Molecular Medicine 6(7): 570–580, 2000

Molecular Medicine


## Original Articles

# Evidence that the β-catenin Nuclear Translocation Assay Allows for Measuring Presenilin 1 Dysfunction

**Geert Van Gassen,[1] Chris De Jonghe,[1] Masaki Nishimura,[2] Gang Yu,[2] Sofie Kuhn,[1] Peter St. George-Hyslop,[2] and Christine Van Broeckhoven[1]**

[1]Molecular Genetics Laboratory, Neurogenetics Group, Flanders Interuniversity Institute for Biotechnology (VIB), Born-Bunge Foundation (BBS), University of Antwerp (UIA), Department of Biochemistry, Antwerpen, Belgium
[2]Centre for Research in Neurodegenerative Diseases, Department of Medicine, University of Toronto and Department of Medicine (Division of Neurology), University Health Network, Toronto, Canada

Accepted May 18, 2000

### Abstract

**Background:** Mutations in the presenilin (PSEN) genes are responsible for the majority of early-onset Alzheimer disease (AD) cases. PSEN1 is a component of a high molecular weight, endoplasmic reticulum, membrane-bound protein complex, including β-catenin. Pathogenic PSEN1 mutations were demonstrated to have an effect on β-catenin and glycogen synthase kinase-3β(GSK-3β), two members of the wingless Wnt pathway. The nuclear translocation and the stability of β-catenin, and the interaction between GSK3β and PSEN1 were influenced.
**Materials and Methods:** Stably transfected human embryonic kidney (HEK) 293 cells overexpressing wild-type (wt) and mutant (mt) PSEN1, treated with and without LiCl, were used to isolate cytoplasmic and nuclear fractions. By Western blot analysis, endogenous β-catenin levels were examined. By analyzing cytosolic fractions of PSEN1, transfected and nontransfected HEK 293 cells, and total brain extracts of AD patients and controls, we evaluated the effect of PSEN1 overexpression on β-catenin stability. Finally, we analyzed the effect of pathogenic PSEN1 mutations on the interaction between PSEN1 and GSK3β by co-immunoprecipitation experiments.
**Results:** We report reduced nuclear translocation of β-catenin in cells stably expressing I143T, G384A, and T113-114ins PSEN1. The G384A PSEN1 mutation showed a similar pronounced effect on nuclear translocation of β-catenin, as reported for processing of amyloid precursor protein (APP) into amyloid β(Aβ). Overexpression of PSEN1 and the presence of pathogenic mutations in PSEN1 had no significant effect on the stability of β-catenin. Nonspecific binding of overexpressed PSEN1 to endogenous GSK3β was observed when GSK3β was immunoprecipitated. Immunoprecipitation of PSEN1 in cells overexpressing PSEN1 and in native cells, however, did not result in co-immunoprecipitation of endogenous GSK3β.
**Conclusion:** Our results further establish the nuclear translocation assay of β-catenin as an adequate alternative for traditional Aβ measurement to evaluate the effect of PSEN1 mutations on biochemical processes. We detected no significant effect of overexpressed wt or mt PSEN1 on the stabilty of β-catenin. Finally, co-immunoprecipitation between PSEN1 and GSK3β was not observed in our experimental setup.

Address for correspondence and reprint requests to: Christine Van Broeckhoven, Ph.D., Dr.Sc., Laboratory of Molecular Genetics, University of Antwerp (U.I.A.), Department of Biochemistry, Universiteitsplein 1, B-2610 Antwerpen, Belgium. Phone: +32 820.26.01; Fax: +32 820.25.41; E-mail: cvbroeck@uia.ua.ac.be

## Introduction

Mutations in the presenilin (PSEN) genes are responsible for the majority of early-onset Alzheimer disease (AD) cases (1). PSEN1 is a

serpentine-like integral protein of mainly the endoplasmic reticulum and early-Golgi membranes (2–4), but it is also observed in the nuclear envelope (4,5). PSEN1 is constitutively cleaved by an unknown protease, termed presenilinase, resulting in the generation of an N-terminal fragment (NTF) of ~30 kDa and a C-terminal fragment (CTF) of ~20 kDa (6,7). PSEN1 NTF and CTF bind to form a stable 250 kDa complex (8–10). The fragments in the protein complex are probably the functional state of PSEN1, since they are resistant to degradation; whereas, the holoprotein is degraded rapidly by the proteasome (11,12). PSEN1 is involved in the processing of amyloid precursor protein (APP) and NOTCH-1 (13–16). Whether this involvement is direct, by a physical interaction and proteolytic activity of PSEN1, or indirect, by transporting APP and NOTCH-1 to a specific cellular compartment where they are processed, is not clear yet.

PSEN1 is also postulated to play a role in the wingless (Wnt) pathway. Pathogenic PSEN1 mutations reduce the nuclear translocation of β-catenin after stimulation of the Wnt/β-catenin signal transduction pathway in human fibroblasts and human embryonic kidney (HEK) 293 cells (17). The Wnt pathway signals inductive events during development, such as segmentation, central nervous system patterning, and control of asymmetric cell division (18). Recently, Wnts secreted by postsynaptic granule cells also were found to be involved in axon and growth cone remodeling of presynaptic mossy fibers (19). Binding of Wnt ligands to their receptors initiates a cytoplasmic signal, resulting downstream in the inactivation of GSK-3β and the translocation of β-catenin to the nucleus. Binding of β-catenin to Tcf/Lef transcription factors results in transcription of sensitive genes in the nucleus. Without Wnt stimulation, β-catenin is phosphorylated by active glycogen synthaser kinase-3β (GSK-3β) and, subsequently, degraded by the 26S proteasome in an ubiquitin-dependent manner. Physical interactions between PSEN1 and, respectively, β-catenin and GSK-3β were demonstrated (8,20). Moreover, using glycerol velocity gradient centrifugation, β-catenin was demonstrated to comigrate with PSEN1 NTF and CTF in the 250 kDa fraction, illustrating that β-catenin was a part of the functional active PSEN1 NTF/CTF complex (8). The PSEN1/β-catenin interaction is lost when PSEN1 CTF is cleaved by caspase after staurosporine-induced cell death (21). Potentially, PSEN1 mutations also reduce the stability of β-catenin and increase its subsequent degradation (22). However, others suggest that mutant (mt) PSEN1 was shown not to promote the association between GSK3β and β-catenin, resulting in increased steady-state levels of endogenous β-catenin in brains of transgenic mice (23). Furthermore, the interaction between PSEN1 and a new member of the catenin family, termed δ-catenin, was identified using the yeast two-hybrid technique (24–26).

We evaluated different aspects of the involvement of PSEN1 and the pathogenic mutation, therein, on Wnt signaling through its interaction with β-catenin. Reduced nuclear translocation of β-catenin by three pathogenic PSEN1 mutations (I143T, G384A and T113-114ins), compared with wild-type (wt) PSEN1, was confirmed. The G384A PSEN1 mutation showed the most pronounced reduction. No significant effect of mt PSEN1 on the stability of β-catenin was observed, neither in cells nor in brain extracts. Nonspecific binding of overexpressed PSEN1 to endogenous GSK3β in HEK 293 cells was demonstrated. At physiological levels, however, PSEN1 and GSK3β did not bind in our experimental setup.

## Materials and Methods

### *Cell Lines*

Monoclonal HEK 293 cell lines, stably expressing wild-type (wt) or I143T, G384A, T113– 114ins PSEN1 were previously established by resistance to 800 μg/ml G418 (Promega, Madison, WI) (27,28) and were cultured in (OPTIMEM 1 culture medium), supplemented with 10% fetal bovine serum (FBS), 100 IU/ml streptomycin, and 100 μg/ml penicillin (Gibco BRL, Gaithersburg, MD). A polyclonal HEK 293 cell line expressing C263R PSEN1 was established following the earlier described protocol (27). The mutated C263R PSEN1 cDNA was obtained by QuikChange® Site-directed mutagenesis (Stratagene, La Jolla, CA).

### *Antibodies*

The mouse monoclonal antibodies against β-catenin and GSK3β were purchased from Transduction Laboratories, San Diego, CA. SB129 is an antiserum raised in rabbits against the synthetic peptide LPAPLSYFQNAQMSE, corre-

sponding to amino acids 3-18 of PSEN1 (28). Anti-PARP poly(ADP-ribose)polymerase was purchased from Pharmingen, Becton Dickinson, and anti-$\beta$-actin from Sigma, St. Louis, MO.

*$\beta$-catenin Nuclear Translocation Assay*

The assay was essentially performed as described previously (17). In brief, HEK 293 cells stably expressing wt, I143T, G384A, or T113–114ins PSEN1 were grown in a 60 mm dish to 90% confluency. Cells were treated with LiCl (25 mM final concentration) for 1 hr or not treated. Nuclear fractions were isolated in a two-step lysis. Cells were harvested in 500 $\mu$l low-salt buffer [10 mM N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES) pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.5 mM DL-Dithiothreitol (DTT), protease inhibitors], centrifuged at 1300 $\times$ g and resuspended in 50 $\mu$l high-salt buffer (20 mM Hepes pH 7.9, 400 mM NaCl, 1 mM EDTA, 10% glycerol, protease inhibitors). Cadherin-bound $\beta$-catenin was removed using Con A sepharose beads (Amarsham Pharmacia Biotech, Buckinghamshire, UK). Protein concentration was determined using BioRad protein assay (BioRad, Hercules, CA) in triplet. 5 $\mu$g of nuclear proteins were loaded onto a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel and blotted onto a polyvinylidenedifluoride(PVDF) membrane. Western blots were probed with 1/4000 anti-$\beta$-catenin and were developed with a peroxidase-conjugated secondary anti-mouse antibody using enhanced chemiluminescence (Renaissance, NEN, Boston, MA).

*Brain Extracts*

Frozen cortical brain tissue was homogenized in radio-immunoprecipitation assay (RIPA) buffer (150 mM NaCl, 1% NP-40, 0.5% Na Deoxycholate, 0.1% SDS, 50 mM Tris pH 8.0) containing protease inhibitors (Complete®, Boehringer Mannheim, Wheaton, Millville, NY) using a Dounce homogenizer. Cell debris was removed by centrifugation for 30 min at 14,000 rpm after sonication. Protein concentration was determined using the BCA system (Pierce, Rockford, IL). Cell lysates were separated by SDS-PAGE on a 10% Tris/Glycine gel and blotted onto PVDF membranes.

*Co-immunoprecipitation*

1-2.$10^6$ HEK 293T cells in a 10 cm culture dish were transfected with wt or mt full-length PSEN1 cDNA using Fugene 6 (Boehringer Mannheim, Mannheim, Germany) or the same amount of stable HEK 293 cells were used 40 hr post transfection or seeding, cells were harvested and the proteins were extracted in 500 $\mu$l lysis buffer (0.2% NP40, 40 mM Tris.HCl pH 7.4, 150 mM NaCl, 2 mM EDTA, 2 mM DTT, 10% glycerol, protease inhibitors (Complete®, Boehringer Mannheim) and phosphatase inhibitors (50 mM NaF and 1 mM Na Orthovanadate ; Sigma). Co-immunoprecipitations were performed essentially as described before (12). Lysates were precleared with anti-mouse immunoglobulin G (IgG) and protein G sepharose when anti-GSK3$\beta$ was used to immunoprecipitate.

*Statistics*

A nonparametric Kruskal-Wallis test, based on a $\chi^2$ distribution, was used to evaluate the differences between nuclear translocation before and after LiCl stimulation in wt and mt PSEN1 HEK 293 cell lines. A correction for ties was made. The experiment-wise error was adjusted for multiple comparisons.

## Results

*Effect of Pathogenic PSEN1 Mutations on Nuclear Translocation of $\beta$-catenin*

HEK 293 cell lines stably expressing either wt or mt PSEN1 were analyzed for PSEN1 expression. Clones that showed comparable PSEN1 expression (Fig. 1A) were selected. HEK 293 cells are highly appropriate to evaluate the effect of PSEN1 mutations on nuclear translocation of $\beta$-catenin, since HEK 293 cells show all cell biological features of AD-associated proteins, observed in primary neuronal cells (9). Stable HEK 293 cells were stimulated with LiCl, which blocks the activity of GSK3$\beta$, a negative regulator of $\beta$-catenin. Total cellular $\beta$-catenin, PSEN1, and GSK3$\beta$ levels were influenced only slightly by LiCl stimulation (Fig. 1A). To correct for protein concentration and loading errors, expression of $\beta$-actin was evaluated. No detectable differences in endogenous $\beta$-actin levels were observed, when comparing wt and mt PSEN1 stable cell lines. LiCl stimulation did not have an effect on total $\beta$-actin levels in either wt or mt cell lines (Fig. 1A).

Cytoplasmic and nuclear fractions were isolated and evaluated for endogenous $\beta$-catenin

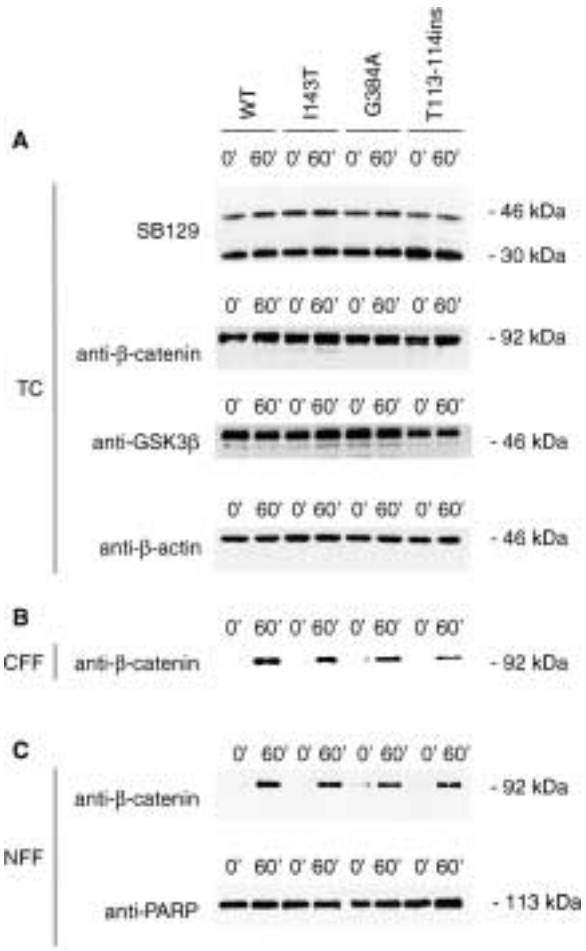



**Fig. 1. Nuclear translocation assay.** (A) Total cell (TC) lysates of stable human embryonic kidney (HEK) 293 cells expressing wild type (wt) or mutant (mt) (I143T, G384A, T113–114ins) PSEN1, were evaluated for presenilin (PSEN1), β-catenin, glycogen synthase kinase-3β (GSK3β), and β-actin expression levels before (0 min) and after (60 min) LiCl stimulation. 10 μg of total proteins were separated on a 10 or 14% Tris/Glycine polyacrylamide gel, blotted onto polyvinylidenedifluoride (PVDF) membranes and developed using the specified antibodies. (B) To obtain cytosolic-free fractions (CFF), cytosolic fractions were incubated overnight with concanavalin A sepharose beads to remove cadherin-bound β-catenin. 5 μg of proteins were separated on a 10% Tris/Glycine polyacrylamide gel and immunoblotting was performed using 1/4000 mouse monoclonal anti-β-catenin. (C) Nuclear free fractions (NFF) were obtained after overnight incubation with concanavalin A sepharose. Immunoblotting was performed using 1/4000 anti-PARP and 1/4000 anti-β-catenin.

expression. Cadherin-bound β-catenin was removed by incubating the fractions with concanavalin A Sepharose beads, potently binding cell surface glycoproteins. Endogenous cytosolic free β-catenin levels were similar in nonstimulated stable wt and mt PSEN1 HEK 293 cells (Fig. 1B). Cytosolic free β-catenin levels increased after 1 hr of LiCl stimulation, indicating that less β-catenin was degraded by the proteasome. The β-catenin signals were analyzed densitometrically and values were normalized for β-actin expression. The relative increase in free cytosolic β-catenin levels after 1 hr of LiCl stimulation of five independent experiments was plotted (Fig. 2A). Overall

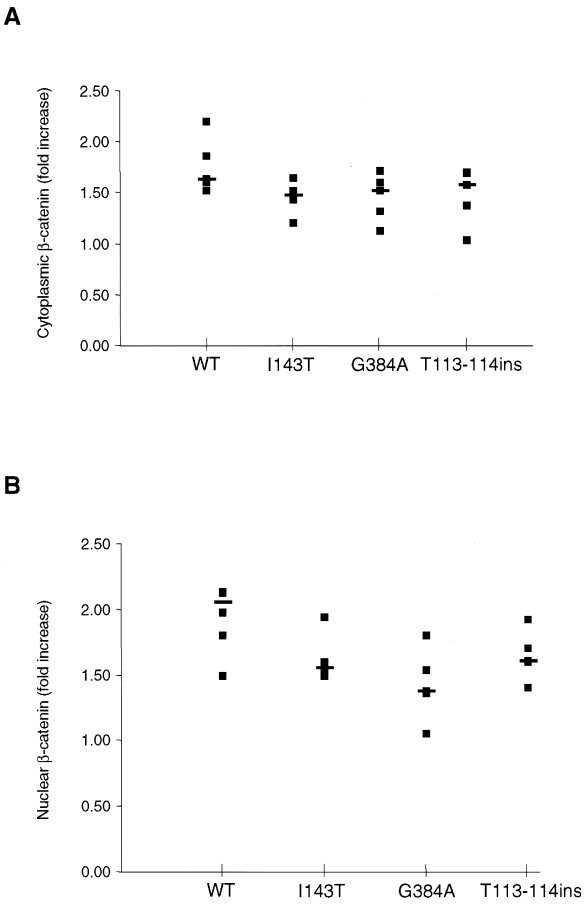



**Fig. 2. Scatter plots of the increase in free β-catenin levels in stable presenilin PSEN1 human embryonic kidney HEK 293 cells.** (A) Plot of the increase in free cytosolic b-catenin levels. The ratio of the amount of normalized cytosolic-free β-catenin after 1 hr of LiCl stimulation to the amount of normalized cytosolic-free β-catenin before LiCl stimulation (fold increase) of five independent experiments were plotted. (B) Plot of the increase in free nuclear β-catenin levels. The ratio of the amount of normalized nuclear-free β-catenin after 1 hr of LiCl stimulation to the amount of normalized nuclear-free β-catenin before LiCl stimulation (fold increase) of six independent experiments were plotted. Symbols: data points are plotted using black boxes (■); the black horizontal bar (—) indicates the median of each set of data. WT, wild type.

evaluation of the median of wt versus mt cell lines showed no significant differences in free cytosolic β-catenin levels (Fig. 2A). Statistical analysis did not result in rejection of null hypothesis ($H_0$) ($p = 0.26$) when evaluating the four different groups.

Nonstimulated, endogenous nuclear β-catenin levels were comparable (Fig. 1C). After 1 hr of LiCl stimulation, however, reduced nuclear translocation was observed in cells expressing mt PSEN1, compared with cells expressing wt PSEN1 (Fig. 1C). The β-catenin signals were analyzed densitometrically and β-catenin values were normalized for (PARP) expression, an exclusively nuclear protein that was not a downstream member of the Wnt pathway (Fig. 1C). The relative increase in free nuclear β-catenin levels of six independent experiments was plotted (Fig. 2B). Overall evaluation of the median of wt versus mt cell lines showed a clear reduction in nuclear translocation of β-catenin in the mt cells, compared with the wt cells. Statistical analysis resulted in rejection of $H_0$ ($p = 0.02$) when comparing the four different groups. Comparison of the wt group with the different mt groups resulted in a significantly reduced nuclear translocation of β-catenin in G384A PSEN1 ($p = 0.001$) and I143T PSEN1 ($p = 0.034$) stable HEK 293 cells. The nuclear translocation in the T113–114ins PSEN1 stable HEK 293 cells was reduced, but not significantly ($p = 0.089$). When the experiment-wise error was adjusted for multiple testing, only the G384A PSEN1 cell line remained significantly different from the wt PSEN1 cell line. Similar data were obtained using different monoclonal HEK 293 cells expressing the same PSEN1 mutations (data not shown). The neutral PSEN1 polymorphism E318G (29) showed nuclear translocation of β-catenin comparable to wt PSEN1 (data not shown), demonstrating that only pathogenic PSEN1 mutations exerted a reduction in nuclear translocation.

To exclude the possibility that the differences in nuclear translocation of β-catenin were due to a difference in the major catabolic pathway of β-catenin, cells were treated with 25 μM N-acetyl-L-leucinal-L-norleucinal (ALLN), an inhibitor of proteasomal activity, for different time periods (0 hr, 3 hr, and 6 hr). Total cell lysates were analyzed for β-catenin expression and normalized for β-actin levels. The relative increases in β-catenin levels after 6 hr of ALLN treatment were similar in wt and mt PSEN stable HEK 293 cells (Fig. 3).

### *Effect of Pathogenic PSEN1 Mutations on the Stability of b-catenin*

In order to evaluate the effect of PSEN1 overexpression on the stability of β-catenin, cytosolic fractions of HEK 293 cells stably expressing wt and mt PSEN1 were analyzed for free β-catenin levels and compared with free β-catenin levels in nontransfected HEK 293 cells. Small differences in free cytosolic β-catenin levels between the nontransfected and transfected cells were observed (Fig. 4A). The free cytsolic β-catenin levels before and after LiCl stimulation were different, but comparable among wt, mt PSEN1 cells and nontransfected HEK 293 cells. Cytosolic free β-catenin levels of each stable PSEN1 cell line and of the nontransfected HEK 293 cells were determined in five independent experiments and the normalized densitometric β-catenin values were plotted (Fig. 4B). Although some variability

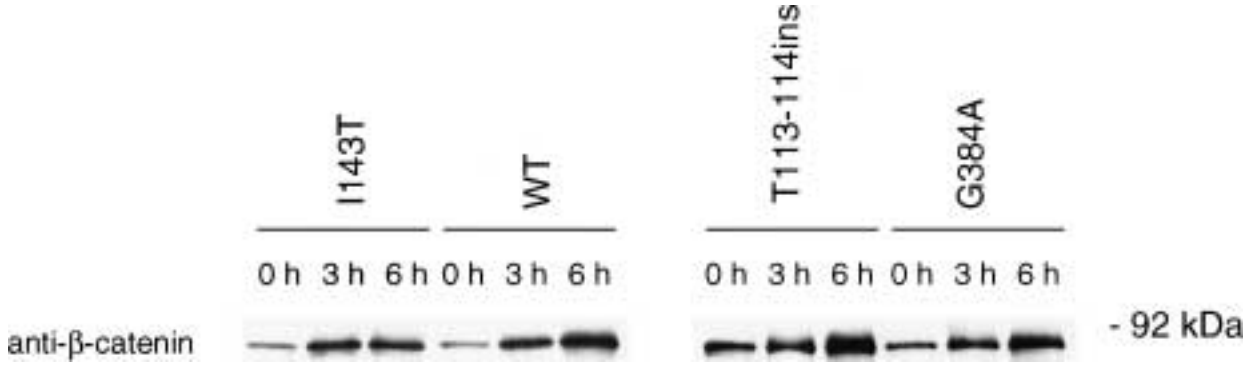



**Fig. 3. Effect of presenilin PSEN1 mutations on the proteasomal degradation of β-catenin.** Stable HEK 293 cells were treated with 25 μM N-acetyl-L-leucinal-L-norleucinal (ALLN), a proteasome inhibitor, for 0 hr, 3 hr, and 6 hr. 10 μg of total cell lysate was separated on a 10% SDS gel and immunoblotted using 1/4000 anti-β-catenin. Endogenous β-catenin levels were normalised for endogenous β-actin levels.

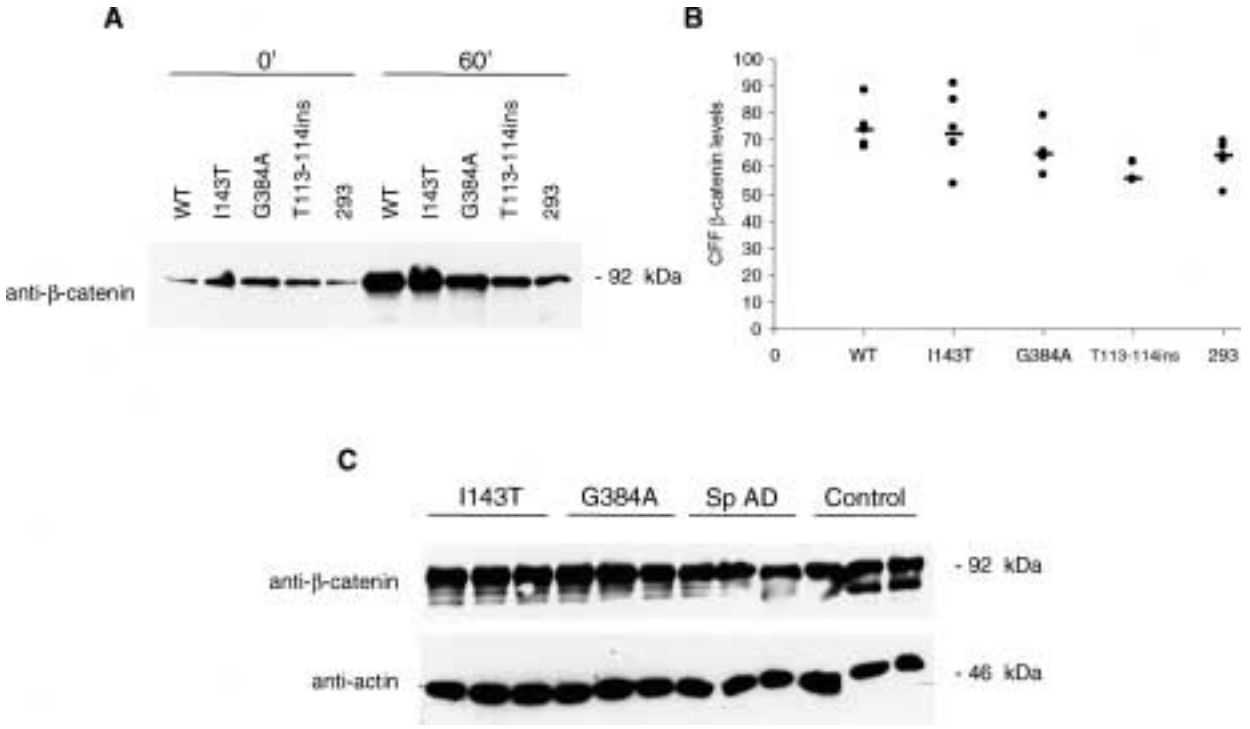


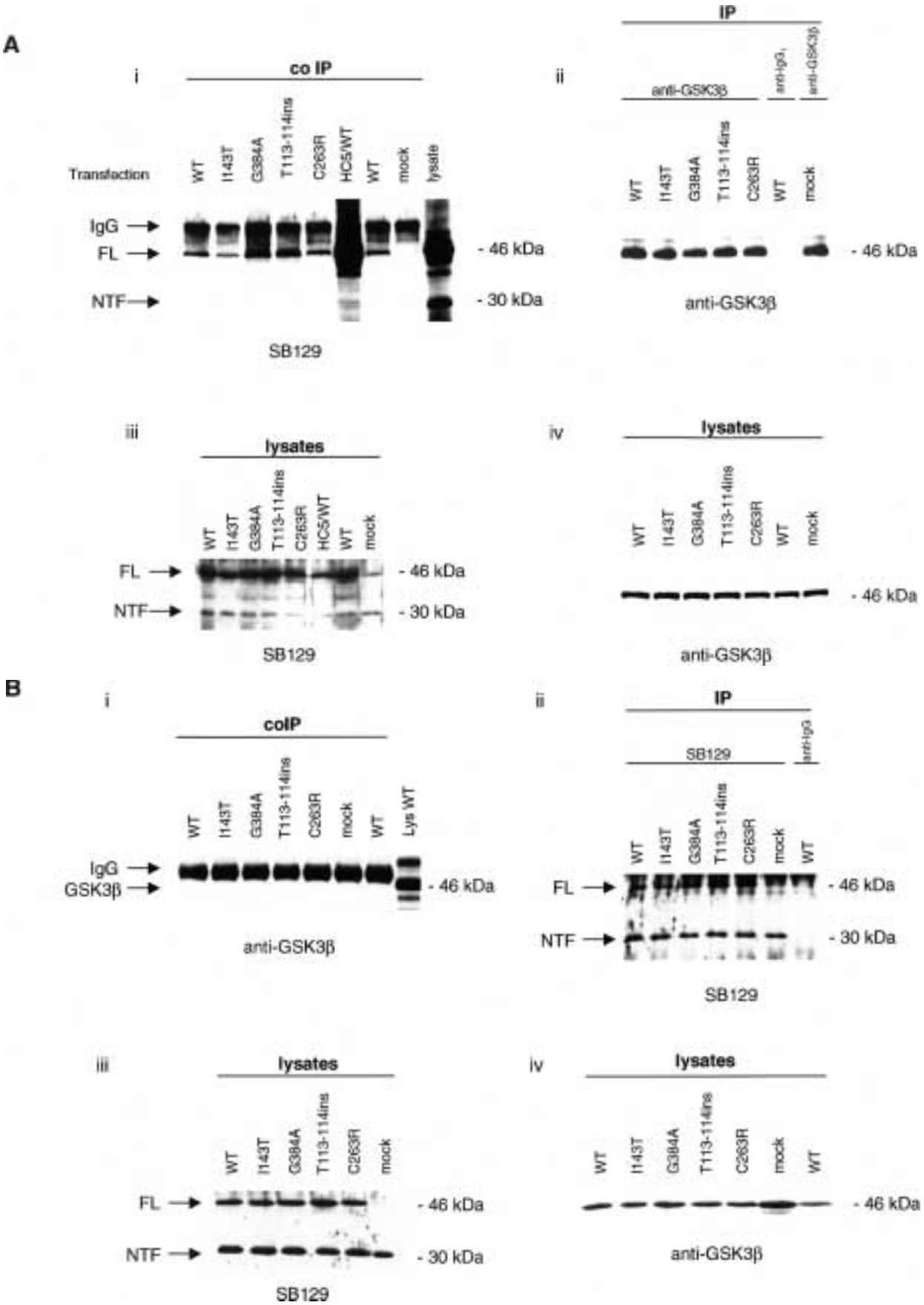

**Fig. 4. Effect of PSEN1 mutations on the stability of β-catenin.** (A) 10 $\mu$g of cystolic-free fraction (CFF) proteins of nonstimulated (0 min) and LiCl-stimulated (60 min) stable transfected wild type (wt) and mutant (mt; I143T, G384A and T113-114ins) presenilin (PSEN1) (HEK) 293 cells and nontransfected HEK 293 cells (293), were separated on a 10% sodium dodecyl sulphate (SDS) gel and evaluated for $\beta$-catenin expression. (B) Plot of normalized cytosolic-free $\beta$-catenin values without LiCl stimulation of five independent experiments. Symbols: data points are plotted using black dots; the black horizontal bar (—) indicates the median of each set of data. (C) 10 $\mu$g of total brain extract of I143T PSEN1 Alzheimer disease (AD) patients, G384A PSEN1 AD patients, sporadic AD patients, and controls were separated on a 10% SDS gel and immunoblotted with anti-$\beta$-catenin.

was observed in $\beta$-catenin values, statistical analysis for independence of the five different groups (4 PSEN1 transfected HEK 293 cells and 1 nontransfected HEK 293), did not result in the rejection of $H_0$ ($p = 0.13$). In conclusion, although small differences in $\beta$-catenin levels between nontransfected and transfected cells and between cells expressing wt and mt PSEN1 occurred, overall statistically significant differences were not observed.

The determination of expression levels of $\beta$-catenin in brain extracts from PSEN1 AD cases, allowed the evaluation of the influence of PSEN1 mutations on $\beta$-catenin stability *in vivo*. Brain extracts from I143T or G384A patients showed similar levels of $\beta$-catenin expression, compared with sporadic AD cases and controls (Fig. 4C). As demonstrated previously, levels of NTF and CTF PSEN1 fragments were indistinguishable in brains from I143T or G384A PSEN1 AD cases, sporadic AD cases, or controls (30).

*Effect of Pathogenic PSEN1 Mutations on PSEN1 and GSK3β Binding*

To evaluate the effect of pathogenic PSEN1 mutations on the binding between PSEN1 and GSK3$\beta$, HEK 293T cells were transfected with wt or mt PSEN1. Since Takashima et al. (20) reported that PSEN1 mutations located in the GSK3$\beta$ binding region of PSEN1 (between amino acids 250–298) increased the PSEN1/GSK3$\beta$ interaction 3-fold, we included cells expressing the C263R PSEN1 cDNA in the co-immunoprecipitation experiments. Comparable amounts of immunoprecipitated GSK3$\beta$ (Fig. 5Aii) resulted in the detection of variable amounts of full-length PSEN1 (Fig. 5Ai), correlating with the amount of full-length PSEN1 expressed in the lysates (Fig. 5Aiii), but no NTF was detected in the immunocomplex. Endogenous levels of GSK3$\beta$ were identical (Fig. 5Aiv). As a negative control, proteins in cells expressing wt PSEN1 were immunoprecipitated using anti-mouse $IgG_1$, surprisingly


A
i
co IP
Transfection
WT
I143T
G384A
T113-114ins
C263R
HC5/WT
WT
mock
lysate
IgG
FL
NTF
- 46 kDa
- 30 kDa
SB129
ii
IP
anti-GSK3β
anti-IgG,
anti-GSK3β
WT
I143T
G384A
T113-114ins
C263R
WT
mock
- 46 kDa
anti-GSK3β
iii
lysates
WT
I143T
G384A
T113-114ins
C263R
HC5/WT
WT
mock
FL
NTF
- 46 kDa
- 30 kDa
SB129
iv
lysates
WT
I143T
G384A
T113-114ins
C263R
WT
mock
- 46 kDa
anti-GSK3β
B
i
coIP
WT
I143T
G384A
T113-114ins
C263R
mock
WT
Lys WT
IgG
GSK3β
- 46 kDa
anti-GSK3β
ii
IP
SB129
anti-IgG
WT
I143T
G384A
T113-114ins
C263R
mock
WT
FL
NTF
- 46 kDa
- 30 kDa
SB129
iii
lysates
WT
I143T
G384A
T113-114ins
C263R
mock
FL
NTF
- 46 kDa
- 30 kDa
SB129
iv
lysates
WT
I143T
G384A
T113-114ins
C263R
mock
WT
- 46 kDa
anti-GSK3β

showing full-length PSEN1 in the immunocomplex (Fig. 5Ai). As a positive control, the interaction between PSEN1 and Flag-tagged HC5 (12), a 20S proteasome subunit, was used. Immunoprecipitation of endogenous GSK3β in the mock-transfected cells did not result in the detection of NTF or full-length PSEN1.

Immunoprecipitating comparable amounts of NTF and full-length PSEN1 in cells stably expressing wt or mt PSEN1 (Fig. 5Bii) did not result in the detection of GSK3β in the immunocomplex (Fig. 5Bi). Levels of overexpressed PSEN1 and endogenous GSK3β detected in the lysates of the different cell lines were comparable (Fig. 5Biii and iv). Proteins in cells expressing wt PSEN1 were immunoprecipitated using anti-rabbit IgG as a negative control. When endogenous PSEN1 was immunoprecipitated from mock cells, no endogenous GSK3β was detected in the immunocomplex.

## Discussion

We investigated the effect of two PSEN1 missense mutation (I143T and G384A), leading to an early age–of–onset and a severe AD phenotype in two autosomal dominant AD families (31) on the nuclear translocation of β-catenin in stable PSEN1 HEK 293 cells. We also analyzed a PSEN1 insertion mutation (T113–114ins), also leading to autosomal dominant early-onset AD (28). Reduced nuclear translocation of β-catenin after chemical induction of the Wnt pathway by LiCl stimulation is observed in cells expressing the three different PSEN1 mutations, compared with wt PSEN1-expressing cells. The differences in β-catenin nuclear translocation cannot be explained by differences in proteasomal degradation of β-catenin. Also, the pool of free cytosolic β-catenin is not significantly different in wt versus mt PSEN1 cells. Finally, Nishimura and colleagues (17) demonstrated that there was no PSEN1 present in the nuclear fraction of either wt or mt HEK 293 cells, indicating that the reduced nuclear translocation in mt PSEN1 cells results from a trafficking effect, rather than a defect in localization of the PSEN1-β-catenin complex. Moreover, the proteolytic release and nuclear translocation of NOTCH-1 is also impaired by pathogenic PSEN1 mutations (16), again indicating that PSEN1 might be involved with trafficking. The G384A PSEN1 mutation shows the most pronounced reduction of nuclear translocation of β-catenin. Interestingly, the same mutation also has the strongest increase reported on Aβ42 secretion (27,32), indicating that PSEN1 mutations can have dramatic effects on different cellular events. Notably, G384A is situated in the arm repeat (amino acids 372–399) in PSEN1, the region required for binding to the armadillo proteins (26). Moreover, G384A is adjacent to D385, a critical residue in terms of PSEN1 endoproteolysis and γ-secretase cleavage of APP (33). Together, our results further establish the nuclear translocation assay of β-catenin as an adequate alternative for the Aβ ELISA to evaluate the effect of PSEN1 mutations. However, we have to admit that the observed reduction of nuclear translocation of β-catenin observed in cells expressing mt PSEN1 is rather small and, therefore, could be an artifact. The exact mechanism behind the translocation of β-catenin to the nucleus is not known, but it was demonstrated to be independent of a nuclear localization signal and importin or karyophin transporter molecules (34). Since β-catenin has a molecular weight of 92 kDa, the nuclear translocation is due presumably to active transport through the nuclear pore complex, although no data are available to support this. The fact that full-length PSEN1 is localized mainly in the nuclear membrane, however (4), is not at least, in contradiction with the observed effect of PSEN1 on β-catenin nuclear translocation.

Contradictory data have been published

**Fig. 5. Effect of PSEN1 mutations on the interaction between PSEN1 and GSK3β.** (A)Proteins were immunoprecipitated from equal amounts of cells ($\pm 2.10^6$) transiently overexpressing presenilin (PSEN1) using 2.5 μg of monoclonal anti-glycogen synthase kinase-3β (GSK3β) or anti-mouse immunoglobulin $G_1$ ($IgG_1$). The immunocomplexes were washed four times in binding buffer, were seperated on a 14% SDS gel and immunoblotted with 1/5000 SB129 (i). 1/720 and 1/360 of the original lysate was evaluated for PSEN1 (iii) and GSK3β (iv) expression, respectively. One-quarter of the immunocomplex was tested for the presence of GSK3β (ii). (B) Proteins were immunoprecipitated from equal amounts of cells ($\pm 2.10^6$) stably overexpressing PSEN1 using approximately 10 μg of polyclonal SB129 or anti-rabbit IgG. The immunocomplexes were washed four times in binding buffer, were seperated on a 14% SDS gel, and immunoblotted with 1/4000 anti-GSK3β (i). 1/720 and 1/360 of the original lysate was evaluated for PSEN1 (iii) and GSK3β(iv) expression, respectively. One-quarter of the immunocomplex was tested for the presence of SB129 (ii). colP, co-immunoprecipitation; IP, immunoprecipitation; FL, full length; NTF, N-terminal fragment; WT, wild type; mock.

concerning a potential effect of pathogenic PSEN1 mutations on the stability of β-catenin (22,23). We evaluated endogenous β-catenin expression in HEK 293 cells, stably expressing wt and mt PSEN1 and in brain extracts from PSEN1 AD patients. Overexpression of wt or mt PSEN1 does not result significantly in pronounced degradation of β-catenin. Also, no significant differences in β-catenin levels are observed among PSEN1 AD cases, sporadic AD cases, and controls.

Conflicting results concerning the interaction between PSEN1 and GSK3β also are reported. One report states that PSEN1 mutations situated in the PSEN1 binding region (amino acids 250–290) enhance the binding between PSEN1 and GSK3β (20). Another report demonstrates binding between GSK3β and wt PSEN1, but no detectable co-immunoprecipitation between GSK3β and PSEN1 bearing the M146L and Δexon9 mutations (23). We, therefore, evaluated the effect of pathogenic PSEN1 mutations on the interaction between PSEN1 and GSK3β by co-immunoprecipitations in cells transiently and stably overexpressing PSEN1. Immunoprecipitation of endogenous GSK3β results in a detectable, but nonspecific interaction between overexpressed full-length PSEN1 and endogenous GSK3β, independent of the presence of mutations in PSEN1. Indeed, full-length PSEN1 also is detected in the anti-mouse $IgG_1$ immunocomplex in cells expressing wt PSEN1. No full-length PSEN1 is detected in the anti-GSK3β immunocomplex in mock-transfected cells, thus, at physiological PSEN1 levels. Therefore, the observed PSEN1-GSK3β interaction is more likely to be an artifact resulting from overexpression of full-length PSEN1, although the interaction at physiological levels could be missed due to Western blot sensitivity limitations. The sticky nature of overexpressed full-length PSEN1 was shown before (12). Also, the C263R PSEN1 mutation, situated in the GSK3β binding region, does not show an increased affinity towards GSK3β. Further, immunoprecipitation of overexpressed PSEN1 shows no co-immunoprecipitation of endogenous GSK3β. Anti-rabbit IgG and protein A sepharose do not nonspecifically bind to PSEN1. In conclusion, no specific binding between PSEN1 and GSK3β could be demonstrated in our experimental setup, neither at overexpressed nor at endogenous expression levels.

In summary, PSEN1 plays a role in Wnt signaling through its interaction with β-catenin. We confirm reduced nuclear translocation of β-catenin in cells overexpressing mt PSEN1. The downstream effect of reduced β-catenin translocation on transcription of Tcf/Lef transcription factors sensitive genes is not addressed. We are unable to confirm the reported effects of PSEN1 mutations on the stability of β-catenin and on the interaction between PSEN1 and GSK3β. An interaction between overexpressed PSEN1 and endogenous GSK3β is demonstrated, but proves to be nonspecific. At physiological levels, no interaction between PSEN1 and GSK3β was detected in our experimental setup, indicating that data obtained from overexpressed proteins need to be interpreted with great care.

## Acknowledgment

The authors want to thank Sofie Van Gestel for statistical assistance. This work was supported by the Fund for Scientific Research-Flanders (FWO-F), the Interuniversity Attraction Poles (IUAP p4/17), the International Alzheimer's Research Foundation (IARF), and the Focused Giving Program of Johnson & Johnson. This work was further supported by grants of the Canadian Genetic Disease Network, the Medical Research Council of Canada, the Howard Hughes Medical Research Foundation (PH St GH), and the Department of Medicine Post-Doctoral fellowship award (MN). GVG is a grant holder of the Institute for Science and Technology (IWT). CDJ is a postdoctoral fellow at the FWO-F.

## References

1. Cruts M, Van Broeckhoven C. (1998) Molecular genetics of Alzheimer's disease. *Ann. Med.* **30:** 560–565.
2. Kovacs DM, Fausett HJ, Page KJ, et al. (1996) Alzheimer-associated presenilins 1 and 2: neuronal expression in brain and localization to intracellular membranes in mammalian cells. *Nat. Med.* **2:** 224–229.
3. Lah JJ, Heilman CJ, Nash NR, et al. (1997) Light and electron microscopic localization of presnilin-1 in primate brain. *J. Neurosci.* **17:** 1971–1980.
4. Annaert WG, Levesque L, Craessaerts K, et al. (1999) Presenilin 1 controls γ-secretase processing of amyloid precursor protein in pre-golgi com-

partments of hippocampal neurons. *J. Cell Biol.* **147:** 277–294.
5. Li J, Xu M, Zhou H, et al. (1997) Alzheimer presenilins in the nuclear membrane, interphase kinetochores, and centrosomes suggest a role in chromosome segregation. *Cell* **90:** 917–927.
6. Thinakaran G, Borchelt DR, Lee MK, et al. (1996) Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo. *Neuron* **17:** 181–190.
7. Thinakaran G, Harris CL, Ratovitski T, et al. (1997) Evidence that levels of presenilins (PS1 and PS2) are coordinately regulated by competition for limiting cellular factors. *J. Biol. Chem.* **272:** 28415–28422.
8. Yu G, Chen F, Levesque G, et al. (1998) The presenilin 1 protein is a component of a high molecular weight intracellular complex that contains (β catenin. *J. Biol. Chem.* **273:** 16470–16475.
9. Capell A, Grunberg J, Pesold B, et al. (1998) The proteolytic fragments of the Alzheimer's disease-associated presenilin-1 form heterodimers and occur as a 100-150-kDa molecular mass complex. *J. Biol. Chem.* **273:** 3205–3211.
10. Thinakaran G, Regard JB, Bouton CM, et al. (1998) Stable association of presenilin derivatives and absence of presenilin interactions with APP. *Neurobiol. Dis.* **4:** 438–453.
11. Steiner H, Capell A, Pesold B, et al. (1998) Expression of Alzheimer's disease-associated presenilin-1 is controlled by proteolytic degradation and complex formation. *J. Biol. Chem.* **273:** 32322–32331.
12. Van Gassen G, De Jonghe C, Pype S, et al. (1999) Alzheimer's disease associated presenilin 1 interacts with HC5 and ZETA, subunits of the catalytic 20S proteasome. *Neurobiol. Dis.* **6:** 376– 391.
13. Borchelt DR, Thinakaran G, Eckman CB, et al. (1996) Familial Alzheimer's disease-linked presenilin 1 variants elevate Aβ1-42/1-40 ratio in vitro and in vivo. *Neuron* **17:** 1005–1013.
14. De Strooper B, Saftig P, Craessaerts K, et al. (1998) Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature* **391:** 387–390.
15. De Strooper B, Annaert W, Cupers P, et al. (1999) A presenilin-1-dependent γ-secretase-like protease mediates release of Notch intracellular domain. *Nature* **398:** 518–522.
16. Song W, Nadeau P, Yuan M, et al. (1999) Proteolytic release and nuclear translocation of Notch-1 are induced by presenilin-1 and impaired by pathogenic presenilin-1 mutations. *Proc. Natl. Acad. Sci. U.S.A.* **96:** 6959–6963.
17. Nishimura M, Yu G, Levesque G, et al. (1999) Presenilin mutations associated with Alzheimer disease cause defective intracellular trafficking of β-catenin, a component of the presenilin protein complex. *Nat. Med.* **5:** 164–169.
18. Wodarz A, Nusse R. (1998) Mechanisms of Wnt signaling in development. *Annu. Rev. Cell Dev. Biol.* **14:** 59–88.
19. Hall AC, Lucas FR, Salinas PC. (2000) Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. *Cell 2000* **100:** 525–535.
20. Takashima A, Murayama M, Murayama O, et al. (1998) Presenilin 1 associates with glycogen synthase kinase-3β and its substrate τ. *Proc. Natl. Acad. Sci. U.S.A.* **95:** 9637–9641.
21. Tesco G, Kim TW, Diehlmann A, et al. (1998) Abrogation of the presenilin 1/β-catenin interaction and preservation of the heterodimeric presenilin 1 complex following caspase activation. *J. Biol. Chem.* **273:** 33909–33914.
22. Zhang Z, Hartmann H, Do VM, et al. (1998) Destabilization of β-catenin by mutations in presenilin-1 potentiates neuronal apoptosis. *Nature* **395:** 698–702.
23. Kang DE, Soriano S, Frosch MP, et al. (1999) Presenilin 1 facilitates the constitutive turnover of β-catenin: differential activity of Alzheimer's disease-linked PS1 mutants in the β-catenin-signaling pathway. *J. Neurosci.* **19:** 4229–4237.
24. Zhou J, Liyanage U, Medina M, et al. (1997) Presenilin 1 interaction in the brain with a novel member of the Armadillo family. *Neuroreport.* **8:** 1489–1494.
25. Tanahashi H, Tabira T. (1999) Isolation of human Δ-catenin and its binding specificity with presenilin 1. *Neuroreport.* **10:** 563–568.
26. Levesque G, Yu G, Nishimura M, et al. (1999) Presenilins interact with armadillo proteins including neural-specific plakophilin-related protein and β-catenin. *J. Neurochem.* **72:** 999– 1008.
27. De Jonghe C, Cras P, Vanderstichele H, et al. (1999) Evidence that Aβ42 plasma levels in presenilin-1 mutation carriers do not allow for prediction of their clinical phenotype. *Neurobiol. Dis.* **6:** 280–287.
28. De Jonghe C, Cruts M, Rogaeva EA, et al. (1999) Aberrant splicing in the presenilin-1 intron 4 mutation causes presenile Alzheimer's disease by increased Aβ42 secretion. *Hum. Mol. Genet.* **8:** 1529–1540.
29. Dermaut B, Cruts M, Slooter AJ, et al. (1999) The Glu318Gly substitution in presenilin 1 is not causally related to Alzheimer disease. *Am. J. Hum. Genet.* **64:** 290–292.
30. Hendriks L, Thinakaran G, Harris CL, et al. (1997) Processing of presenilin 1 in brains of patients with Alzheimer's disease and controls. *Neuroreport.* **8:** 1717–1721.
31. Cruts M, Backhovens H, Wang SY, et al. (1995) Molecular genetic analysis of familial early-onset Alzheimer's disease linked to chromosome 14q24.3. *Hum. Mol. Genet.* **4:** 2363–2371.
32. Murayama O, Tomita T, Nihonmatsu N, et al.

(1999) Enhancement of amyloid $\beta$ 42 secretion by 28 different presenilin 1 mutations of familial Alzheimer's disease. *Neurosci. Lett.* **265:** 61–63.
33. Wolfe MS, Xia W, Ostaszewski BL, et al. (1999) Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and $\gamma$-secretase activity. *Nature* **398:** 513–517.
34. Fagotto F, Gluck U, Gumbiner BM. (1998) Nuclear localization signal-independent and importin/karyopherin-independent nuclear import of $\beta$-catenin. *Curr. Biol.* **8:** 181–190.