to examine the effects of the Arctic (E22G) and artificial (L17P) mutations on intracellular distribution and toxicities of A $\beta$ 42 generated from the full-length APP.

In summary, our results lead us to predict two issues. First, the partial prevention of A $\beta$ 42 amyloidogenesis by aggregation inhibitors may result in qualitative shifts in the pathogenic effects of A $\beta$ 42. Second, the tendency of A $\beta$ 42, a natively unfolded polypeptide consist primarily of random-coil structure in their native and soluble states [36,37], to aggregate may be affected by a combination of genetic [38], environmental [39], and aging factors [40], and the resultant A $\beta$ 42 conformers or species may contribute to the heterogeneous pathogenesis of AD [41]. The existence of different “A $\beta$  species” has been recently verified both *in vitro* [12] and *in vivo* [42].

## Materials and Methods

### *Drosophila* genetics and stocks

cDNA fragments encoding the human A $\beta$ 42, A $\beta$ 42Arc, and A $\beta$ 42art peptides were amplified by PCR from human APP cDNA, fused to the rat pre-proenkephalin signal peptide, cloned into the pUAST *Drosophila* transformation vector and microinjected into fly embryos of the *w*<sup>1118</sup> (*isoC71*) genotype. Several transgenic lines for each A $\beta$  construct were established. The flies were raised and maintained at 25°C, under conditions of 70% humidity and a 12 h:12 h light:dark cycle. The transgenic *UAS-CD8::GFP::OK107* line was a kind gift from Dr. L. Luo [26]. *UAS-nlsGFP* and *elav-GAL4<sup>155</sup>* flies were obtained from the Bloomington *Drosophila* Stock Center. For Pavlovian olfactory conditioning, the *elav-GAL4<sup>155</sup>* line was outcrossed with *w*<sup>1118</sup> (*isoC71*) flies, an isogenic line, for 5 generations. Genotypes of flies used in this study are as follows: Control; *elav-GAL4<sup>155</sup>/Y*; A $\beta$ 42; *elav-GAL4<sup>155</sup>/Y::UAS-A $\beta$ 42/+*; A $\beta$ 42Arc; *elav-GAL4<sup>155</sup>/Y::UAS-A $\beta$ 42Arc/+* and A $\beta$ 42art; *elav-GAL4<sup>155</sup>/Y::UAS-A $\beta$ 42art/+*. In figure 3G, H and I, flies' genotypes are as follows: Control; *w/Y::UAS-CD8::GFP/+;OK107/+*, A $\beta$ 42; *w/Y::UAS-CD8::GFP/+;UAS-A $\beta$ 42/+;OK107/+*, A $\beta$ 42Arc; *w/Y::UAS-CD8::GFP/+;UAS-A $\beta$ 42Arc/+;OK107/+* and A $\beta$ 42art; *w/Y::UAS-CD8::GFP/+;UAS-A $\beta$ 42art/+;OK107/+*.

### Western blot and dot blot analysis

For sequential extractions, fly heads were homogenized in RIPA buffer (50 mM Tris-HCl, pH 8.0, 0.5% sodium deoxycholate, 1% Triton X-100, 150 mM NaCl) containing 1% SDS. Lysates were centrifuged at 100,000 g for 1h, and supernatants were collected (SDS-soluble fraction). SDS-insoluble pellets were further homogenized in 70% formic acid (Sigma) followed by centrifugation at 13,000 rpm for 20 min and the supernatants were collected (FA fraction). Formic acid was evaporated by Speed Vac (Savant, SC100) and protein was resuspended in dimethyl sulfoxide (Sigma).

Protein extracts were immunoprecipitated with the anti-A $\beta$  antibody 6E10 (Signet), separated on 10–20% Tris-Tricine gels (Invitrogen), and transferred to nitrocellulose membranes (Invitrogen). The membranes were boiled in phosphate buffered saline (PBS) for 3 min, blocked with 5% non-fat dry milk (Nestlé) and

blotted with the 6E10 antibody or anti-tubulin antibody (Sigma). To quantify levels of expression of the A $\beta$  peptide, heads from 1–2 dae flies were homogenized in Tris-Tricine sample buffer (Invitrogen), centrifuged at 13,000 rpm for 20 min and the supernatants were subjected to Western blotting, as described above. The signal intensity was quantified using ImageJ (NIH).

For dot blot analysis, fly heads were homogenized in 2% SDS followed by centrifugation at 13,000 rpm for 10 min. The supernatants were applied to a nitrocellulose membrane and air-dried. The membranes were blocked with 5% non-fat dry milk (Nestlé) and blotted with the A11 (Invitrogen) or 6E10 antibodies (Signet).

### Immunoprecipitation and mass spectrometric analysis

Two hundred A $\beta$  fly heads were homogenized in 70% formic acid (Sigma) followed by centrifugation at 13,000 rpm for 20 min. The supernatant was evaporated using a Speed Vac (Savant, SC100) and protein was resuspended in dimethyl sulfoxide (Sigma). The A $\beta$  peptides were immunoprecipitated with the 6E10 antibody (Signet) and Protein-G Sepharose beads. The beads were washed three times with washing buffer (140 mM NaCl, 10 mM Tris-HCl, pH 8.0) containing 0.1% Octyl  $\beta$ -D-glucopyranoside, and the peptides were eluted with 70% formic acid and subjected to MALDI-TOF mass spectrometry.

### Climbing assay

Approximately 25 flies were placed in an empty plastic vial. The vial was gently tapped to knock the flies to the bottom and the number of flies at the top, middle, or bottom of the vial was scored after 10 seconds under red light (Kodak, GBX-2, Safelight Filter). Experiments were repeated more than three times, and a representative result was shown.

### Survival assay

Food vials containing 25 flies were placed on their sides at 25°C, under conditions of 70% humidity and a 12 h:12 h light:dark cycle. Food vials were changed every 2–3 days, and the number of dead flies was counted each time. At least four vials for each genotype were prepared. Experiments were repeated more than three times, and a representative result was shown.

### Pavlovian olfactory associative learning

Approximately 100 flies were trained by exposure to electroshock paired with one odour [octanol (OCT, 10<sup>-3</sup>(v/v)) or methylcyclohexanol (MCH, 10<sup>-3</sup>(v/v))] for 60 s and subsequent exposure to the other odour without electroshock for 60 s [23]. Immediately after training, learning was measured by allowing flies to choose between the two odours for 120 s. For one hour memory, trained flies were transferred to food vials, which were placed on their side in the dark at 25°C and 70% humidity, and tested after one hour. The performance index (PI) was calculated by subtracting the number of flies making the incorrect choice from those making the correct one, dividing by the total number of flies, and multiplying by 100. Absolute odour avoidance was quantified by a T-maze with one of the two odours (octanol [10<sup>3</sup> (vol/vol)] or methylcyclohexanol [10<sup>3</sup> (vol/vol)]) coming from one side and air from the other side. Naïve flies avoid odours, and the performance index was calculated by subtracting the number of flies that chose the odour side of the T-maze from those in the air side, dividing by the total number of flies and multiplying by 100. Electric shock reactivity was tested by putting approximately 100 flies in a T-maze having one arm with electric shock and one arm without electric shock. The performance index was calculated by

subtracting the number of flies that chose the electric shock arm of the T-maze from those in the arm without shock, dividing by the total number of flies and multiplying by 100.

### Quantification of neurodegeneration

Heads were fixed in 4% paraformaldehyde (Electron Microscopy Sciences), processed to embed in paraffin blocks, and sectioned at a thickness of 6  $\mu$ m. Sections were placed on slides, stained with haematoxylin and eosin (Vector), and examined by bright-field microscopy. To quantify neurodegeneration in the cell body and neuropil of the mushroom body structures, images of the sections which included the Kenyon cell body and/or Calyx were captured, and the area of the vacuoles in the Kenyon cell body or Calyx region was measured in each image. The ratio was calculated by dividing the sum of the vacuole areas by the total area of the Kenyon cell body or Calyx region. Seven to nine hemispheres from five flies were analyzed for each genotype. To quantify the atrophy of dendritic and axonal structures of the mushroom body neurons, the GFP signal in whole fly brains carrying *UAS-CD8::GFP::OK107* was analyzed using confocal microscopy (Carl Zeiss LSM 510). The area of Calyxes (dendritic structures of the Kenyon cells), Lobes (axon bundles of the Kenyon cells) and the size of the brains was measured using LSM Image software. Six hemispheres from three flies were quantified for each genotype.

### Whole-mount immunostaining and Thioflavin S staining

Fly brains were dissected in cold PBS and fixed in PBS containing 4% paraformaldehyde (EMS), and then placed under vacuum in PBS containing 4% paraformaldehyde and 0.25% Triton X-100. After permeabilization with PBS containing 2% Triton X-100, the brains were treated with 70% formic acid (Sigma), and stained with a mouse monoclonal anti-A $\beta$  antibody (Chemicon) followed by detection with biotin-XX goat anti-mouse IgG and streptavidin-Oregon Green 488 conjugate (Molecular Probes). Nuclei were counterstained with propidium iodide (Molecular Probes). To detect nuclei of glial cells, fly heads were stained with an anti-Repo antibody (DSHB) followed by detection with Texas Red goat anti-mouse IgG (Molecular Probes). The brains were analyzed using a confocal microscope (Carl Zeiss LSM 510). For Thioflavin S (TS) staining, the brains were permeabilized and incubated in 50% EtOH containing 0.1% TS (Sigma) overnight. After washing in 50% EtOH and PBS, the brains were analyzed using a confocal microscope. TS-positive deposits and neurites were quantified from six hemispheres from three flies per genotype.

### Congo-Red staining

Heads were fixed in 4% paraformaldehyde, processed to embed in paraffin blocks, and sectioned at a thickness of 10  $\mu$ m. Sections were placed on slides and stained with an amyloid stain, Congo Red kit (Sigma), following the manufacturer's protocol. Apple-green birefringence was examined by bright-field microscopy with a polarizing filter (Leica). Slides of human kidney tissue containing intracellular amyloid (Sigma) were processed at the same time as positive controls.

### Immuno-gold labelling and electron microscopy

Proboscis were removed from decapitated heads, which were then immersion-fixed overnight in 4% glutaraldehyde and 2% paraformaldehyde in 0.1 M PBS. Samples were post-fixed 1 h in ferrocyanide-reduced osmium tetroxide (1% osmium tetroxide and 1.5% potassium ferrocyanide). Fixation was followed by dehydration in a graded alcohol series and infiltration with LR White resin (2 h in 50% LR White in ethanol and 24 h in 100% LR White)



using constant rotation. After transferring the samples to gelatin capsules with fresh LR White resin, the samples were polymerized overnight at 60°C. Thin sections (100 nm) of Kenyon cells and neuropil regions of the mushroom body were collected on nickel grids (100 mesh, Veco-EMS). For immunogold labelling of A $\beta$ 42 transgenic and control fly heads, thin sections were first incubated for 2 minutes in 10% hydrogen peroxide for antigen retrieval, jet-rinsed in distilled water, and then placed on drops of 1% deacetylated BSA in PBS for 5 min. The grids were then transferred to drops of a rabbit antibody specific for human A $\beta$ 42 (Chemicon-Millipore) diluted 1:10 in PBS and incubated for 2 h at room temperature. Unbound primary antibody was removed by rinsing the grids through 5 drops of PBS. Antibody was detected by incubating grids for 1 h in 10 nm colloidal gold conjugated goat anti-rabbit H&L (GE Healthcare) diluted 1:10 in PBS. Grids were then rinsed in 10 drops of distilled water and air-dried. Thin sections were counterstained for 5 minutes in 3% uranyl acetate dissolved in 30% ethanol and then rinsed in distilled water.

### Drosophila S2 cell culture

*Drosophila* Schneider's cells (S2 cells) were maintained in Schneider's *Drosophila* Medium (Gibco) supplemented with 10% FBS (GEMINI) and an Antibiotic-Antimycotic mixture (Gibco). The cells were transiently transfected with *Actin-Gal4* and *UAS-A $\beta$*  plasmid constructs using a calcium phosphate transfection kit (Invitrogen). Culture medium was replaced at 12 h post-transfection, and cells were cultured for an additional 24 h. The cells and culture medium were then harvested and subjected to immunoprecipitation followed by Western blot analysis as described above.

### Supporting Information

**Figure S1** MS/IP analysis of A $\beta$  peptides expressed in fly brains. Each A $\beta$  peptide was immunoprecipitated using the anti-A $\beta$  antibody and subjected to MALDI-TOF mass spectrometry. A $\beta$ 42 (A), A $\beta$ 42Arc (B), and A $\beta$ 42art (C) were each detected at their predicted mass.

Found at: doi:10.1371/journal.pone.0001703.s001 (0.98 MB PDF)

**Figure S2** Secretion of A $\beta$  peptides expressed in *Drosophila* S2 cells. The levels of A $\beta$ 42 (blue), A $\beta$ 42Arc (magenta), and A $\beta$ 42art (green) in the culture medium were detected by Western blotting, normalized to intracellular A $\beta$  levels, and shown as a ratio relative to that of A $\beta$ 42. Each A $\beta$  peptide was secreted at different levels. Asterisks indicate a significant difference from A $\beta$ 42 ( $n=3$ ,  $P<0.05$ , Student's t-test).

Found at: doi:10.1371/journal.pone.0001703.s002 (0.08 MB PDF)

**Figure S3** Glial cells accumulate A $\beta$ 42 peptide produced in neurons in the *Drosophila* brain. ImmunoEM analysis detected A $\beta$ 42 (gold particles, arrowhead) in glial cells in brains of 25 dae flies with A $\beta$ 42 expression driven by *elav-Gal4c155* (D). Scale bar in D: 1  $\mu$ m. Confocal analysis revealed that *elav-Gal4c155* does not drive the expression of transgene in glial cells. All nuclei of neurons in the fly brain were labeled by GFP fused to a nuclear localization signal driven by *elav-Gal4c155* (A, green). The brain was counterstained with anti-Repo, a marker for *Drosophila* glial cells (B, magenta). The overlay image showed no significant overlap between the two signals (C). Scale bar in A, 50  $\mu$ m.

Found at: doi:10.1371/journal.pone.0001703.s003 (3.82 MB AI)

**Figure S4** Behavioral defects induced by the expression of A $\beta$ 42, A $\beta$ 42Arc, and A $\beta$ 42art peptides were dose dependent. A, C and E, Locomotor dysfunction in independent transgenic lines (W; weak, M; moderate, S; strong expression) of A $\beta$ 42 (A), A $\beta$ 42Arc (C), and A $\beta$ 42art (E). The percent of flies at the top (yellow,

middle (magenta), or bottom (blue) of the vial at 10 seconds after knocking flies to the bottom are shown (average $\pm$ SEM ( $n=10$ )). B, D and F, The percent survivorship of independent transgenic lines (W, M, and S) of A $\beta$ 42 (B), A $\beta$ 42Arc (D), and A $\beta$ 42art (F) was plotted against the age (dae). The expression levels of A $\beta$  peptides in all transgenic lines are shown in Figure 1B, and indicated as S (strong), M (moderate) or W (weak). The results are summarized in table S1. In the main text, the data from A $\beta$ 42 M, A $\beta$ 42Arc M and A $\beta$ 42art M (asterisks in Figure 1B) are presented.

Found at: doi:10.1371/journal.pone.0001703.s004 (0.40 MB TIF)

**Figure S5** No A11-positive A $\beta$  oligomers in A $\beta$ 42, A $\beta$ 42Arc, and A $\beta$ 42art fly brains. Fly brain lysates (1 or 2  $\mu$ l) were applied on membrane and probed with 6E10 (top) or oligomer-specific antibody, A11 (bottom). No specific signal was observed with A11 antibody (top, compare Control and A $\beta$ 42Arc, A $\beta$ 42 or A $\beta$ 42art), while 6E10 detected A $\beta$  in fly brains (bottom, compare Control and A $\beta$ 42Arc, A $\beta$ 42 or A $\beta$ 42art). *elav-Gal4c155* flies were used as control.

Found at: doi:10.1371/journal.pone.0001703.s005 (1.61 MB TIF)

**Figure S6** Learning defects in A $\beta$ 42, A $\beta$ 42Arc, and A $\beta$ 42art flies. Learning ability was assessed by Pavlovian olfactory conditioning at 10 dae. Asterisks indicate a significant difference from control ( $n=6-8$ ,  $\alpha<0.05$ , Tukey-Kramer significant difference). Average learning scores $\pm$ SEM are shown.

Found at: doi:10.1371/journal.pone.0001703.s006 (0.04 MB TIF)

**Figure S7** Brain sizes of control, A $\beta$ 42, A $\beta$ 42Arc, and A $\beta$ 42art flies were not significantly different. The thickness of fly brains was measured as indicated, and presented as average $\pm$ SEM ( $n=5$  individual flies). The age of the flies is indicated at the bottom.

Found at: doi:10.1371/journal.pone.0001703.s007 (0.62 MB TIF)

**Figure S8** The majority of A $\beta$  aggregates in fly brains are not stained by Congo-Red. Paraffin sections of brains of 25 dae control (B), A $\beta$ 42 (C), A $\beta$ 42Arc (D) and A $\beta$ 42art (E) flies were stained with Congo-Red. Sections from human kidney tissue containing intracellular amyloid were used as a positive control (A). Amyloid was observed as pink signals under bright field, and  $\beta$ -pleated structures were detected as birefringent apple-green signals using a polarizing filter (arrows in (A)). None of A $\beta$  fly brains were stained with Congo-Red (C-E). The numerous vacuoles in (C-E) indicate neurodegeneration. For this analysis, transgenic lines with the highest expression of each A $\beta$  peptide (A $\beta$ 42 S, A $\beta$ 42Arc M and A $\beta$ 42art S) were used.

Found at: doi:10.1371/journal.pone.0001703.s008 (7.67 MB PDF)

**Table S1** Summary of the characterization of A $\beta$  transgenic lines. Asterisks (\*) indicate transgenic lines primarily used in this study.

Found at: doi:10.1371/journal.pone.0001703.s009 (0.08 MB PDF)

**Table S2** Shock reactivity and olfactory acuity of transgenic flies at 10 dae. A $\beta$ 42, A $\beta$ 42Arc, and A $\beta$ 42art flies did not show any significant differences from control flies ( $n=6$ ,  $\alpha<0.05$ , Tukey-Kramer significant difference). Average scores $\pm$ SEM are shown. In MCH olfactory acuity of males, there were no differences relative to controls, but A $\beta$ 42Arc and A $\beta$ 42art flies were significantly different from each other (\*).

Found at: doi:10.1371/journal.pone.0001703.s010 (0.18 MB PDF)

### Acknowledgments

We thank L. Bianco and the CSHL animal facility for preparation of paraffin sections, M. Myers for Mass spectrometry analysis. We also thank J. Jaynes and D. Merry for helpful comments on the manuscript, L. Luo, the Bloomington stock center and the fly community at CSHL for *Drosophila* stocks.

## Author Contributions

Conceived and designed the experiments: KI KI-A. Performed the experiments: IH KI HC SH AL KI-A AG LG CS. Analyzed the data:

YZ KI HC SH KI-A. Contributed reagents/materials/analysis tools: KI. Wrote the paper: YZ KI KI-A.

## References

- Selkoe DJ (2001) Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* 81: 741–766.
- Thal DR, Capetillo-Zarate E, Del Tredici K, Braak H (2006) The development of amyloid beta protein deposits in the aged brain. *Sci Aging Knowledge Environ* 2006: re1.
- Tanzi RE, Bertram L (2005) Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. *Cell* 120: 545–555.
- Nilsberth C, Westlund-Danielsson A, Eckman CB, Condron MM, Axelman K, et al. (2001) The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced A $\beta$  protofibril formation. *Nat Neurosci* 4: 887–893.
- Johansson AS, Berglind-Dehlin F, Karlsson G, Edwards K, Gellerfors P, et al. (2006) Physicochemical characterization of the Alzheimer's disease-related peptides A $\beta$  1-42Arctic and A $\beta$  1-42wt. *Febs J* 273: 2618–2630.
- Lansbury PT, Lashuel HA (2006) A century-old debate on protein aggregation and neurodegeneration enters the clinic. *Nature* 443: 774–779.
- Gestwicki JE, Crabtree GR, Graef IA (2004) Harnessing chaperones to generate small-molecule inhibitors of amyloid beta aggregation. *Science* 306: 865–869.
- Yankner BA, Dawes LR, Fisher S, Villa-Komaroff L, Oster-Granite ML, et al. (1989) Neurotoxicity of a fragment of the amyloid precursor associated with Alzheimer's disease. *Science* 245: 417–420.
- Murakami K, Irie K, Morimoto A, Ohgashi H, Shindo M, et al. (2003) Neurotoxicity and physicochemical properties of A $\beta$  mutant peptides from cerebral amyloid angiopathy: implication for the pathogenesis of cerebral amyloid angiopathy and Alzheimer's disease. *J Biol Chem* 278: 46179–46187.
- Caughey B, Lansbury PT (2003) Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders. *Annu Rev Neurosci* 26: 267–298.
- Klein WL, Stine WB Jr, Teplow DB (2004) Small assemblies of unmodified amyloid beta-protein are the proximate neurotoxin in Alzheimer's disease. *Neurobiol Aging* 25: 569–580.
- Petkova AT, Leapman RD, Guo Z, Yau WM, Mattson MP, et al. (2005) Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils. *Science* 307: 262–265.
- Slow EJ, Graham RK, Hayden MR (2006) To be or not to be toxic: aggregations in Huntington and Alzheimer disease. *Trends Genet* 22: 408–411.
- Iijima K, Liu HP, Chiang AS, Hearn SA, Konsolaki M, et al. (2004) Dissecting the pathological effects of human A $\beta$ 40 and A $\beta$ 42 in *Drosophila*: a potential model for Alzheimer's disease. *Proc Natl Acad Sci U S A* 101: 6623–6628.
- Whalen BM, Selkoe DJ, Hartley DM (2005) Small non-fibrillar assemblies of amyloid beta-protein bearing the Arctic mutation induce rapid neuritic degeneration. *Neurobiol Dis* 20: 254–266.
- Cheng IH, Palop JJ, Esposito LA, Bien-Ly N, Yan F, et al. (2004) Aggressive amyloidosis in mice expressing human amyloid peptides with the Arctic mutation. *Nat Med* 10: 1190–1192.
- Lord A, Kalimo H, Eckman C, Zhang XQ, Lannfelt L, et al. (2006) The Arctic Alzheimer mutation facilitates early intraneuronal A $\beta$  aggregation and senile plaque formation in transgenic mice. *Neurobiol Aging* 27: 67–77.
- Morimoto A, Irie K, Murakami K, Masuda Y, Ohgashi H, et al. (2004) Analysis of the secondary structure of beta-amyloid (A $\beta$ 42) fibrils by systematic proline replacement. *J Biol Chem* 279: 52781–52788.
- Fay DS, Fluet A, Johnson CJ, Link CD (1998) In vivo aggregation of beta-amyloid peptide variants. *J Neurochem* 71: 1616–1625.
- Brand AH, Perrimon N (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118: 401–415.
- Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, et al. (2003) Common Structure of Soluble Amyloid Oligomers Implies Common Mechanism of Pathogenesis. *Science* 300: 486–489.
- Ganetzky B, Flanagan JR (1978) On the relationship between senescence and age-related changes in two wild-type strains of *Drosophila melanogaster*. *Exp Gerontol* 13: 189–196.
- Tully T, Quinn WG (1985) Classical conditioning and retention in normal and mutant *Drosophila melanogaster*. *J Comp Physiol [A]* 157: 263–277.
- Selkoe DJ (2002) Alzheimer's disease is a synaptic failure. *Science* 298: 789–791.
- Heisenberg M (2003) Mushroom body memoir: from maps to models. *Nat Rev Neurosci* 4: 266–275.
- Watts RJ, Hoopfer ED, Luo L (2003) Axon pruning during *Drosophila* metamorphosis: evidence for local degeneration and requirement of the ubiquitin-proteasome system. *Neuron* 38: 871–885.
- Ryman D, Lamb BT (2006) Genetic and environmental modifiers of Alzheimer's disease phenotypes in the mouse. *Curr Alzheimer Res* 3: 465–473.
- Small SA, Gandy S (2006) Sorting through the cell biology of Alzheimer's disease: intracellular pathways to pathogenesis. *Neuron* 52: 15–31.
- Greeve I, Kretschmar D, Tschape JA, Beyn A, Brellinger C, et al. (2004) Age-dependent neurodegeneration and Alzheimer-amyloid plaque formation in transgenic *Drosophila*. *J Neurosci* 24: 3899–3906.
- Busciglio J, Gabuzda DH, Matsudaira P, Yankner BA (1993) Generation of beta-amyloid in the secretory pathway in neuronal and nonneuronal cells. *Proc Natl Acad Sci U S A* 90: 2092–2096.
- Cook DG, Forman MS, Sung JC, Leight S, Kolson DL, et al. (1997) Alzheimer's A $\beta$  (1-42) is generated in the endoplasmic reticulum/intermediate compartment of NT2N cells. *Nat Med* 3: 1021–1023.
- Lee SJ, Liyanage U, Bickel PE, Xia W, Lansbury PT Jr, et al. (1998) A detergent-insoluble membrane compartment contains A $\beta$  in vivo. *Nat Med* 4: 730–734.
- Skovronsky DM, Doms RW, Lee VM (1998) Detection of a novel intraneuronal pool of insoluble amyloid beta protein that accumulates with time in culture. *J Cell Biol* 141: 1031–1039.
- Wild-Bode C, Yamazaki T, Capell A, Leimer U, Steiner H, et al. (1997) Intracellular generation and accumulation of amyloid beta-peptide terminating at amino acid 42. *J Biol Chem* 272: 16085–16088.
- Cruz JC, Kim D, Moy LY, Dobbin MM, Sun X, et al. (2006) p25/cyclin-dependent kinase 5 induces production and intraneuronal accumulation of amyloid beta in vivo. *J Neurosci* 26: 10536–10541.
- Kelly JW (2005) Attacking amyloid. *N Engl J Med* 352: 722–723.
- Rochet JC, Lansbury PT Jr (2000) Amyloid fibrillogenesis: themes and variations. *Curr Opin Struct Biol* 10: 60–68.
- DeMattos RB, Cirrito JR, Parsadanian M, May PC, O'Dell MA, et al. (2004) ApoE and clusterin cooperatively suppress A $\beta$  levels and deposition: evidence that ApoE regulates extracellular A $\beta$  metabolism in vivo. *Neuron* 41: 193–202.
- Cherny RA, Atwood CS, Xilinas ME, Gray DN, Jones WD, et al. (2001) Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron* 30: 665–676.
- Cohen E, Bieschke J, Percivalle RM, Kelly JW, Dillin A (2006) Opposing activities protect against age-onset proteotoxicity. *Science* 313: 1604–1610.
- Cummings JL (2000) Cognitive and behavioral heterogeneity in Alzheimer's disease: seeking the neurobiological basis. *Neurobiol Aging* 21: 845–861.
- Meyer-Luchmann M, Coomaraswamy J, Bolmont T, Kaeser S, Schaefer C, et al. (2006) Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. *Science* 313: 1781–1784.
- Kretschmar D, Hasan G, Sharma S, Heisenberg M, Benzer S (1997) The swiss cheese mutant causes glial hyperwrapping and brain degeneration in *Drosophila*. *J Neurosci* 17: 7425–7432.