Mutation of the amyloid precursor protein (APP), presenilin-1, or presenilin-2 results in the development of early onset autosomal dominant forms of Alzheimer disease (AD). These mutations lead to an increased Aβ42/Aβ40 ratio that correlates with the onset of disease. However, it remains unknown how these mutations affect γ-secretase, a protease that generates the termini of Aβ40 and Aβ42. Here we have determined the reaction mechanism of γ-secretase with wild type and three mutated APP substrates. Our findings indicate that despite the overall outcome of an increased Aβ42/Aβ40 ratio, these mutations each display rather distinct reactivity to γ-secretase. Intriguingly, we found that the ratio of Aβ42/Aβ40 is variable with substrate concentration; increased substrate concentrations result in higher ratios of Aβ42/Aβ40. Moreover, we demonstrated that reduction of γ-secretase substrate production may introduce a practical therapeutic modality for treatment of AD. However, despite intensive studies on γ-secretase, the mechanism of cleavage specificity for γ-secretase is still unknown.

APP was the first gene found to be linked with inherited AD (7). Each mutation surrounding the γ-secretase cleavage site appears to alter the production of Aβ40 and Aβ42. Suzuki et al. (8) demonstrated that mutating APP at Val-46 to Phe or Ile increased the ratio of secreted Aβ42 to Aβ40 in transfected cells. An increased ratio of Aβ42/Aβ40 was also observed with other mutations (9–11). De Jonghe et al. (9) found certain mutations enhanced the stability of the γ-secretase substrates known as C-terminal fragments (βCTF and αCTF), and Ancolio et al. (12) reported that the V44M mutation influences α-secretase cleavage. Therefore, secretion of Aβ from APP mutation-transfected cells is affected by a multitude of factors. How these mutations affect APP binding and reaction with γ-secretase remains unknown.

In this study, we have developed a simplified system using small peptide substrates that allow for the mechanistic characterization of APP FAD mutations. Kinetic analyses (kcat/Km) suggest that all of these APP mutations enhance the preference of γ-secretase for the 42-site over the 40-site cleavage and these changes are generally caused by kcat rather than Km. Importantly, we have revealed that the amount of γ-secretase substrate governs the ratio of Aβ42/Aβ40 in vitro and in cells. Increased substrate concentrations result in higher ratios of Aβ42/Aβ40.

**Experimental Procedures**

Peptide and Compound Synthesis—Wild type (WT) and mutant APP-TM peptides were synthesized and purified by Midwest Biotech (Fig. 1). Biotinylated standard peptides (P40 and P42) were synthesized in our laboratory using standard Fmoc (N-(9-fluorenylethoxycarbonyl) solid phase chemistry on a peptide synthesizer (Protein Technologies, Inc.). Peptides were purified by reversed-phase high performance liquid chro-
matography using a semi-preparative C18 column. The identity of these peptides was confirmed by liquid chromatography tandem mass spectrometry analysis (Agilent Technologies). Compound 3 was synthesized as described (13).

**In Vitro **/H9253-**Secretase Assay**—/H9253-secretase assay was the same as described previously (14) except for substrate identity. Briefly, the wild type or mutant APP-TM peptides were incubated with HeLa cell membrane in the presence of CHAPSO (0.25%) in buffer A (50 mM PIPES, pH 7.0, 5 mM MgCl2, 5 mM CaCl2, 150 mM KCl) at 37 °C for 2.5 h. The reactions were stopped by adding radioimmune precipitation buffer (150 mM NaCl, 1.0% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris-HCl, pH 8.0). Then the reaction mixtures were incubated with ruthenylated G2–10 or G2–11 antibody, which specifically recognizes products P40 and P42, respectively. The antibody reactions were detected by electrochemiluminescence (ECL). The G2–10 and G2–11 antibodies were ruthenylated with ruthenium (II) tris-bipyridine N-hydroxysuccinimide ester (Biovarice Inc.) according to the manufacturer’s instructions. The concentrations of P40 and P42 were determined using synthetic peptide standards. The Km and Vmax were determined from the Michaelis-Menten equation Michaelis-Menten kinetics (\( \nu = V_m [S]/(K_m + [S]) \)) where \( \nu \) is initial rate, \( V_m \) is maximum velocity, \( K_m \) is the Michaelis-Menten constant, and \( S \) is substrate). p values were calculated from Student’s t-test.

**Cell-based Assay for Aβ Production**—N2A cells that stably express APP Swedish mutation were incubated with /H9253-secretase or BACE1 inhibitors. Twenty-four hours later, the conditioned medium was removed and assayed for Aβ production. Aβ40 and Aβ42 were detected with biotinylated 6E10 paired with ruthenylated G2–10 or G2–11 antibodies, respectively. Aβx40 and Aβx42 were detected with biotinylated 4G8 paired with ruthenylated G2–10 or G2–11 antibodies, respectively (15). Concentration of Aβ peptides is calculated from standard curves that are generated using synthetic Aβ40 and Aβ42 peptides using the ECL assay.

**RESULTS AND DISCUSSION**

βC100, a β-secretase-generated C-terminal fragment of APP, has been utilized as a substrate of γ-secretase to biochemically characterize this protease (14, 16). Prior studies suggest that this substrate binds to the active site and docking site of γ-secretase (17). To elucidate the reaction mechanism, we developed a sensitive in vitro assay in which the substrate solely interacts with the active site of γ-secretase. We chose a biotinylated peptide substrate that contains a transmembrane domain (APP-TM) plus three lysine residues of APP (Fig. 1A).

---

**FIGURE 1. Transmembrane domain of APP is a good substrate of γ-secretase. A, sequences of APP wild type (WT) and mutation substrates. Substrates contain biotin, transmembrane domain of APP, and three Lys at the C terminus. The mutated residue is in bold and underlined at 44–46 positions. The cleavage sites of 40 and 42 and the TM domain are indicated. P40 and P42 represent γ-secretase cleavage products at 40 and 42 sites, respectively.**, B, double reciprocal plots for inhibition of γ-secretase by L-685,458. Inhibition of γ-secretase by L-685,458 at [I] = 0 (filled circle), [I] = 0.3 nM (open circle), [I] = 1 nM (filled triangle), and [I] = 3 nM (open triangle). C, double reciprocal plots for inhibition of γ-secretase by Compound E. Inhibition of γ-secretase by Compound E at [I] = 0 (filled circle), [I] = 0.3 nM (open circle), and [I] = 1 nM (filled triangle). D, schematic models for the interaction of γ-secretase and the TM substrate and C100. The TM substrate only binds the active site, whereas C100 interacts with both the active site and the docking site.
Similar experimental conditions as used for the C100 FLAG assay were used in this APP-TM assay (14). Our peptide substrate was incubated with HeLa cell membrane in the presence of 0.25% CHAPSO, and the P40 and P42 products (reference to products resulting from the cleavage at the 40 and 42 sites) were detected with G2–10 and G2–11 antibodies, respectively. The γ-secretase activity against this substrate was characterized to determine optimal conditions (supplemental Fig. S1). In our assay both the substrate and products are biotinylated. To eliminate the possibility of signal saturation under high concentrations of substrate due to the lack of streptavidin magnetic beads, we assayed 5, 10, and 25 μl of total γ-secretase reaction mixture, each containing increasing amounts of biotinylated peptide. All three assay conditions resulted in the same substrate dependence (supplemental Fig. 1C), demonstrating that streptavidin beads are not limited under our assay conditions. Therefore, the γ-secretase cleavage reaction with APP-TM as a substrate displayed a steady state kinetic mechanism. P42 production was 11.5% of the total sum of P40 and P42. γ-Secretase activity for production of both P40 and P42 was inhibited by L-685,458, with an IC50 of 1.5 and 1.3 nM, respectively. The three products, P38, P40, and P42, were confirmed by liquid chromatography tandem mass spectrometry analysis (supplemental Fig. 2). Under current assay conditions, our mass spectrometry analysis was not able to detect ε- and ε-cleavages (18, 19). Nevertheless, this study focuses on γ-secretase sites (P40 and P42) that have been well characterized in association with AD. Hence, this novel assay using the APP-TM substrate recapitulates native characteristics and is a suitable assay to characterize γ-secretase activity for Aβ40 and Aβ42 production.

We examined the inhibition patterns of L-685,458 and Compound E bind to different sites on γ-secretase, as previously reported (20, 21). This study provides experimental evidence that L-685,458 is a transition state inhibitor, further validating prior conclusions that the presenilins contain the active site of γ-secretase (22).

We next evaluated the effect of APP mutations on P40 and P42 production. Three APP mutant substrates were selected (Fig. 1). Among them, two were FAD mutants that reside at positions 44 (V44M, named French mutation V715M) and 45 (I45V, named Florida mutation I716V) (Fig. 1A). The V45F mutation is a non-natural mutant discovered by phenylalanine mutagenesis of the transmembrane domain of APP (23). At a concentration of 0.5 μM, each substrate was incubated with HeLa cell membrane and the initial rate of P40 and P42 production was determined (Table 1). Our findings indicate that two mutations (I45F and I45V) reduced P40 production and increased P42 production, whereas the V44M mutation augmented the production of both P40 and P42. Interestingly, the I45F mutation led to production of higher quantities of P42 than P40, which is in contrast to other mutations. Nevertheless, the overall outcome of each of these mutations is an increased ratio of P42/P40.

To elucidate the mechanism of γ-secretase catalysis, the kinetic parameters of γ-secretase for these substrates were determined (Table 2). Surprisingly, there were different Km values for P40 and P42 production and all these substrates exhibited a higher apparent Km for P42 than that for P40. If these differences truly reflect the affinity between substrate and γ-secretase, we predict that the ratio of P42 to P40 will be altered as substrate concentration is varied. In other words, a substrate concentration that saturates for P40 production, but not for P42, will lead to a higher ratio of P42 to P40. Thus, we determined the ratio of P42 to P40 at different substrate concentrations of WT and I45F-mutated APP-TM. Ratios of P42 to total product for WT substrate were 13 and 22% at concentra-

### Table 1

| Substrate | P40 rate* | P42 rate | Ratio (P42/P40) |
|-----------|-----------|----------|----------------|
| WT        | 9.33 ± 0.20 | 1.40 ± 0.07 | 0.15 |
| I45F      | 2.27 ± 0.07** | 3.00 ± 0.07*** | 1.32 |
| V44M      | 15.90 ± 0.67*** | 5.13 ± 0.60* | 0.33 |
| I45V      | 7.00 ± 0.07*** | 3.93 ± 0.33* | 0.56 |

* The amounts of P40 and P42 were determined by the ECL assay using standard curves that were generated with synthetic P40 and P42 peptides. Mean ± S.E.; n ≥ 3.

### Table 2

| Substrates | Vmax | Km | kcat/Km (mutant) | kcat/Km (WT) |
|------------|------|----|-----------------|--------------|
| P40        |      |    |                 |              |
| P42        |      |    |                 |              |
| WT         | 27.64 ± 1.16 | 15.85 ± 0.05 | 0.97 ± 0.06 | 5.16 ± 1.25 |
| I45F       | 6.98 ± 0.37** | 31.09 ± 5.25* | 1.04 ± 0.15 | 4.69 ± 0.35 |
| V44M       | 31.76 ± 3.1** | 72.23 ± 8.09** | 0.53 ± 0.22 | 6.53 ± 0.54 |
| I45V       | 19.48 ± 0.87*** | 43.22 ± 1.31** | 0.89 ± 0.20 | 5.00 ± 1.51 |

* p < 0.05; ** p < 0.01.

Substrate Regulation of γ-Secretase Cleavage
Substrate Regulation of γ-Secretase Cleavage

**FIGURE 2.** The biochemical relationship between the P42/P40 ratio and substrate concentration. A, in vitro assay. γ-Secretase activity for P40 and P42 production was assayed at the substrate concentrations of 0.5 and 2 μM for substrate WT and I45F (mean ± S.D., n = 3). B, calculated P42/P40 ratio based on $V_{\text{max}}$ and $K_m$ parameters from Table 2. Inset, substrate concentration from 0.01 to 2 μM.

**FIGURE 3.** The cellular relationship between the P42/P40 ratio and substrate concentration. A, structure of Compound 3. B, effect of Compound 3 on Aβ40 and Aβ42 production. The N2A cells that stably express APP Swedish mutation were treated with Compound 3, and secreted Aβ40 and Aβ42 were determined with biotinylated 6E10/ruthylylated G2–10 or biotinylated 6E10/ruthylylated G2–11, respectively (n = 6). C, effect of Compound 3 on Aβx40 and Aβx42 production. Secreted Aβx40 and Aβx42 were determined with biotinylated 4G8/ruthylylated G2–10 or biotinylated 4G8/ruthylylated G2–11, respectively (n = 6). D, relative ratio of Aβx42/Aβ40 or Aβx42/Aβx42. *, p < 0.05; **, p < 0.01.

Substrate Regulation of γ-Secretase Cleavage

- The biochemical relationship between the P42/P40 ratio and substrate concentration was investigated. γ-Secretase activity for P40 and P42 production was assayed at substrate concentrations of 0.5 and 2 μM for WT and I45F. The calculated P42/P40 ratio based on $V_{\text{max}}$ and $K_m$ parameters from Table 2 is shown in Figure 2B.

- The cellular relationship between the P42/P40 ratio and substrate concentration was also examined. Figure 3A shows the structure of Compound 3. The effect of Compound 3 on Aβ40 and Aβ42 production is shown in Figure 3B. Secreted Aβ40 and Aβ42 were determined with biotinylated 6E10/ruthylylated G2–10 or biotinylated 6E10/ruthylylated G2–11, respectively (n = 6). Figure 3C shows the effect of Compound 3 on Aβx40 and Aβx42 production. Secreted Aβx40 and Aβx42 were determined with biotinylated 4G8/ruthylylated G2–10 or biotinylated 4G8/ruthylylated G2–11, respectively (n = 6). Figure 3D shows the relative ratio of Aβx42/Aβ40 or Aβx42/Aβx42. * indicates p < 0.05; ** indicates p < 0.01.

- This observation strongly suggests that γ-secretase has distinct affinities to the same substrate for P40 and P42 production. There was no significant difference between $K_m$ values of WT and the mutated substrates for P40 and P42 activities. These mutations significantly influence $V_{\text{max}}$ (maximum velocity) for both P40 and P42 production (Table 2).

- Importantly, we next examined the catalytic efficiency ($k_{\text{cat}}/K_m$ or $V_{\text{max}}/K_m$) of γ-secretase against each substrate. $k_{\text{cat}}/K_m$ is a second-order rate constant that informs how the enzyme performs when the substrate concentration is low and indicates an enzyme’s preference for different substrates (also called specificity constant). Considering that $V_{\text{max}} = k_{\text{cat}} * E_{\text{total}}$ (total concentration of γ-secretase) and the same concentrations of γ-secretase were used for each substrate, the ratio of $V_{\text{max}}/K_m$ (mutation) to $V_{\text{max}}/K_m$ (WT) are equal to the ratio of their $k_{\text{cat}}/K_m$. We analyzed the $V_{\text{max}}/K_m$ of these substrates from two perspectives. First, we compared mutated substrate versus WT. For P40 production V44M was a better substrate (2.12-fold) than WT, while both I45F (0.24-fold) and I45V (0.78-fold) were poorer substrates. For P42, all three mutations were better substrates, ranging from 2.12- to 3.60-fold compared with WT. Although each mutation displays a unique effect on P40 and P42 production, the overall tendency for all three mutations was to increase the relative specificity for P42 production. Secondly, we determined the relative $k_{\text{cat}}/K_m$ for each substrate for P40 and P42 production. The relative specificity for P40 over P42 is 9.21, 1.01, 5.42, and 2.53 for...
WT, I45F, V44M, and I45V, respectively. These studies indicate that γ-secretase generally prefers the 40-site cleavage when the WT substrate concentration is low and suggest that γ-secretase is responsible for the 40-site processing under physiological conditions. Mutations alter the preference of γ-secretase for 40- versus 42-site cleavage, and these changes are generally attributable to $k_{cat}$ rather than $K_m$. Moreover, we calculated the overall effect on the rate of γ-secretase for P40 and P42 production based on the $V_{max}$ and $K_m$ values of WT (Fig. 2B). This analysis revealed that an increase in substrate concentration not only results in generation of more total amount of Aβ but also leads to an increased ratio of Aβ42/Aβ40, which may be directly related to the pathogenesis of AD.

The next critical question is whether substrate concentration correlates with the ratio of Aβ42/Aβ40 at the cellular level. BACE1 is responsible for generation of βCTF, a γ-secretase substrate; therefore, we chose to control the βCTF level through inhibition of BACE1 activity. N2A cells that stably express APP Swedish mutation were treated with a BACE1 inhibitor, Compound 3 (13) (Fig. 3A) at 1 and 3 μM. The amounts of secreted Aβ40, Aβx40 (that includes Aβ1–40 and Aβ17–40), Aβ42, and Aβx42 were estimated using an ECL assay with synthetic peptides as standards. Compound 3 at 1 and 3 μM inhibits 33 and 39% of Aβ40 and 47 and 55% of Aβ42, respectively (Fig. 3B). Similarly, this inhibitor at 1 and 3 μM suppresses 44 and 52% of Aβx40 and 61 and 72% of Aβx42, respectively. Treatment of APP Swedish mutation cells with BACE1 inhibitor at 1 and 3 μM reduced the relative ratio (compared with “no treatment” assigned a value of 1) of Aβ42/Aβ40 to 0.81 and 0.73 and Aβx42/Aβx40 to 0.70 and 0.56, respectively (Fig. 3C). Western analysis confirmed that this compound has no effect on the level of APP (supplemental Fig. S3). These cellular studies therefore reinforce our assertion that the Aβ42/Aβ40 ratio is dependent on the substrate concentration of γ-secretase. This finding corroborates other studies showing that up-regulation of BACE1 activity by Par4 elevates the ratio of Aβ42/Aβ40 (24). Our discovery illustrates that the Aβ42/Aβ40 ratio is variable with γ-secretase substrate concentration and provides critical insight into the pathogenesis of sporadic AD. Any biological or environmental factors that promote increased levels of γ-secretase substrate likely will result in an increase in the Aβ42/Aβ40 ratio, which is similar to the effect of presenilin-1, presenilin-2, and APP mutations (25–27). Recent studies have shown that BACE1 activity is increased in sporadic AD brains and is correlated with Aβ load (28, 29). Our studies suggest that higher BACE1 activity in AD patients increases the production of βCTF and ultimately leads to a higher ratio of Aβ42/Aβ40, which is associated with the pathological state of the disease. A slight increase in βCTF production resulting from BACE1 cleavage leads to a mild elevation of the Aβ42/Aβ40 ratio (see Fig. 2B) and could chronically be detrimental to neuronal cells. Multiple factors have been found to regulate BACE1 expression and activity, such as Par4 (24) and HIF-1α (30). The role of these proteins in regulation of BACE activity in AD patients needs to be investigated.

In summary, this study demonstrates that in addition to the clinical mutations of presenilin-1, presenilin-2, and APP, γ-secretase substrate concentration can increase the ratio of Aβ42/Aβ40, which may play a critical role in the pathogenesis of sporadic AD (Fig. 4). Moreover, BACE inhibitors that reduce βCTF production are capable of lowering the ratio of Aβ42/Aβ40 in addition to reducing the total amount of Aβ. Partial inhibition of BACE1 activity could reduce the Aβ42/Aβ40 ratio and represent a practical strategy for AD therapies.

ACKNOWLEDGEMENTS—We thank Christopher Chad Shelton and Lisa Placanica for discussions and suggestions regarding this manuscript.

REFERENCES

1. Hardy, J., and Allsop, D. (1991) Trends Pharmacol. Sci. 12, 383–388
2. Jarrett, J. T., Berger, E. P., and Lansbury, P. T., Jr. (1993) Annu. N. Y. Acad. Sci. 695, 144–148
3. Iijima, K., Liu, H. P., Chiang, A. S., Hearn, S. A., Konsolaki, M., and Zhong, Y. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 6623–6628
4. McGowan, E., Pickford, F., Kim, J., Onstead, L., Eriksen, J., Yu, C., Skipper, L., Murphy, M. P., Beard, J., Das, P., Jansen, K., Delucia, M., Lin, W. L., Dolios, G., Wang, R., Eckman, C. B., Dickson, D. W., Hutton, M., Hardy, J., and Golde, T. (2005) Neuron 47, 199–119
5. Deng, Y., Tarassishin, L., Kallhoff, V., Peethumongsin, E., Wu, L., Li, Y. M., and Zheng, H. (2006) J. Neurosci. 26, 3845–3854
6. Kim, J., Onstead, L., Randle, S., Price, R., Smithson, L., Zwisinski, C., Dickson, D. W., Golde, T., and McGowan, E. (2007) J. Neurosci. 27, 627–633
7. Goate, A., Chartier-Harlin, M. C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L., Mant, R., Newton, P., Rooke, K., Roques, P., Talbot, C., Pericak-Vance, M., Roses, A., Williamson, R., Rossor, M., Owen, M., and Hardy, J. (1991) Nature 349, 704–706
8. Suzuki, N., Cheung, T. T., Cai, X. D., Odaka, A., Otvos, L., Jr., Eckman, C., Golde, T. E., and Younkin, S. G. (1994) Science (N. Y.) 264, 1336–1340
9. De Jonghe, C., Esselens, C., Kumar-Singh, S., Craessaerts, K., Semeens, S., Checler, F., Annaert, W., Van Broeckhoven, C., and De Strooper, B. (2001) Hum. Mol. Genet. 10, 1665–1671
10. Lichtenthaler, S. F., Ida, N., Multhaup, G., Masters, C. L., and Beyreuther, K. (1997) Biochemistry 36, 15396–15403
11. Maruyama, K., Tomita, T., Shinozaki, K., Kume, H., Asada, H., Saito, T. C., Ishiura, S., Iwatsubo, T., and Obata, K. (1996) Biochem. Biophys. Res. Comm. 227, 730–735
12. Ancolio, K., Dumanchin, C., Barelli, H., Warter, J. M., Brice, A., Campion, D., Frebourg, T., and Checler, F. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4119–4124
13. Stachl, S. J., Coburn, C. A., Steele, T. G., Jones, K. G., Loutsenifer, E. S., Gregor, A. R., Rajapakse, H. A., Lai, M. T., Crouthamel, M. C., Xu, M., Tugusheva, K., Lineberger, J. E., Pietrak, B. L., Expeseth, A. S., Shi, X. P., Chen-Dodson, E., Holloway, M. K., Munshi, S., Simon, A. J., Kou, L., and Vacca, J. P. (2004) J. Med. Chem. 47, 6447–6450

FIGURE 4. Proposed role of higher γ-secretase substrate concentration in AD. Mutations of presenilin-1 (PS1), presenilin-2 (PS2), and APP appear to cause an increase in the Aβ42/Aβ40 ratio and ultimately lead to an early onset of AD. This work found that higher γ-secretase substrate concentrations result in a higher ratio of Aβ42/Aβ40, which is reminiscent of genetic mutations. Previous reports showed that BACE1 activity is elevated in sporadic AD brains (28, 29). Accordingly, higher BACE1 activity generates more βCTF and leads to a higher ratio of Aβ42/Aβ40.
Substrate Regulation of γ-Secretase Cleavage

14. Li, Y. M., Lai, M. T., Xu, M., Huang, Q., DiMuzio Mower, J., Sardana, M. K., Shi, X. P., Yin, K. C., Shafer, J. A., and Gardell, S. J. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 6138–6143
15. Lai, M. T., Chen, E., Crouthamel, M. C., DiMuzio-Mower, J., Xu, M., Huang, Q., Price, E., Register, R. B., Shi, X. P., Donoviel, D. B., Bernstein, A., Hazuda, D., Gardell, S. J., and Li, Y. M. (2003) J. Biol. Chem. 278, 22475–22481
16. Kimberly, W. T., LaVoie, M. J., Ostaszewski, B. L., Ye, W., Wolfe, M. S., and Selkoe, D. J. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 6382–6387
17. Tian, G., Sobotka-Briner, C. D., Zysk, J., Liu, X., Birr, C., Sylvester, M. A., Edwards, P. D., Scott, C. D., and Greenberg, B. D. (2002) J. Biol. Chem. 277, 31499–31505
18. Zhao, G., Mao, G., Tan, J., Dong, Y., Cui, M. Z., Kim, S. H., and Xu, X. (2004) J. Biol. Chem. 279, 50647–50650
19. Sastre, M., Steiner, H., Fuchs, K., Capell, A., Multhaup, G., Condron, M. M., Teplow, D. B., and Haass, C. (2001) EMBO Rep. 2, 835–841
20. Tian, G., Ghanekar, S. V., Aharony, D., Shenvi, A. B., Jacobs, R. T., Liu, X., and Greenberg, B. D. (2003) J. Biol. Chem. 278, 28968–28975
21. Clarke, E. E., Churcher, I., Ellis, S., Wrigley, J. D., Lewis, H. D., Harrison, T., Shearman, M. S., and Beher, D. (2006) J. Biol. Chem. 281, 31279–31289
22. Li, Y. M., Xu, M., Lai, M. T., Huang, Q., Castro, J. L., DiMuzio-Mower, J., Harrison, T., Lellis, C., Nadin, A., Neduvelil, J. G., Register, R. B., Sardana, M. K., Shearman, M. S., Smith, A. L., Shi, X. P., Yin, K. C., Shafer, J. A., and Gardell, S. J. (2000) Nature 405, 689–694
23. Lichtenthaler, S. F., Wang, R., Grimm, H., Uljon, S. N., Masters, C. L., and Beyreuther, K. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 3053–3058
24. Xie, J., and Guo, Q. (2005) J. Biol. Chem. 280, 13824–13832
25. Selkoe, D. J. (2001) Physiol. Rev. 81, 741–766
26. De Strooper, B. (2007) EMBO Rep. 8, 141–146
27. Kumar-Singh, S., Theuns, J., Van Broeck, B., Pirici, D., Vennekens, K., Cosmit, E., Cruts, M., Dermaut, B., Wang, R., and Van Broeckhoven, C. (2006) Hum. Mut. 27, 686–695
28. Yang, L. B., Lindholm, K., Yan, R., Citron, M., Xia, W., Yang, X. L., Beach, T., Sue, L., Wong, P., Price, D., Li, R., and Shen, Y. (2003) Nat. Med. 9, 3–4
29. Fukumoto, H., Cheung, B. S., Hyman, B. T., and Irizarry, M. C. (2002) Arch. Neurol. 59, 1381–1389
30. Zhang, X., Zhou, K., Wang, R., Cui, J., Lipton, S. A., Liao, F.-F., Xu, H., and Zhang, Y.-W. (2007) J. Biol. Chem. 282, 10873–10880