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Mutant Presenilin 1 Increases the Expression and Activity of BACE1*[S]

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Mutations of the presenilin 1 (PS1) gene are the most common cause of early onset familial Alzheimer disease (FAD). PS1 mutations alter the activity of the γ-secretase on the β-amyloid precursor protein (APP), leading to selective overproduction of β-amyloid (Aβ) 42 peptides, the species that forms oligomers that may exert toxic effects on neurons. Here we show that PS1 mutations, expressed both transiently and stably, in non-neuronal and neuronal cell lines increase the expression and the activity of the β-secretase (BACE1), the rate-limiting step of Aβ production. Also, BACE1 expression and activity are elevated in brains of PS1 mutant knock-in mice compared with wild type littermates as well as in cerebral cortex of FAD cases bearing various PS1 mutations compared with in sporadic AD cases and controls. The up-regulation of BACE1 by PS1 mutations requires the γ-secretase cleavage of APP and is proportional to the amount of secreted Aβ42. Aβ42, and not AICD (APP intracellular domain), is indeed the APP derivative that mediates the overexpression of BACE1. The effect of PS1 mutations on BACE1 may contribute to determine the wide clinical and pathological phenotype of early onset FAD.

The β-amyloid peptide (Aβ)[2] that accumulates in vulnerable brain regions in Alzheimer disease (AD) is released from the β-amyloid precursor protein (APP) by sequential cleavages by β-secretase and γ-secretase. A single protein called BACE1 is responsible for β-secretase activity, whereas γ-secretase involves at least four proteins including a catalytic subunit called presenilin-1 (PS1) (1, 2). Mutations of PS1 are the most common cause of early onset familial AD (FAD). The known effect of PS1 mutations on APP processing is the increased production of Aβ species ending at residue 42, which aggregates faster than the Aβ40 isoform and accumulates in the brain in the state of soluble low molecular weight oligomers (3, 4). Small, soluble, and diffusible aggregates composed of a mixture of full-length and N-terminal-truncated Aβ42 species appear early in the cerebral cortex of subjects at risk of Alzheimer disease pathology (5). The rate of accumulation as well as the properties of aggregation and toxicity of cerebral soluble Aβ depend on the ratio of the three major Aβ species, 1–42, pyroglutamate 3–42, and pyroglutamate 11–42 (6). We have shown that, in the cerebral cortex of FAD cases with mutations of PS1, the relative percentage of the two N-terminal-truncated Aβ species is significantly increased in comparison to sporadic AD cases (7). A relative increase of N-terminal-truncated Aβ peptides also occurs in the brain of transgenic mice bearing a double PS1 mutation (8). When overexpressed in cell lines, BACE1 increases the production of Aβ 11-x (9) and Aβ 3-x peptides (10) *in vitro*. BACE1 has been found to be transcriptionally regulated by several different mechanisms involving various transcription factors and pathways (11–15), and given the complexity and richness in transcription factor recognition sites of its gene promoter, it is likely to be a highly regulated protein (16). We and other research groups have shown that oxidative stress up-regulates the expression of BACE1 through the activity of the γ-secretase (14, 17). Moreover, we showed that the presence of presenilins is necessary for obtaining the increase of BACE1 expression under stress conditions (17, 18). On this basis, we predicted that mutant PS1 might augment the expression of BACE1 by altering the activity of the γ-secretase.

EXPERIMENTAL PROCEDURES

Cell Culture, Transfection, and Treatments—HEK-293 cells were cultured in high glucose Dulbecco's modified Eagle's medium, with 10% (v/v) fetal bovine serum, 100 units/ml pen-

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental "Experimental Procedures," Table 1, and Figs. 1 and 2.

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[2] The abbreviations used are: Aβ, β-amyloid peptide; AD, Alzheimer disease; FAD, familial AD; APP, β-amyloid precursor protein; PS1, presenilin-1; MEF, mouse embryonic fibroblast; AICD, APP intracellular domain; wt, wild type; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; siRNA, small interfering RNA; ANOVA, analysis of variance; CTF, C-terminal fragment.

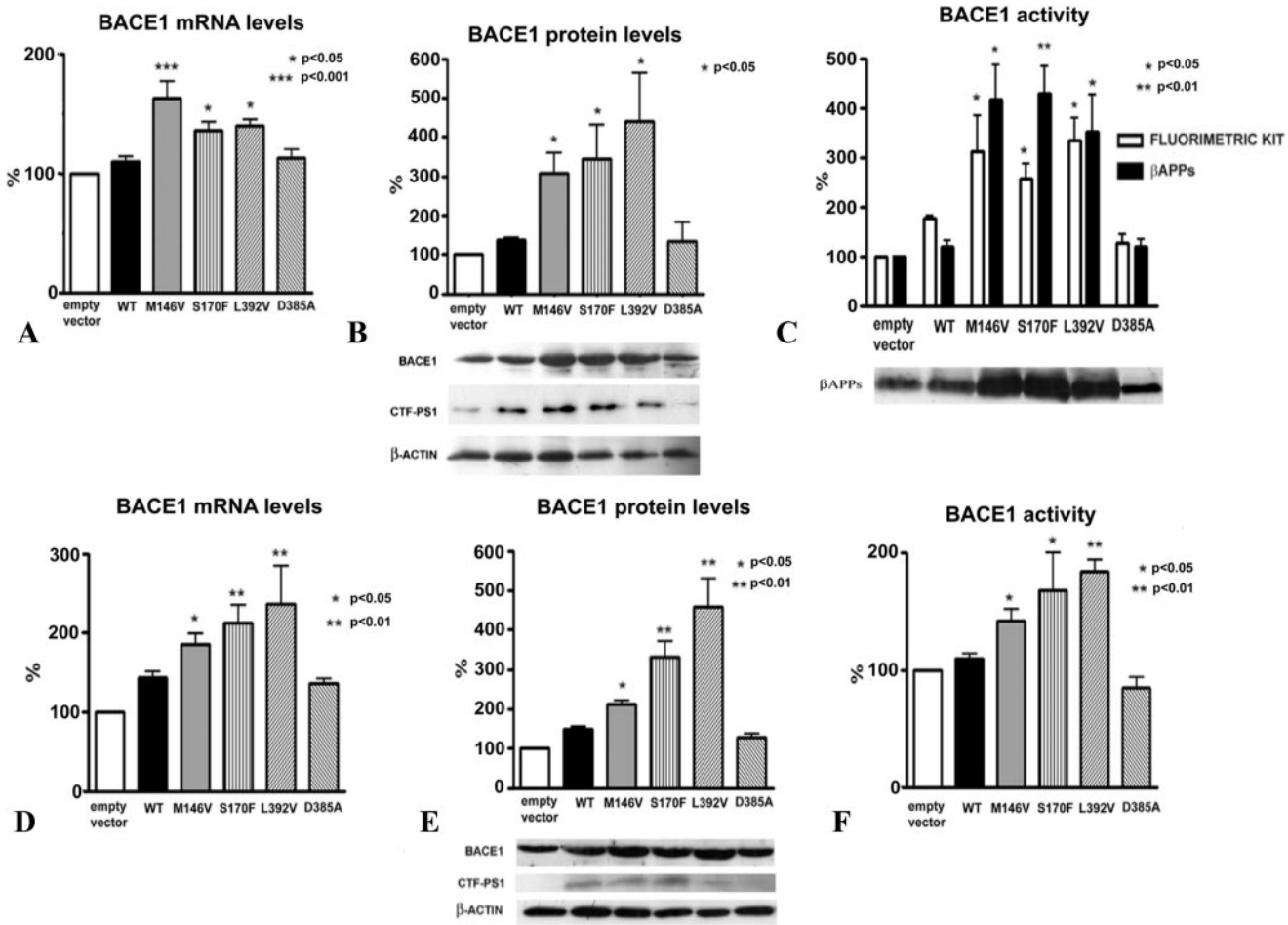


FIGURE 1. **PS1 mutations induce the transcription and activity of BACE1.** *A–C*, HEK-293 APPwt cells were transfected with empty vector, wild type PS1, or mutant PS1 constructs. BACE1 mRNA expression (*A*), BACE1 protein levels (*B*) and activity (*C*) were analyzed; also, indication of BACE1 activity is given by the analysis of βAPPs on cell culture media. *D–F*, MEFs deficient for PS1 were transfected with control, wild type, and mutant plasmids. Quantification of BACE1 mRNA (*D*), protein levels (*E*), and activity (*F*) was performed. Data represent the mean and S.E. of three or more experiments; significance is relative to empty vector control. Statistical analysis was performed with ANOVA and Bonferroni post-test.

icillin, 100 μg/ml streptomycin, 2 mM L-glutamine (Euroclone, Milano, Italy). HEK-293 APPwt stable cells (provided by Dr. Luciano D'Adamio, Albert Einstein College of Medicine, Bronx, NY) were cultured similarly, except for the addition of puromycin at 5 μg/ml. Mouse embryonic fibroblast (MEF) wt, $PS1^{-/-}$, and PS dko (provided by Dr. Bart De Strooper, Center for Human Genetics, VIB4 and KULeuven, Leuven, Belgium) (19), APP/APLP2 dko (provided by Dr. Ulrike Mueller, Max Planck Institute for Brain Research, Frankfurt, Germany) (20, 21), and Fe65 ko (provided by Dr. Tommaso Russo, CEINGE, Napoli, Italy) were cultured in low glucose Dulbecco's modified Eagle's medium with 10% (v/v) fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine (Euroclone, Milano, Italy). SH-SY5Y and SKNBE(2c) cells were cultured in RPMI 1640 supplemented with 10% (v/v) fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 1% nonessential amino acids, and 1% sodium pyruvate (all from Invitrogen). M17 cells were obtained from Dr. Xinglong Wang and Dr. Xiongwei Zhu at the Department of Pathology, Case Western Reserve University, Cleveland, Ohio 44106 and cultured in Opti-MEM with 10% fetal bovine serum and 1% penicillin/streptomycin and with 50 μg/ml G418. Transient transfection of empty vector (pcDNA3.1, Invitrogen), wild type, and mutant PS1 cDNA (M146V, S170F, L392V, and D385A) and BACE1 in HEK-293 and HEK-293 APPwt cells was carried out with polyethyleneimine (Sigma) at 12 μl/20 μg of DNA according to the manufacturer's instructions. Transient transfection of empty vector, wild type, and mutants PS1 cDNA (M146V, S170F, L392V, and D385A) in MEFs and SKNBE(2c) was carried out with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. In particular, to improve transfection efficiency in MEFs, cells were transfected in penicillin/streptomycin-free medium at 70% optical confluence. Efficiency of transfection was checked with the use of a green fluorescent protein coding plasmid. APP reconstitution in MEFs APP/APLP2 dko was performed by transfecting a human APP 695 construct together with pcDNA3.1, PS1 wt, or PS1 S170F using Lipofectamine 2000 as described above. For mRNA analysis, cells were collected 4 h after transfection; for protein analysis, cells were collected 12 h after transfection. The γ-secretase inhibitor XIX (Calbiochem) was added at 25 nM to the cells at the time of transfection and replenished every 4 h for 12 h. The BACE1 Inhibitor IV (22) was added to the cells overnight before trans-

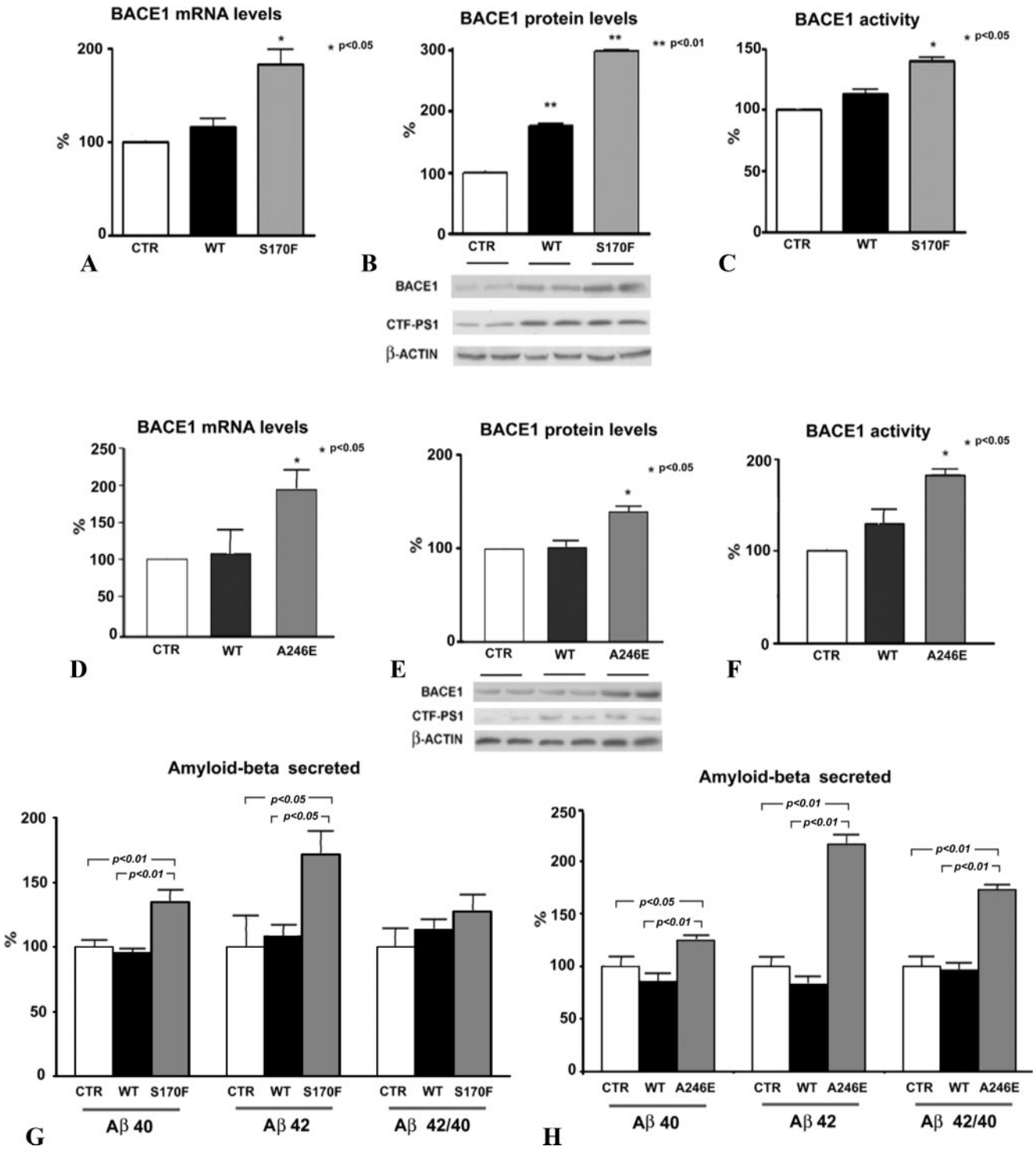


FIGURE 2. **PS1 mutations stably expressed in neuronal cell lines determine BACE1 up-regulation.** SH-SY5Y, stably transfected with pcDNA3.1, PS1 wt, and PS1 mutant S170F, were analyzed for BACE1 mRNA (*A*), BACE1 protein levels (*B*), and BACE1 activity (*C*). Similarly, M17 neuronal cells, stably expressing PS1 A246E mutant, PS1 wt, and pcDNA3.1, were analyzed: *D*, BACE1 mRNA; *E*, BACE1 protein levels; *F*, BACE1 activity. *G* and *H*, sandwich enzyme-linked immunosorbent assay for Aβ40 and -42, performed analyzing conditioned medium of SH-SY5Y stable cells (*G*) and M17 stable cells (*F*). The Aβ42/40 ratio is also presented. Data represent the mean and S.E. of three or more experiments, performed on two different stable cell clones; significance is relative to empty vector control (*CTR*); for enzyme-linked immunosorbent assay, *p* values are specified in the figure. Statistical analysis was performed with ANOVA and Bonferroni post-test.

fection and after transfection until cell harvest at 15 nM (Calbiochem).

Generation of SH-SY5Y PS1 S170F Stable Cell Lines—Cells were transfected with SspI linearized and purified pcDNA3.1, PS1 wt, and PS1 S170F constructs using Lipofectamine 2000 according to the manufacturer's protocol. G418 was used as mean of selection up to 800 μg/ml in culture medium until resistant clones could be identified. Clones were picked, trypsinized, and transferred to 96 wells in triplicate. DNA was then extracted by NaCl-ethanol precipitation. PCR was conducted with

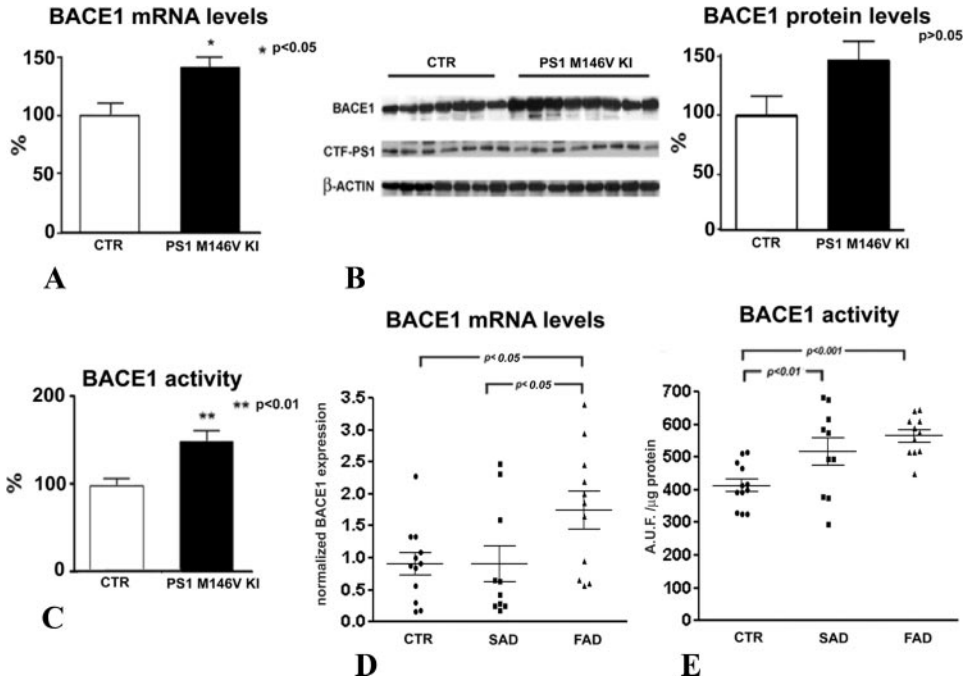


FIGURE 3. **BACE1 expression and activity are significantly increased in PS1 mutant knock-in mice and in PS1 mutant FAD cases.** *A* and *B*, M146V knock in (PS1 M146V KI) mice (4 males and 4 females) and age-matched littermates (4 males and 3 females) were analyzed. mRNA was extracted from one dissected brain hemisphere, whereas protein extraction was performed on the other hemisphere. *A*, BACE1 mRNA. *B*, BACE1 protein levels. *C*, an aliquot of brain protein lysates was also used to perform BACE1 activity assay. *D* and *E*, frozen brains from 11 FAD patients carrying 10 different mutations, 10 sporadic AD (*SAD*) patients, and 12 control (*CTR*) subjects were processed likewise. *D*, BACE1 mRNA levels; *E*, BACE1 activity. Statistical analysis was performed with *t* test (*A–C*) and ANOVA (*D–E*) and the Bonferroni post-test.

the following primers: PS1 277-S 5′-gcc acc ctg agc cat tat ct-3′ and Universal primer bovine growth hormone primer sequence as antisense primer. Positive clones were sequenced to ascertain the presence or not of the mutation using the primer PS1 291–313 S: 5′-ggc tac cat taa gtc agt cag c-3′. At least two different clones for each stable cell line were analyzed.

Treatment of Cultured Cells with Aβ Peptides—Treatments of SH-SY5Y and SKNBE(2c) with Aβ peptides (1–40, 1–42 scramble, and 1–42, Anaspec, San Jose, CA) were started 16–18 h after plating the cells in serum-free medium. Primary neuronal cultures were treated likewise at 14 or 21DIV. The lyophilized commercial peptides were dissolved as a stock solution in 1,1,1,3,3,3-hexafluoro-2-propanol (Sigma) and stored at −80 °C in aliquots. The desired amount was left under the cell culture hood to allow for 1,1,1,3,3,3-hexafluoro-2-propanol to evaporate and brought to 1 μM with sterile double-distilled water.

Evaluation of BACE1 activity, PS1, BACE1, and APP Protein Levels—BACE1 enzymatic activity was measured in frozen samples of human, mouse brain tissues, and in cultured cells using a fluorometric reaction kit (R&D Systems, Inc., Minneapolis, MN) according to the manufacturer's protocol. More details are in the supplemental "Experiment Procedures." For quantification of PS1, BACE1, and APP protein levels, 50 μg of total proteins (obtained by cell lysis R&D buffer, R&D Systems, according to the suggested protocol) were loaded on 9% Tris-glycine SDS-PAGE gels (unless otherwise specified) and transferred onto polyvinylidene difluoride membranes (Amersham Biosciences). Blots were probed with an anti-PS1 polyclonal antibody recognizing a band of about 20 kDa, corresponding to the C-terminal fragment of the protein (1:100; Cell Signaling Inc., Beverly, MA) with an anti-BACE1 polyclonal antibody (1:300; Calbiochem) and with a monoclonal antibody anti-APP (clone 22C11, 1:250 Chemicon International, Temecula, CA); the reactive bands were revealed with ECL plus (Amersham Biosciences). To normalize protein levels of PS1, APP, and of the mature form of BACE1 (75 kDa), membranes were stripped with Restore Western blot stripping reagent (Pierce) and probed with a monoclonal antibody against β-actin (1:6000; Sigma). Western blot determination of PS1 CTFs and Aβ peptides was performed by running protein samples on 10–18% Tris-Tricine gel and transferring the proteins on a 0.20 μm nitrocellulose membrane (Bio-Rad). The density of the specific bands was quantified using Quantity One software system (Bio-Rad).

RNA Interference—APP 695 RNA silencing was accomplished using the SMARTpool siRNA (Dharmacon, Lafayette, CO) consisting of four duplexes, all designed to target distinct sites within the APP gene. A non-targeting siRNA pool was used as a negative control. siRNAs (100 nM) were transfected in HEK-293 cells according to the manufacturer's instructions using DharmaFect One transfection reagent (Dharmacon); cells were harvested 48 h after siRNA transfection for mRNA analysis and after 72 h for protein analysis. To verify the inhibition rate after RNA, silencing levels of APP were evaluated by reverse transcription-PCR (forward, 5′-gtt ccg agg ggt aga gtt tg-3′; reverse 5′-cct ggg aca ttc tct ctc gg-3′) and by Western blot (monoclonal antibody 22C11; Chemicon International).

More details on plasmids, Aβ peptides, primary neuronal cultures, Aβ peptides aggregation and Western blot, transmission electron microscopy imaging, anti-Aβ antibody in-culture treatment of cells, PS1 M146V knock-in mice generation, mouse and human brain tissue, β-secretase assays, APP intracellular domain (AICD) semiquantitative PCR, quantitative real time PCR analysis, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, reactive oxygen species, and GSSG/GSH assays, AICD transgenic mice and statistical analysis can be found in the supplemental "Experiment Procedures."

RESULTS

Presenilin 1 Mutations Determine an Increase in BACE1 Activity, Based on Its Augmented Transcription—HEK-293 cells, stably overexpressing APP wild type, were transiently transfected (10, 23) with empty vector control (pcDNA3.1), PS1 wild type, and constructs coding for three PS1 mutations

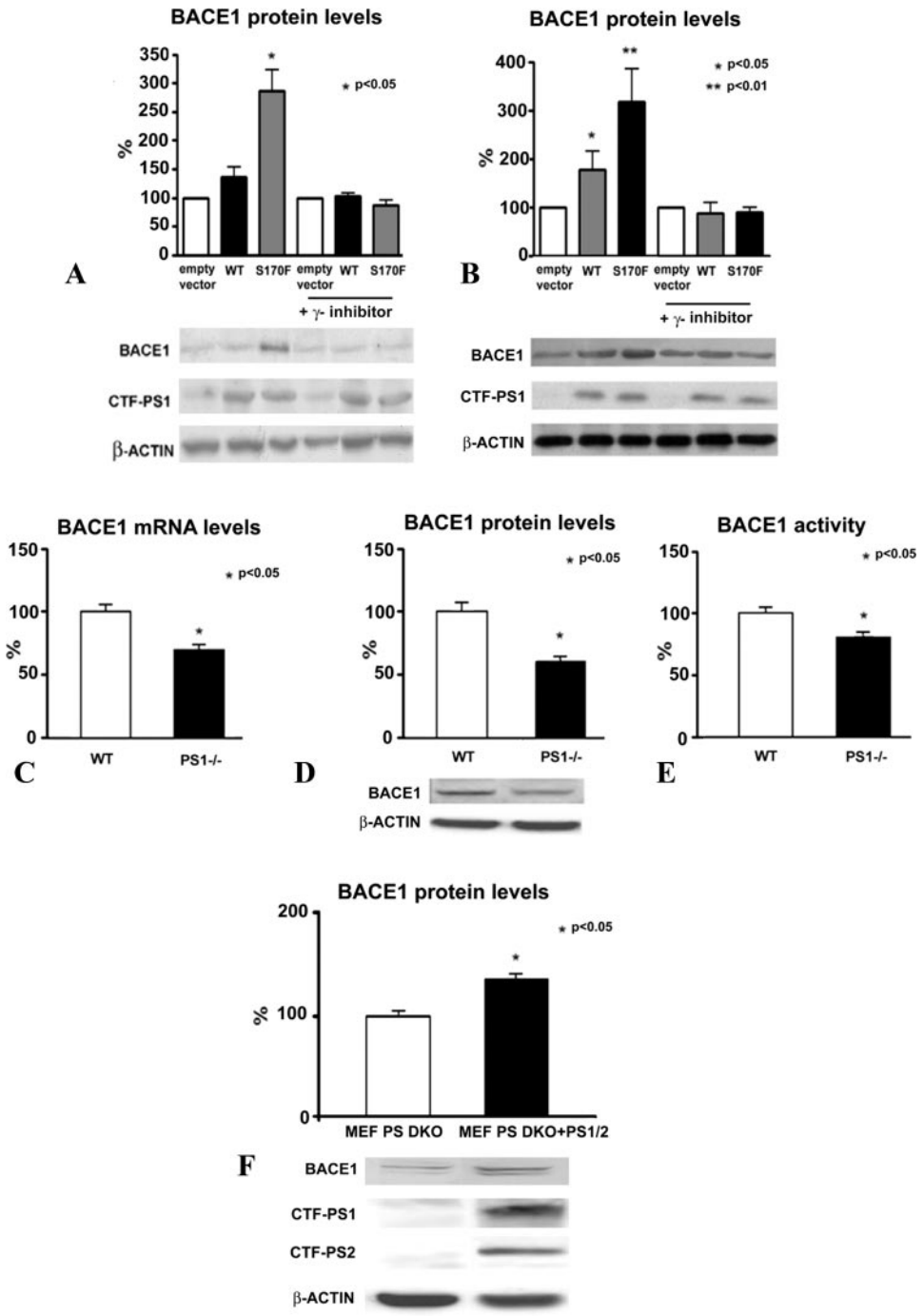


FIGURE 4. **The up-regulation of BACE1 induced by PS1 mutations requires the activity of the γ-secretase.** *A*, HEK-293 APPwt cells were treated with a transition analogue inhibitor of γ-secretase at the moment of transfection with empty vector control, PS1 wild type, and PS1 S170F and replenished twice every 4 h; BACE1 protein levels were analyzed. *B*, MEFs PS1$^{-/-}$ were treated similarly, and BACE1 protein levels were analyzed. Basal levels of BACE1 mRNA (*C*), protein levels (*D*), and activity (*E*) were evaluated in MEFs PS1$^{-/-}$, all resulting lower than in wild type cells. *F*, MEFs deficient for both PS1 and PS2 were transfected with empty vector control or with PS1 and PS2: reconstitution of PS1/2 expression determined an increased expression of BACE1; PS1/2 CTFs are produced as expected. Data represent the mean and S.E. of three or more experiments; significance is relative to the empty vector control or to control cells. Statistical analysis was performed with ANOVA (*A* and *B*) and Bonferroni post-test and *t* test (*C–F*).

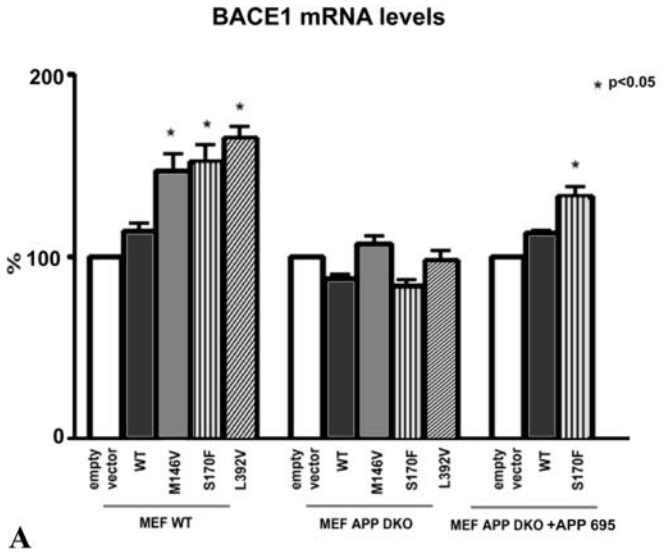
(M146V, S170F, L392V) linked to FAD (11, 24–27), known to increase the production of Aβ42, and with the loss of function mutation D385A (28). PS1 mutations determined a significant elevation of BACE1 mRNA (27–53%), protein levels (172–302%), and activity (81–160%) compared with wild type PS1 expressed at a similar level (Fig. 1, *A–C*). The increase of BACE1 activity was paralleled by an increase (230–300%) of the secreted APP derivative (βAPPs) resulting from the β-secretase cleavage (Fig. 1*C*), found in the culture medium. As expected, the PS1 D385A mutant did not determine any significant increase in BACE1 parameters.

We then asked whether the presence of endogenous wild type PS1 could affect the extent of BACE1 gene transcription. Mouse embryonic fibroblasts lacking PS1 (MEFs PS1$^{-/-}$) transfected with PS1 mutants showed an increase of BACE1 mRNA (42–93%) compared with cells transfected with wild type PS1, higher than that observed in HEK-293 APPwt cells, indicating that the up-regulation of BACE1 determined by the PS1 mutations was not compensated by the presence of the wild type PS1; also, they showed an increase of BACE1 protein levels (65–311%) and activity (32–73%) compared with cells transfected with wild type PS1 (Fig. 1, *D–F*). Again, the PS1 aspartate mutant did not have any influence on BACE1.

As shown in Fig. 1, *B* and *E*, the PS1 D385A CTF is barely visible, as this mutant undergoes little endoproteolysis (28). Full-length D385A PS1 was detected, ascertaining the efficiency of transfection for this mutant as well (not shown).

To verify the specificity of BACE1 antibody on Western blot, we transfected HEK-293 cells either with the empty vector pcDNA3.1 or with a plasmid coding for the full-length BACE1. Western blot analysis (supplemental Fig. 1*A*) showed the same pattern for transfected and endogenous BACE1. Also, to verify the specificity of the BACE1 activity assay, we pretreated HEK-293 APPwt cells with a BACE1 inhibitor (22), then transfected them with either wild type PS1 or the S170F mutant. As predicted, the inhibitor prevented the PS1-mediated increase of BACE1 activity observed in non-treated cells (supplemental Fig. 1*B*).

Several reports have recently shown how BACE1 levels can be controlled by post-translational and post-transcriptional mechanisms (11–15, 17, 18, 29–33). To further confirm the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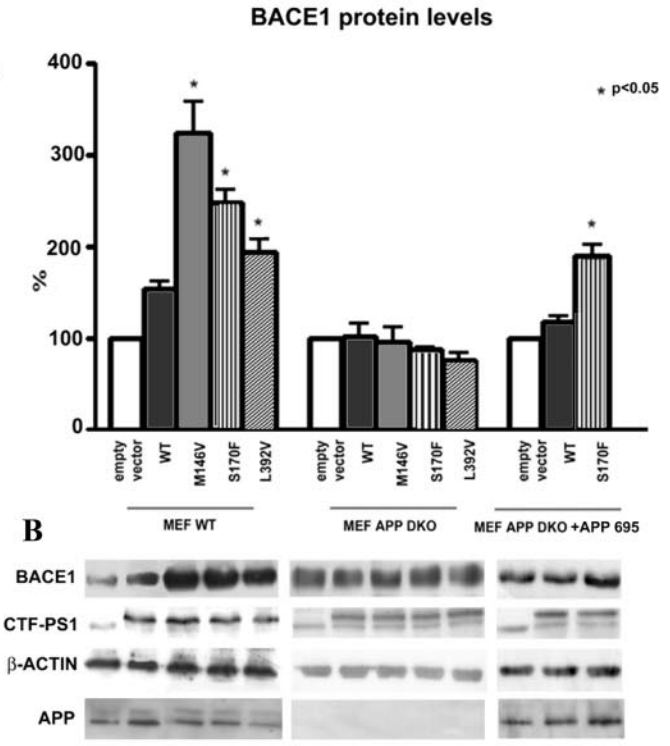
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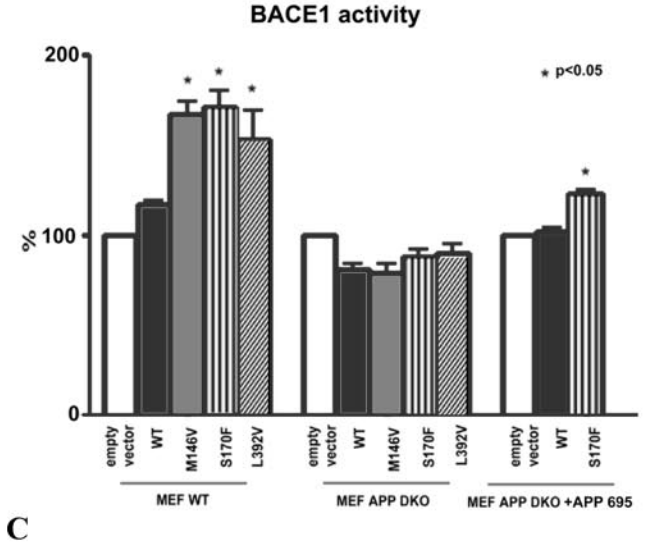
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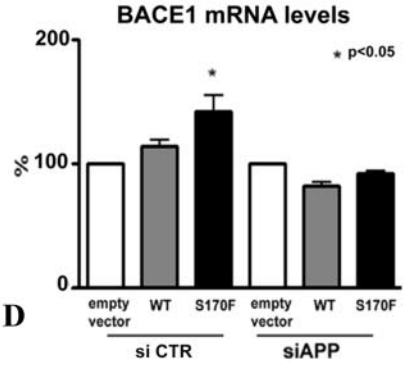
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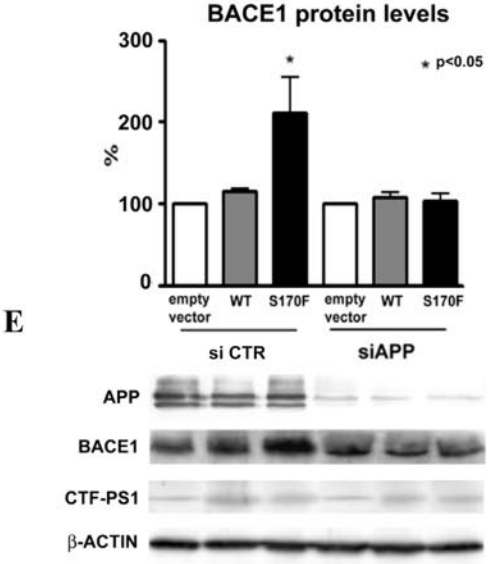
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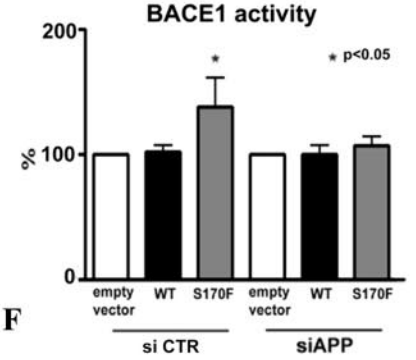
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effect of mutant PS1 on BACE1 expression, we generated a line of stable neuroblastoma cells (SHSY-5Y), expressing wild type PS1 and the S170F mutation (34). Also, we utilized a similar pre-established line of stable cells, M17, carrying wild type PS1 and the A246E mutation. PS1 A246E stable cells showed (Fig. 2*H*) a little increase of soluble secreted Aβ40 and a much higher increase of Aβ42 (+24 and 115%, respectively). PS1S170F and A246E mutations determined a significant increase in the levels of BACE1 mRNA (+82.74 and +94.14%), protein levels (+196 and +40.7%), and activity (+40 and + 81%) compared with control cells. (Fig. 2, *A–E*). As shown by Western blot analysis, SH and M17 stable clones show adequate levels and pattern of PS1 expression and maturation (Fig. 2, *B* and *E*). Of note, stable SH-SY5Y cells have levels of PS1 mRNA, measured by Taqman real time PCR, of 1.3–1.9× those of non-transfected cells; similarly, M17 show levels of 1.9–3.0× those of wild type M17 cells (data not shown). PS1 S170F stable cells showed (Fig. 2*G*) an increase of soluble secreted Aβ40 and -42 (+34 and +71%, respectively), as also reported before (35). Both cell lines showed an increase of the Aβ42/40 ratio (S170F, +27%; A246E, +73%), although this was less evident in the S170F mutant, where Aβ40 levels are higher.

Mutant PS1 Up-regulates BACE1 in Vivo—To investigate if the same effect observed *in vitro* is also present *in vivo*, we first evaluated BACE1 parameters in the brains of mutant PS1 knock-in mice (PS1 M146V KI) that express physiological levels of the human mutant protein. The levels of BACE1 mRNA and activity were significantly increased by 41 and 51% in the brain of mutant PS1 knock-in mice compared with the wild type littermates (Fig. 3, *A* and *C*), whereas the 47% elevation of BACE1 protein levels did not reach statistical significance (Fig. 3*B*). Up-regulation of BACE1 was also detected in brains of FAD subjects bearing various PS1 mutations (supplemental Table 1). Levels of BACE1 mRNA and activity were significantly increased on average by 80 and 36%, respectively, in cerebral cortices of 11 FAD cases bearing 10 different PS1 mutations compared with age-matched normal controls (Fig. 3, *D* and *E*). Interestingly, in sporadic AD, levels of BACE1 activity, and not mRNA, significantly differ (+24%) from controls, as previously reported (10, 36, 37). supplemental Fig. 1, *C* and *D*, show BACE1 mRNA and activity levels in each FAD case described, expressed as relative values of the mean of the control cases average.

The Up-regulation of BACE1 Requires the Presenilins and the Activity of the γ-Secretase—As we have recently shown (17, 18), PS1/2 and the activity of the γ-secretase are required to obtain the up-regulation of BACE1 induced by oxidative stress. Thus, we asked if the action of the PS1 mutants on BACE1 is also mediated by their involvement in the γ-secretase activity. In HEK-293 APPwt (Fig. 4*A*) and MEFs PS1$^{-/-}$ (Fig. 4*B*) cells, transiently transfected with PS1 mutant S170F, BACE1 protein levels were not altered in the presence of a transition state analogue inhibitor of the γ-secretase, suggesting that the up-regulation of BACE1 induced by the PS1 mutations depends on the activity of the γ-secretase. Indeed, in MEFs PS1$^{-/-}$ the basal levels of BACE1 mRNA, protein levels, and activity are lower than in wild type MEFs (−30, −38, and −20%, respectively) (Fig. 4, *C–E*). As shown before (Fig. 4*B*, empty vector *versus* PS1 wt in cells not treated with γ-inhibitor), reconstitution of PS1 in these cells reestablishes the basal values of BACE1 protein levels. Furthermore, in MEFs lacking both presenilins (MEFs PS dko), BACE1 protein levels increase upon reconstitution of PS1/2 by transient transfection (Fig. 4*F*), as shown by the detection of PS1/2 CTFs.

The Up-regulation of BACE1 Requires APP—Because PS1 mutations increase the expression of BACE1 by a mechanism that requires the γ-secretase activity, we asked whether this effect depends on the presence of APP, one major substrate of γ-secretase. MEFs deficient for APP and APP-like protein 2 (MEFs APP/APLP2 dko, or APP dko), also naturally lacking APLP1, were transfected with empty vector, wild type PS1, and PS1 mutants. They did not show any variation of BACE1 mRNA, protein levels, and activity compared with wild type MEFs (Fig. 5, *A–C*, *first* and *second set* of *columns*). When APP 695 was reconstituted into MEFs APP dko cells, the concomitant transfection of PS1 S170F mutant determined a significant increase of BACE1 parameters. (Fig. 5, *A–C*, *third set* of *columns*). The requirement of APP for the up-regulation of BACE1 was confirmed by another approach. Silencing of APP by RNA interference in HEK-293 cells, subsequently transfected with the PS1 S170F mutant, prevented the increase of BACE1 mRNA, protein levels, and activity (Fig. 5, *D–F*).

Aβ42 Is the APP Derivative That Mediates the Up-regulation of BACE1—The APP derivatives that result from the γ-secretase cleavage are the Aβ peptides and the AICD. We investigated which derivative is responsible for BACE1 up-regulation. We first analyzed the role of AICD, which is generated by the γ- or ϵ-cleavage of APP together with the Aβ peptides (38). We cloned several AICD fragments into a pcDNA3.1 vector; AICD 57 and 59 are the theoretical APP derivatives resulting form the γ-cleavage of APP and corresponding to Aβ42 and -40, respectively; AICD 50 and 51 are the ϵ-cleavage derivatives resulting from the processing of APP (39–41). Transfection of several cell lines (SKNBE-2c, SH-SY5Y, HEK-293, and MEFs; data from at least three experiments for each cell line, all showing similar outcomes, were pulled together) with constructs coding for AICD 50, 51, 57, and 59 determined no change of BACE1 mRNA compared with the empty vector control (Fig. 6*A*). To test for AICD expression, we performed a semiquantitative PCR, starting from the cDNA of the same cells transfected with AICD. The *lower panel* in *A* shows how AICD is amplified only in transfected cells, whereas the endogenous APP-derived

FIGURE 5. **The activation of BACE1 by mutant PS1 requires APP.** MEFs from APP/APLP2-deficient mice were transfected with empty vector control, wild type, and all the mutants considered in Fig. 1. Furthermore, MEFs APP DKO were transfected with human APP 695, reconstituting its expression, and with a representative PS1 mutant (S170F). Quantification of BACE1 mRNA (*A*), protein levels (*B*), and activity (*C*) were carried out as previously described. *D–F*, APP knock down (*E*, Western blot shown, performed with APP N-terminal-specific antibody) was performed on HEK-293 cells. Cells were transfected with non-targeting siRNA or *Homo sapien* APP-specific siRNA in 2 pulses, at times 0 and 24 h later; empty vector control, PS1 wild type, and PS1 S170F mutant were transfected subsequently, and quantification of BACE1 mRNA (*D*), protein levels (*E*), and activity (*F*) were performed. Data represent the mean and S.E. of three or more experiments; significance is relative to empty vector control. Statistical analysis was performed with ANOVA and the Bonferroni post-test. *CTR*, control.

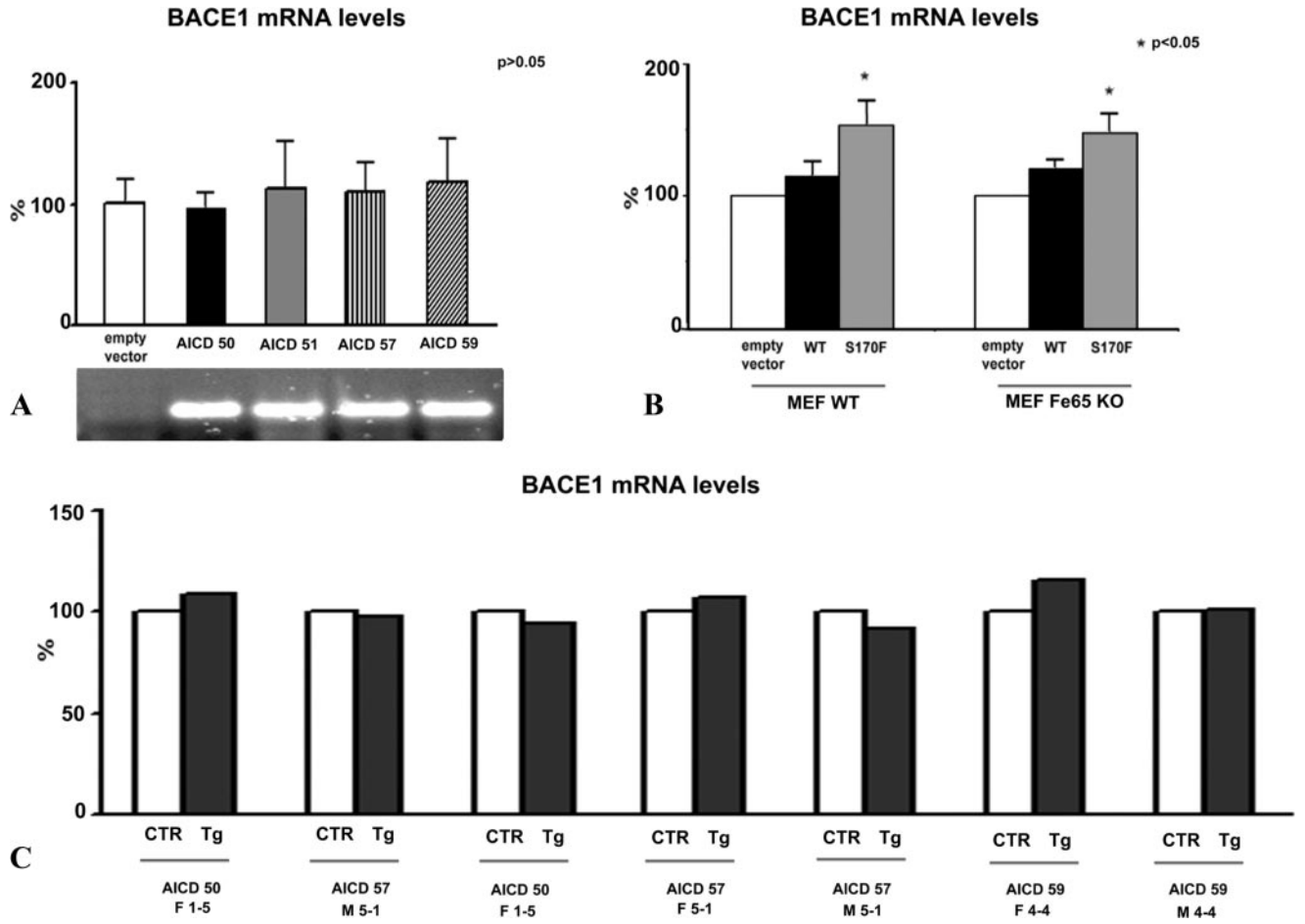

FIGURE 6. **APP intracellular derivatives do not play a role in BACE1 transcriptional control.** *A*, a series of neuronal and non neuronal cell lines (SKNBE(2c), SH-SY5Y, HEK-293, and MEFs) was transiently transfected with AICD derived from γ- or ϵ-secretase cleavage of APP. BACE1 transcription levels were determined. Data are a pool of several experimental repeats, performed at least in three repeats for each different cell line, which yielded analogous results. Transfected AICD transcript was detected by PCR conducted on the same cDNAs used for BACE1 real time PCR experiments, and a representative panel of semiquantitative PCR is shown. *B*, wild type and Fe65-deficient MEFs were transfected with empty vector, PS1 wt, or PS1 S170F. BACE1 transcription levels were determined. Data represent the mean and S.E. of three or more experiments; significance is relative to empty vector control. *C*, seven 4-week-old AICD transgenic mice (*tg*) and their littermates were analyzed for BACE1 mRAN expression. AICD length (50, 57, or 59 amino acids), mouse sex (*F* or *M*) and tg line (*1-5*, *5-1*, *4-4*) are indicated. Data from tg mice are compared with each corresponding littermate control (each control (*CTR*) = 100). Statistical analysis was performed with ANOVA and Bonferroni post-test.

amplicon appears as a faint band. More permissive PCR conditions allowed for the APP template to be amplified to more detectable levels (not shown). AICD is thought to act, whether in the nucleus or not, through the binding with chaperones Fe65 and Tip60 (42, 43). We tested whether MEFs lacking Fe65 showed different behavior upon transfection with PS1 mutant S170F compared with wild type cells. As shown in Fig. 6*B*, both cell lines yielded an increase in BACE1 mRNA levels upon transfection with the cited mutant, thus ruling out a role for the AICD-Fe65 complex in the control of BACE1 transcription. A further confirmation that AICD does not have a role in BACE1 transcription control resulted from the analysis of brain tissue of transgenic mice (tg) overexpressing AICD. Mice were generated on an FVB background, with the transgene placed under the control of calmodulin kinase II-α promoter (driving the expression in the forebrain) and have been recently described and characterized (44). Seven tg and 7 littermates carrying the AICD 50, 57, or 59 transgene were analyzed at 4 weeks of age for forebrain BACE1 mRNA expression; we did not detect any difference between the tg and littermate mice (Fig. 6*C*).

We, thus, concentrated our attention on Aβ peptides. It is thought that the most toxic Aβ species are low molecular weight oligomers, which form when soluble monomers are let in aqueous milieu at for a certain period of time (45). Before using Aβ on our cells, we checked the aggregation state of the used preparations. As shown in supplemental Fig. 2*A*, preparations of Aβ-(1–42) (similar results not shown for Aβ-(1–40) and -(1–42) scramble Aβ42s), at the concentration of 1 μM and for the times used on cultured cells, 1 and 6 h, do not show appreciable high molecular weight aggregates under transmission electron microscopy, contrary to the oligomeric and fibrillar preparations. Similar data are shown in supplemental Fig. 2*B*, where peptides, kept in culture conditions for 1 and 6 h at 1 μM, only show Aβ monomers and very low molecular aggregates (dimers-trimers) in Western blot analysis with 6E10 antibody. We treated primary neuronal cultures (supplemental Fig. 2*C*) at 21 days *in vitro* with 1 μM Aβ-(1–40), -(1–42), -(1–42) scramble or medium only for 1 h; only Aβ-(1–42) determined an increase in BACE1 mRNA production of +51% (Fig. 7*A*; similar data for 14 days *in vitro* neurons; not shown). Similarly,

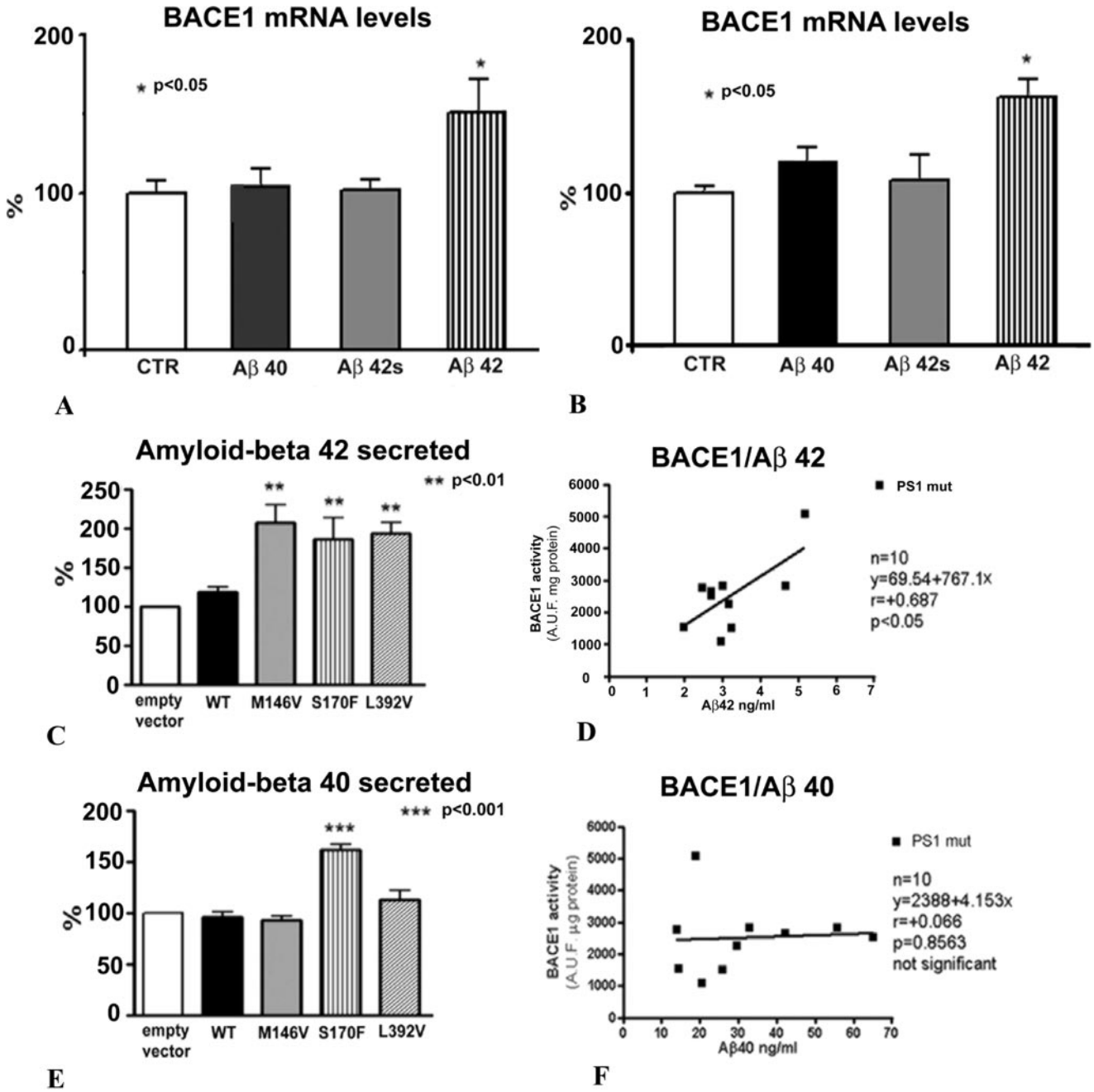

FIGURE 7. **Aβ-(1–42) peptide is the APP derivative responsible for the up-regulation of BACE1.** *A*, primary neurons, obtained from a 17.5 pregnant FVB mouse, and cultured for 21 days (similar data for 14 days *in vitro* neurons not shown) were treated for 1 h with freshly dissolved 1 μM Aβ peptides or with medium alone. BACE1 transcription levels were determined. Nine wells for each condition from three different neuronal cultures were analyzed as single experimental points. *B*, a series of neuronal cell lines (SKNBE(2c) and SH-SY5Y) were treated similarly at least 18 h after plating with 1 μM Aβ peptides or with medium alone for 1 h. mRNA was extracted as described, and TaqMan Real Time PCR for BACE1 was performed. Data are a pool of several experimental repeats, performed at least in three repeats for each different cell line, which yielded analogous results; they represent the mean and S.E. *C* and *E*, HEK-293 APPwt cells were transfected with empty vector, wild type PS1, and mutant PS1 constructs. Aβ species were quantified in conditioned media by enzyme-linked immunosorbent assay. *D* and *F*, BACE1 activity was also measured in these cells and correlated to the levels of Aβ40 and Aβ42. Statistical analysis was performed with ANOVA and the Bonferroni post-test and the study of linear correlation (*D* and *F*). *CTR*, control.

SH-SY5Y and SKNBE-2c cells (data from at least three experiments for each cell line, all showing similar outcome, were pulled together) were treated with the same peptides (Fig. 7*B*); again, only Aβ-(1–42) determined a significant increase (+62%) in BACE1 mRNA.

To rule out a possible toxic effect of Aβ, we have evaluated cell viability (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay) and oxidative stress (reactive oxygen species production, GSH depletion) in SH-SY5Y cells exposed to Aβ species under the same experimental conditions which determine the up-regulation of BACE1. We employed 20 μM H_2O_2 as positive controls for oxidative stress production and cell damage. Compared with Aβ free controls, at 1 h of incubation we detected only a 13% decrease of cell viability with Aβ42 and a 31% decrease with H_2O_2; Aβ42 determined a doubling of reactive oxygen species production but only 15% decrease of

GSH, as compared with a 4× increase in reactive oxygen species and 80% decrease of GSH induced by H_2O_2. Non-significant modifications from controls were obtained with Aβ40 and Aβ42s. These data argue against a role for toxicity and oxidative stress in Aβ42-induced BACE1 up-regulation. Further data confirmed these results. HEK-293 APPwt cells were transfected with the three PS1 mutants, and secreted Aβ was measured by enzyme-linked immunosorbent assay. PS1 mutants showed altogether an increased secretion of Aβ42, which significantly correlated with BACE1 activity (Fig. 7, *C* and *D*). Of note, the levels of Aβ40 did not correlate with those of BACE1 activity (Fig. 7, *E* and *F*). As PS1 mutations determine an increase of Aβ-(1–42) secreted in the extracellular medium (Fig. 7*C*), it is likely that secreted Aβ is responsible for the effect on BACE1. We, thus, predicted that an antibody directed toward Aβ42 could sequester the peptide so as to inhibit its signaling/pathologic action, as already suggested (46). Indeed, HEK-293 APPwt transfected with PS1 S170F mutant did not show an increase of BACE1 mRNA upon incubation with an antibody specific for Aβ42, whereas neither anti-Aβ40 nor anti β-actin antibodies showed this effect (supplemental Fig. 2*D*).

DISCUSSION

The known effect of PS1 mutations is the alteration of the γ-secretase cleavage on APP, leading to the relative increased production of Aβ42 species (3, 4). We showed that PS1 mutations also augment the expression of BACE1 and that this effect is dependent on the presence of APP and is proportional to the amount of Aβ42 produced and secreted in the extracellular milieu. This novel effect of PS1 mutations implies the existence of a positive feedback loop from the γ- to the β-secretase cleavages of APP in which Aβ42 is the APP derivative that acts on BACE1 transcription, as previously hinted under stress conditions (14, 17).

The activity of BACE1 is increased in familial early onset (the present study) as well as in sporadic late-onset AD (32, 36, 37, 47). In the latter conditions, various factors such as oxidative stress (10, 48, 49), hypoxia (50, 51), and ischemia (52) have been proposed as causes of BACE1 up-regulation, and different underlying mechanisms have been described, including the activation of hypoxia-inducible factor 1 (53), the decrease of GGA3 activity (30), and the loss of micro RNA specific clusters (32).

BACE1 gene promoter has a complex structure, divided in two distinct promoter regions, carrying several transcription factor binding sites, such as for SP1, AP1, AP2, CREB (cAMP-response element-binding protein), glucocorticoid receptor, Zeste, NFκB, and GC boxes and cAMP response element-like sequence sites (16), many of which organized in repeats, typical of an inducible protein. Different signaling pathways, such as JNK/AP1 (18), NFκB (14) p25/cdk5/STAT3 (13) have been suggested to control BACE1 transcription.

Additional non-transcriptional mechanisms have been hypothesized to account for increased BACE1 protein levels and activity in sporadic AD: maturation of BACE1 may be controlled by PS1 itself (33); expression of a BACE1 antisense transcripts (54), which respond to cellular stresses and Aβ itself, seem to stabilize BACE1 mRNA, thus increasing its protein expression; alternative splicing of BACE1 pre-mRNA has been found to be a control system as well (31); under ischemia, stabilization of BACE1 protein levels, because of defective degradation, is obtained by depletion of GGA3 (30). In all these cases BACE1 mRNA production is not necessarily augmented.

We show that the primary mechanism by which PS1 mutants act is through an increase of BACE1 transcript. The main actor seems to be Aβ42, but the signaling pathway, starting form the action of secreted Aβ42 down to nuclear effectors, has still to be elucidated. We have previously shown that a potential pathway is a c-Jun NH_2-terminal kinase (JNK)/AP1 cascade, at least in stressful conditions (18). Hypothetically, soluble oligomers of Aβ42 may act through the binding with a cellular receptor, located either on the plasma membrane or at intracellular sites (55–57), activating a BACE1-inducing signaling, or more in general, membrane damage generated by oligomers (58, 59) may trigger a stress cellular response leading to enhanced BACE1 expression.

FAD PS1 mutations result in a wide and heterogeneous clinical phenotype that includes atypical presentations, such as ataxia, paraparesis, and epilepsy (35, 60, 61). The up-regulation of BACE1 determined by PS1 mutations may significantly contribute to determine the phenotype of FAD. Indeed, the overexpression of BACE1 increases the production of N-terminal-truncated Aβ species (9). We and others (62) have shown that the composition of soluble Aβ reflects the pathological and clinical phenotype of Aβ amyloidosis and that the prevalence of N-terminal-truncated Aβ42 peptides is correlated with the rate of aggregation and with the degree of toxicity of the mixture of Aβ species (6). Moreover, recent data obtained with different animal models of Aβ amyloidosis support the hypothesis that the composition of Aβ species, indicated as "Aβ strains" (63–65), dictate the conformation of Aβ-soluble aggregates, which in turn produces different pathological phenotypes.

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REFERENCES

1. Haass, C., Schlossmacher, M. G., Hung, A. Y., Vigo-Pelfrey, C., Mellon, A., Ostaszewski, B. L., Lieberburg, I., Koo, E. H., Schenk, D., Teplow, D. B., and Selkoe, D. J. (1992) *Nature* **359,** 322–325
2. Wolfe, M. S., Xia, W., Ostaszewski, B. L., Diehl, T. S., Kimberly, W. T., and Selkoe, D. J. (1999) *Nature* **398,** 513–517

3. Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T. D., Hardy, J., Hutton, M., Kukull, W., Larson, E., Levy-Lahad, E., Viitanen, M., Peskind, E., Poorkaj, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D., and Younkin, S. (1996) *Nat. Med.* **2,** 864–870
4. Xia, W., Zhang, J., Kholodenko, D., Citron, M., Podlisny, M. B., Teplow, D. B., Haass, C., Seubert, P., Koo, E. H., and Selkoe, D. J. (1997) *J. Biol. Chem.* **272,** 7977–7982
5. Teller, J. K., Russo, C., DeBusk, L. M., Angelini, G., Zaccheo, D., Dagna-Bricarelli, F., Scartezzini, P., Bertolini, S., Mann, D. M., Tabaton, M., and Gambetti, P. (1996) *Nat. Med.* **2,** 93–95
6. Piccini, A., Russo, C., Gliozzi, A., Relini, A., Vitali, A., Borghi, R., Giliberto, L., Armirotti, A., D'Arrigo, C., Bachi, A., Cattaneo, A., Canale, C., Torrassa, S., Saido, T. C., Markesbery, W., Gambetti, P., and Tabaton, M. (2005) *J. Biol. Chem.* **280,** 34186–34192
7. Russo, C., Schettini, G., Saido, T. C., Hulette, C., Lippa, C., Lannfelt, L., Ghetti, B., Gambetti, P., Tabaton, M., and Teller, J. K. (2000) *Nature* **405,** 531–532
8. Casas, C., Sergeant, N., Itier, J. M., Blanchard, V., Wirths, O., van der Kolk, N., Vingtdeux, V., van de Steeg, E., Ret, G., Canton, T., Drobecq, H., Clark, A., Bonici, B., Delacourte, A., Benavides, J., Schmitz, C., Tremp, G., Bayer, T. A., Benoit, P., and Pradier, L. (2004) *Am. J. Pathol.* **165,** 1289–1300
9. Liu, K., Doms, R. W., and Lee, V. M. (2002) *Biochemistry* **41,** 3128–3136
10. Borghi, R., Patriarca, S., Traverso, N., Piccini, A., Storace, D., Garuti, A., Cirmena, G., Odetti, P., and Tabaton, M. (2007) *Neurobiol. Aging* **28,** 1009–1014
11. Rossner, S., Sastre, M., Bourne, K., and Lichtenthaler, S. F. (2006) *Prog. Neurobiol.* **79,** 95–111
12. Christensen, M. A., Zhou, W., Qing, H., Lehman, A., Philipsen, S., and Song, W. (2004) *Mol. Cell. Biol.* **24,** 865–874
13. Wen, Y., Yu, W. H., Maloney, B., Bailey, J., Ma, J., Marie, I., Maurin, T., Wang, L., Figueroa, H., Herman, M., Krishnamurthy, P., Liu, L., Planel, E., Lau, L. F., Lahiri, D. K., and Duff, K. (2008) *Neuron* **57,** 680–690
14. Buggia-Prevot, V., Sevalle, J., Rossner, S., and Checler, F. (2008) *J. Biol. Chem.* **283,** 10037–10047
15. Cho, H. J., Jin, S. M., Youn, H. D., Huh, K., and Mook-Jung, I. (2008) *Aging Cell* **7,** 137–147
16. Sambamurti, K., Kinsey, R., Maloney, B., Ge, Y. W., and Lahiri, D. K. (2004) *FASEB J.* **18,** 1034–1036
17. Tamagno, E., Guglielmotto, M., Aragno, M., Borghi, R., Autelli, R., Giliberto, L., Muraca, G., Danni, O., Zhu, X., Smith, M. A., Perry, G., Jo, D. G., Mattson, M. P., and Tabaton, M. (2008) *J. Neurochem.* **104,** 683–695
18. Tamagno, E., Guglielmotto, M., Giliberto, L., Vitali, A., Borghi, R., Autelli, R., Danni, O., and Tabaton, M. (2008) *Neurobiol. Aging*, in press
19. De Strooper, B., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J. S., Schroeter, E. H., Schrijvers, V., Wolfe, M. S., Ray, W. J., Goate, A., and Kopan, R. (1999) *Nature* **398,** 518–522
20. Herreman, A., Van Gassen, G., Bentahir, M., Nyabi, O., Craessaerts, K., Mueller, U., Annaert, W., and De Strooper, B. (2003) *J. Cell Sci.* **116,** 1127–1136
21. Herreman, A., Hartmann, D., Annaert, W., Saftig, P., Craessaerts, K., Serneels, L., Umans, L., Schrijvers, V., Checler, F., Vanderstichele, H., Baekelandt, V., Dressel, R., Cupers, P., Huylebroeck, D., Zwijsen, A., Van Leuven, F., and De Strooper, B. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 11872–11877
22. Stachel, S. J., Coburn, C. A., Steele, T. G., Jones, K. G., Loutzenhiser, E. F., Gregro, A. R., Rajapakse, H. A., Lai, M. T., Crouthamel, M. C., Xu, M., Tugusheva, K., Lineberger, J. E., Pietrak, B. L., Espeseth, A. S., Shi, X. P., Chen-Dodson, E., Holloway, M. K., Munshi, S., Simon, A. J., Kuo, L., and Vacca, J. P. (2004) *J. Med. Chem.* **47,** 6447–6450
23. Pardossi-Piquard, R., Petit, A., Kawarai, T., Sunyach, C., Alves da Costa, C., Vincent, B., Ring, S., D'Adamio, L., Shen, J., Muller, U., St George Hyslop, P., and Checler, F. (2005) *Neuron* **46,** 541–554
24. Clark, R. F., Cruts, M., Korenblat, K. M., He, C., Talbot, C., Van Broeckhoven, C., and Goate, A. M. (1995) *Hum. Mol. Genet.* **4,** 1347–1354
25. Snider, B. J., Norton, J., Coats, M. A., Chakraverty, S., Hou, C. E., Jervis, R., Lendon, C. L., Goate, A. M., McKeel, D. W., Jr., and Morris, J. C. (2005) *Arch. Neurol.* **62,** 1821–1830
26. Campion, D., Flaman, J. M., Brice, A., Hannequin, D., Dubois, B., Martin, C., Moreau, V., Charbonnier, F., Didierjean, O., Tardieu, S., Penet, C., Puel, M., Pasquier, F., Le Doze, F., Bellis, G., Calenda, A., Heilig, R., Martinez, M., Mallet, J., Bellis, M., Clerget-Darpoux, F., Agid, Y., and Frebourg, T. (1995) *Hum. Mol. Genet* **4,** 2373–2377
27. Moehlmann, T., Winkler, E., Xia, X., Edbauer, D., Murrell, J., Capell, A., Kaether, C., Zheng, H., Ghetti, B., Haass, C., and Steiner, H. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 8025–8030
28. Kim, H., Ki, H., Park, H. S., and Kim, K. (2005) *J. Biol. Chem.* **280,** 22462–22472
29. Gong, B., Cao, Z., Zheng, P., Vitolo, O. V., Liu, S., Staniszewski, A., Moolman, D., Zhang, H., Shelanski, M., and Arancio, O. (2006) *Cell* **126,** 775–788
30. Tesco, G., Koh, Y. H., Kang, E. L., Cameron, A. N., Das, S., Sena-Esteves, M., Hiltunen, M., Yang, S. H., Zhong, Z., Shen, Y., Simpkins, J. W., and Tanzi, R. E. (2007) *Neuron* **54,** 721–737
31. Mowrer, K. R., and Wolfe, M. S. (2008) *J. Biol. Chem.* **283,** 18694–18701
32. Hebert, S. S., Horre, K., Nicolai, L., Papadopoulou, A. S., Mandemakers, W., Silahtaroglu, A. N., Kauppinen, S., Delacourte, A., and De Strooper, B. (2008) *Proc. Natl. Acad. Sci. U. S. A.* **105,** 6415–6420
33. Kuzuya, A., Uemura, K., Kitagawa, N., Aoyagi, N., Kihara, T., Ninomiya, H., Ishiura, S., Takahashi, R., and Shimohama, S. (2007) *J. Neurosci. Res.* **85,** 153–165
34. Zhang, J., Kang, D. E., Xia, W., Okochi, M., Mori, H., Selkoe, D. J., and Koo, E. H. (1998) *J. Biol. Chem.* **273,** 12436–12442
35. Piccini, A., Zanusso, G., Borghi, R., Noviello, C., Monaco, S., Russo, R., Damonte, G., Armirotti, A., Gelati, M., Giordano, R., Zambenedetti, P., Russo, C., Ghetti, B., and Tabaton, M. (2007) *Arch. Neurol.* **64,** 738–745
36. Fukumoto, H., Cheung, B. S., Hyman, B. T., and Irizarry, M. C. (2002) *Arch. Neurol.* **59,** 1381–1389
37. Zhao, J., Fu, Y., Yasvoina, M., Shao, P., Hitt, B., O'Connor, T., Logan, S., Maus, E., Citron, M., Berry, R., Binder, L., and Vassar, R. (2007) *J. Neurosci.* **27,** 3639–3649
38. Kakuda, N., Funamoto, S., Yagishita, S., Takami, M., Osawa, S., Dohmae, N., and Ihara, Y. (2006) *J. Biol. Chem.* **281,** 14776–14786
39. Passer, B., Pellegrini, L., Russo, C., Siegel, R. M., Lenardo, M. J., Schettini, G., Bachmann, M., Tabaton, M., and D'Adamio, L. (2000) *J. Alzheimers Dis.* **2,** 289–301
40. Tagami, S., Okochi, M., Fukumori, A., Jiang, J., Yanagida, K., Nakayama, T., Morihara, T., Tanaka, T., Kudo, T., and Takeda, M. (2008) *Neurodegener. Dis.* **5,** 160–162
41. Fukumori, A., Okochi, M., Tagami, S., Jiang, J., Itoh, N., Nakayama, T., Yanagida, K., Ishizuka-Katsura, Y., Morihara, T., Kamino, K., Tanaka, T., Kudo, T., Tanii, H., Ikuta, A., Haass, C., and Takeda, M. (2006) *Biochemistry* **45,** 4907–4914
42. Kimberly, W. T., Zheng, J. B., Guenette, S. Y., and Selkoe, D. J. (2001) *J. Biol. Chem.* **276,** 40288–40292
43. Telese, F., Bruni, P., Donizetti, A., Gianni, D., D'Ambrosio, C., Scaloni, A., Zambrano, N., Rosenfeld, M. G., and Russo, T. (2005) *EMBO Rep.* **6,** 77–82
44. Giliberto, L., Zhou, D., Weldon, R., Tamagno, E., De Luca, P., Tabaton, M., and D'Adamio, L. (2008) *Mol. Neurodegener.* **3,** 12
45. Walsh, D. M., Hartley, D. M., Kusumoto, Y., Fezoui, Y., Condron, M. M., Lomakin, A., Benedek, G. B., Selkoe, D. J., and Teplow, D. B. (1999) *J. Biol. Chem.* **274,** 25945–25952
46. Tampellini, D., Magrane, J., Takahashi, R. H., Li, F., Lin, M. T., Almeida, C. G., and Gouras, G. K. (2007) *J. Biol. Chem.* **282,** 18895–18906
47. 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
48. Tamagno, E., Bardini, P., Guglielmotto, M., Danni, O., and Tabaton, M. (2006) *Free Radic. Biol. Med.* **41,** 202–212
49. Lee, H. G., Perry, G., Zhu, X., Nunomura, A., and Smith, M. A. (2006) *Free Radic. Biol. Med.* **41,** 188–189
50. Xue, S., Jia, L., and Jia, J. (2006) *Neurosci. Lett.* **405,** 231–235
51. Sun, X., He, G., Qing, H., Zhou, W., Dobie, F., Cai, F., Staufenbiel, M., Huang, L. E., and Song, W. (2006) *Proc. Natl. Acad. Sci. U. S. A.* **103,** 18727–18732
52. Lee, P. H., Hwang, E. M., Hong, H. S., Boo, J. H., Mook-Jung, I., and Huh, K. (2006) *Neurochem. Res.* **31,** 821–827

53. 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
54. Faghihi, M. A., Modarresi, F., Khalil, A. M., Wood, D. E., Sahagan, B. G., Morgan, T. E., Finch, C. E., St Laurent, G., III, Kenny, P. J., and Wahlestedt, C. (2008) *Nat. Med.* **14,** 723–730
55. Shaked, G. M., Kummer, M. P., Lu, D. C., Galvan, V., Bredesen, D. E., and Koo, E. H. (2006) *FASEB J.* **20,** 1254–1256
56. Du Yan, S., Zhu, H., Fu, J., Yan, S. F., Roher, A., Tourtellotte, W. W., Rajavashisth, T., Chen, X., Godman, G. C., Stern, D., and Schmidt, A. M. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 5296–5301
57. Kuner, P., Schubenel, R., and Hertel, C. (1998) *J. Neurosci. Res.* **54,** 798–804
58. Kagan, B. L., Hirakura, Y., Azimov, R., Azimova, R., and Lin, M. C. (2002) *Peptides* **23,** 1311–1315
59. Murray, I. V., Liu, L., Komatsu, H., Uryu, K., Xiao, G., Lawson, J. A., and Axelsen, P. H. (2007) *J. Biol. Chem.* **282,** 9335–9345
60. Assini, A., Terreni, L., Borghi, R., Giliberto, L., Piccini, A., Loqui, D., Fogliarino, S., Forloni, G., and Tabaton, M. (2003) *Neurology* **60,** 150–151
61. Takao, M., Ghetti, B., Murrell, J. R., Unverzagt, F. W., Giaccone, G., Tagliavini, F., Bugiani, O., Piccardo, P., Hulette, C. M., Crain, B. J., Farlow, M. R., and Heyman, A. (2001) *J. Neuropathol. Exp. Neurol.* **60,** 1137–1152
62. Schilling, S., Lauber, T., Schaupp, M., Manhart, S., Scheel, E., Bohm, G., and Demuth, H. U. (2006) *Biochemistry* **45,** 12393–12399
63. Meyer-Luehmann, M., Coomaraswamy, J., Bolmont, T., Kaeser, S., Schaefer, C., Kilger, E., Neuenschwander, A., Abramowski, D., Frey, P., Jaton, A. L., Vigouret, J. M., Paganetti, P., Walsh, D. M., Mathews, P. M., Ghiso, J., Staufenbiel, M., Walker, L. C., and Jucker, M. (2006) *Science* **313,** 1781–1784
64. Tabaton, M., and Gambetti, P. (2006) *J. Alzheimers Dis.* **9,** 127–132
65. Petkova, A. T., Leapman, R. D., Guo, Z., Yau, W. M., Mattson, M. P., and Tycko, R. (2005) *Science* **307,** 262–265