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The Presenilin 1 C92S Mutation Increases Aβ 42 Production

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Although wild-type human presenilin 1 (PS1) rescues the *C. elegans* egg-laying (*egl*) phenotype that is caused by a loss of function mutation in the *C. elegans* presenilin homologue *sel12,* most familial Alzheimer's disease (FAD)-linked PS1 mutants only partially rescue this phenotype. To investigate the effects of the loss of function *sel12* mutation on Aβ production in mammalian cells, we analyzed Aβ production in transfected H4 neuroglioma cells expressing the PS1 homologue of the sel12 C60S mutant, PS1 C92S. This analysis revealed that PS1 C92S increased Aβ42 levels in a similar fashion to other pathogenic Alzheimer's disease (AD) PS1 mutations. Significantly, the PS1 C92S mutation has recently been identified as the pathogenic mutation in an Italian family with FAD. Thus, placing a mutation that results in loss of function in *C. elegans* into a context whereby its effect on mammalian cells can be evaluated suggests that all FAD-linked PS1 mutants result in increased Aβ42 production through a partial loss of function mechanism.

Key Words: **presenilin; Alzheimer's disease; *sel12; C. elegans;* Aβ; amyloid protein precursor.**

Mutations in the human presenilin 1 and 2 (PS1, PS2) genes have been shown to cause up to 60% of early onset familial Alzheimer's disease (FAD), and evidence from a number of studies indicates that the FAD-linked PS mutations cause AD by increasing production of Aβ42 from the amyloid precursor protein (APP) (reviewed in (2)). The *C. elegans* homologue of PS1 is *sel12,* and it has been show that mutations disrupting this locus cause an egg-laying phenotype (*egl*) through a loss of function mechanism, with one of these mutations being a missense variant, C60S (3). While wild-type human PS1 can rescue the *egl* phenotype, all pathogenic missense PS1 mutations tested only partially rescue the phenotype suggesting that they have reduced activity in this assay (1). *Sel12* and PS1 have about 50% sequence identity and the *sel12* residue C60 is conserved with a homologous residue occurring at position 92 in PS1 (3). Therefore, we have sought to assess whether the PS1 homologue of the *sel12* loss of function mutation (PS1 C92S) had any affects on Aβ production in order to gain some insight into the genetic mechanisms underlying the pathogenic functions of PS1. Interestingly, after carrying out these studies, linkage of this mutation to an Italian family with FAD was reported suggesting that expression of this mutation was likely to alter Aβ production.

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MATERIALS AND METHODS

In these experiments we assessed the effects of expression of PS1-wild-type, the FAD-linked mutant PS1 M139V; and PS1 C92S (the human homologue of the *sel12 egl* mutation) on Aβ production. Stable H4 human neuroglioma derived cells expressing these constructs were generated as previously described (4). To facilitate measurement of Aβ, these stable lines were then transiently transfected with pcDNA3 APP 695NL, and total Aβ, Aβ40 Aβ42, and sAPP analyzed in the conditioned media by ELISA as previously described (4). For Western blot analysis, 48 h after transfection the cells were lysed and processed for immunodetection of PS1 as previously described (4).

RESULTS

Expression of both PS1 M139V and PS1 C92S mutation significantly increased the relative percentage of Aβ42 in the conditioned media by approximately twofold as compared to cells stably overexpressing PS1 wt (Fig. 1). The increase seen in the C92S mutant was slightly less than the increase in the M139V, an FAD-linked PS1 point mutant that has a very strong effect on Aβ42 production. Western blot analysis of these stable cell lines indicates that the level of expression of PS1wt, M139V, and C92S were quite similar (Fig. 2). Although attempts were made to directly express epitope tagged versions of *sel12* and *sel12* C60S in H4 cells, expression of the *C. elegans* PS1 homologue could

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not be detected in the cells; thus, these experiments were not pursued any farther.

DISCUSSION

The fact that PS1 C92S increases Aβ42 production in a similar fashion to other FAD-linked PS1 mutants is of considerable interest. In the *egl* model, the *sel12* C60S mutant is a loss of function mutation, the phenotype resulting because the mutation precludes appropriate Notch signaling (3). Therefore, by analogy, we might suppose that PS1 C92S is also a loss of function mutation, and that the increase in Aβ42 production is a result of this loss of function, and not a pathologic gain of function as has often been proposed. One implication of these data is that all FAD-linked PS1 mutations may result in partial loss of function of PS1.

The findings that in PS1 knockout animals both Aβ40 and Aβ42 are reduced to an equivalent extent and that a combined PS1 and presenilin 2 (PS2) knockout abolishes Aβ production have been interpreted as suggesting that loss of function of PS1 is not a likely mechanism for the pathogenic action of FAD-linked PS1 mutations that selectively increase the ratio of Aβ42 to Aβ40 (5–7). However, complete knockouts of PS1 lead to embryonic death and thus, complete functional knockouts of presenilin would not be expected to occur, even with pathogenic mutations (8). In PS1 antisense knockdown experiments Refolo and colleagues (9) suggested that partial knockdown of function had a similar effect on APP processing to pathogenic mutations. The results we report here are compatible with that suggestion, indicating that pathogenic mutations in PS1 lead to disease though a partial reduction in the biological activity of PS1 and a subtle alteration in APP processing.

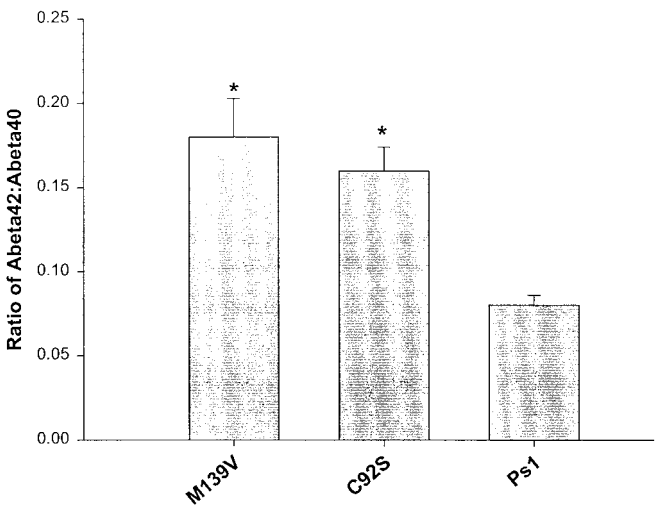

FIG. 1. PS1 C92S expression increases the relative level of Aβ42 expression. Stable H4 glioma lines expressing either PS1 wt, PS1 M139V, or PS1 C92S were transiently transfected with APP695NL, and the amount of Aβ40 and Aβ42 in the conditioned media analyzed by ELISA. The ratio of Aβ42:Aβ40 is shown (*$P < 0.04$ compared to PS1 wt by Mann–Whitney).

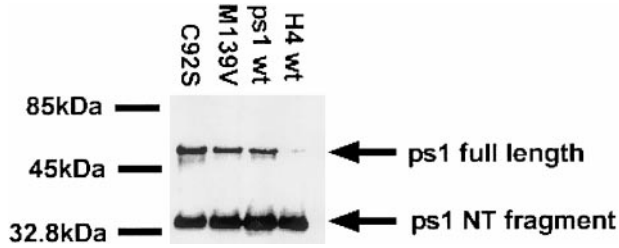

FIG. 2. Western blot analysis of the PS1 stable H4 glioma lines. Expression of PS1 in stable H4 glioma lines overexpressing PS1 wt, PS1 M139V, or PS1 C92S was evaluated with anti-PS1-N (15).

Recent evidence from a number of laboratories indicates that PS1 and presenilin (PS2) are novel aspartic protease, and that PSs function as γ-secretases within the context of a high molecular weight complex (10–13). Based on the knockout studies described above, both PS1 and PS2 appear to catalyze cleavage of Aβ40 and Aβ42; yet, the γ-secretase activities that generate Aβ40 and Aβ42 can be shown to be pharmacologically distinct (14). Because of the later observation, we have previously proposed that altered conformations of PSs could account for the distinct activities (4). If this is the case, we would propose that the partial loss of function associated with FAD-linked presenilin mutations alters the conformation of PSs themselves or the interaction of PSs with other protein in a manner that favors γ-secretase cleavage at Aβ42. We are currently exploring this possibility using an *in vitro* assay to monitor the rates of both Aβ40 and Aβ42 production from partially purified γ-secretase complexes that contain either PS1 wt or FAD-linked PS1 mutants. Based on cell culture studies with PS1 C92S, we predict that FAD-linked mutations will decrease the relative rate of Aβ40 production and have little effect on the rate of Aβ42 production.

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