COMMENTARY

Dominant negative mechanism of *Presenilin-1* mutations in FAD

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Alzheimer's disease (AD) is the most common form of dementia, afflicting more than 5 million people in the United States alone. Mutations in the *Presenilin* genes (*PSEN1* and *PSEN2*) are highly penetrant and account for ∼90% of all mutations identified in familial AD (FAD), highlighting their importance in the pathogenesis of AD. The presenilin proteins (PS1 and PS2) are broadly expressed and serve as the catalytic subunit of the γ-secretase complex, an intramembranous aspartyl protease that cleaves a variety of type 1 transmembrane proteins, including the amyloid precursor protein (APP) and Notch. The β- and γ-secretase–mediated cleavages of APP release β-amyloid (Aβ) peptides of varying lengths and C-terminal heterogeneity. More than 200 distinct mutations in *PSEN1* have been reported (www.alzforum.org), and they are dominantly inherited and mostly missense mutations. Despite extensive studies on the effects of *PSEN1* mutations, the pathogenic mechanism is still being debated. In PNAS, Zhou et al. (1) take advantage of their expertise in expression and purification of γ-secretase complexes in vitro to evaluate the effects of *PSEN1* mutations on the activity of γ-secretase containing wild-type PS1. Their findings show that γ-secretase complexes containing mutant PS1 exert dominant negative effects on wild-type γ-secretase in the production of Aβ, providing compelling evidence and further insight into how dominantly inherited missense mutations in *PSEN1* impair γ-secretase activity and cause FAD.

The dominant negative mechanism of *PSEN* mutations in FAD was initially proposed by the presenilin hypothesis to explain the dominant inheritance and missense nature of large numbers of *PSEN1* pathogenic mutations distributed throughout the coding sequence and the absence of pathogenic nonsense or frame-shift mutations (2). The presenilin hypothesis, which posits that *PSEN* mutations cause FAD via a loss of essential presenilin functions in the brain, was prompted by the age-dependent cortical neurodegeneration and dementia observed in *Psen* conditional double-knockout mice (3–5), along with the lack of neurodegeneration reported in transgenic mice overexpressing *PSEN1* mutations (6, 7). The hypothesis was further supported subsequently by genetic findings in the *Drosophila* and mouse brain showing that partial loss of PS function also results in age-dependent neurodegeneration (8, 9), and by studies in cultured cells and knock-in mouse brains demonstrating that *PSEN1* mutations cause loss of γ-secretase activity and impair essential PS functions in learning and memory, synaptic function, and neuronal survival (10–13). The findings from these mammalian studies are consistent with genetic data obtained in *Caenorhabditis elegans* and *Drosophila* showing that relative to wild-type PS1, mutant PS1 exhibited reduced biological activities (14, 15).

The most extensive analysis of *PSEN1* mutations on γ-secretase activity was recently reported by Sun et al. (16). In a tour de force, the authors used biochemically purified γ-secretase complexes containing each of the 138 distinct *PSEN1* mutations to assess the impact of these mutations on the generation of Aβ40 and Aβ42 (16). The mutations examined include all of the 121 PS1 residues affected by FAD-linked mutations. They discovered that ∼90% of the analyzed mutations impaired γ-secretase–dependent cleavage of APP, as shown by reduced production of Aβ40 and Aβ42 (16). More strikingly, ∼30% of the analyzed *PSEN1* mutations abolished γ-secretase activity, as evidenced by the lack of Aβ40 and Aβ42 detected beyond the background level (16).

Dominant Negative Effects of *PSEN1* Mutations

To reconcile the dominant inheritance trait and the missense nature of *PSEN* mutations, the presenilin hypothesis proposed that *PSEN* mutations are likely to be antimorphic, causing not only an intrinsic loss of presenilin function *in cis* but also a negative impact on wild-type γ-secretase *in trans* (2). The dominant negative effect of mutant PS1 on the activity of wild-type γ-secretase was first demonstrated by

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Heilig et al. (11) in *Psen*-null cells expressing wild-type PS1 alone or together with varying amounts of mutant PS1, which showed that the presence of mutant PS1 impaired the γ-secretase–mediated cleavage of APP and Notch by wild-type PS1 in a dose-dependent manner.

In the new study by Zhou et al. (1), the authors use purified γ-secretase complexes to examine whether γ-secretase containing a loss-of-function *PSEN1* mutation acts in a dominant negative manner to inhibit the activity of wild-type γ-secretase on the production of Aβ. The γ-secretase complex was purified to homogeneity from HEK293 cells overexpressing either wild-type or mutant PS1, along with the other integral subunits of γ-secretase, Nicastrin, Aph-1, and Pen-2. Purified γ-secretase complexes were incubated with APP-C99, a γ-secretase substrate that can be cleaved to produce Aβ, in the presence of the detergent 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO), which is known to provide an optimal γ-secretase reaction milieu (17).

Zhou et al. (1) first determined the optimal range of the enzyme and substrate concentrations for the in vitro γ-secretase assay, so that the enzyme (2–64 nM) was in the linear range, while the substrate (5 μM) was in excess. Indeed, increasing amounts of the mutant γ-secretase complex, in which the catalytic aspartate residues of PS1 were replaced with alanine (D257A/D385A) inhibited production of Aβ40 and Aβ42 by wild-type γ-secretase in a dose-dependent manner. Thus, the catalytically inactive γ-secretase mutant exerts a dominant negative effect on wild-type γ-secretase in Aβ production. The authors went further to demonstrate that mutant γ-secretase containing the pathogenic loss-of-function PS1 mutation Y115H, L166P, C410Y, or L435F also exerts dominant negative effects on the activity of wild-type γ-secretase in a dose-dependent manner (1). These *PSEN1* mutations have been shown previously to exhibit little γ-secretase activity in vitro and in cultured cells and mouse brains, and the L435F mutation causes the most severe loss of function phenotypes, consistent with the closest proximity of the L435 residue to the D257 and D385 catalytic sites (10–13, 16, 18). In contrast to these severe loss-of-function mutations, the PS1 S365A mutation, which was shown to be catalytically comparable with wild-type PS1 (16), resulted in increased production of Aβ in a dose-dependent manner, compared with wild-type γ-secretase alone. The pathogenicity of the S365A mutation, however, is unclear, as it was reported as a novel PS1 variant found in one patient (19). It would be interesting to explore additional *PSEN1* mutations with proven pathogenicity that do not cause loss of γ-secretase activity *in cis* to determine their effects on the activity of wild-type γ-secretase *in trans*.

Mechanism of Dominant Negative Effects of *PSEN1* Mutations

A direct physical interaction between mutant and wild-type γ-secretase complexes would provide the most straightforward mechanism for the dominant negative effect of mutant PS1 on wild-type γ-secretase. Heilig et al. (11) previously demonstrated that wild-type and mutant PS1 can stably interact in cells in the context of the γ-secretase complex, as shown by coimmunoprecipitation of differentially epitope-tagged mutant and wild-type PS1. In the study by Zhou et al. (1), direct physical interaction between mutant and wild-type γ-secretase complexes was investigated using an in vitro pull-down assay, which showed mutual interaction between differentially tagged mutant PS1 (D257A/D385A, Y115H, C410Y, or ΔE9) and wild-type PS1. Furthermore, the interaction is not restricted to wild-type and mutant γ-secretase complexes, and it also occurs between wild-type γ-secretase complexes or mutant γ-secretase complexes. These results provide strong supporting evidence for physical interaction between γ-secretase complexes, and suggest that mutant γ-secretase interacts with wild-type γ-secretase directly to inhibit its function.

Notably, the activity of γ-secretase measured by Aβ production and the physical interaction between γ-secretase complexes are dependent upon the presence of CHAPSO, whereas digitonin (a relatively mild nonionic detergent) or amphipol (an amphipathic polymer) impairs γ-secretase activity and disrupts the physical interaction between γ-secretase complexes (1). These results suggest that the physical interaction between γ-secretase complexes is required for optimal activity. Zhou et al. (1) further performed analytical ultracentrifugation and electron microscopy to examine the effect of different detergents on oligomerization of γ-secretase, and found that wild-type γ-secretase complexes appear in multiple forms of oligomerization in the presence of CHAPSO but mostly as monomers in the presence of digitonin. Furthermore, γ-secretase activity correlates with the presence of γ-secretase oligomers, whereas the monomeric state of γ-secretase exhibits little activity. These findings are consistent with earlier reports of large γ-secretase complexes isolated from cells in the presence of CHAPSO (17, 20), and provide a mechanistic basis for how mutant γ-secretase complexes carrying *PSEN1* mutations could exert dominant negative effects on wild-type γ-secretase and impair its function.

The present study by Zhou et al. sheds additional light on the pathogenic mechanism of FAD-bearing *PSEN1* mutations, and provides further support for the hypothesis that loss of presenilin function and γ-secretase activity plays an important role in FAD pathogenesis.

The present study by Zhou et al. (1) sheds additional light on the pathogenic mechanism of FAD-bearing *PSEN1* mutations, and provides further support for the hypothesis that loss of presenilin function and γ-secretase activity plays an important role in FAD pathogenesis (2). Indeed, the dominant missense nature and loss-of-function effects of clinical *PSEN* mutations point to a dominant negative mechanism (2). The results of Zhou et al. (1) are consistent with the prior report that mutant PS1 can exert dominant negative effects on wild-type PS1 via physical interaction in cultured cells (11), and extend these findings further by showing that this dominant negative effect is mediated by oligomerization of the purified γ-secretase complex. Interestingly, this oligomeric assembly appears to represent the catalytically competent form of the enzyme, thereby rendering it vulnerable to dominant negative inhibition. Novel therapeutic strategies aimed at overcoming the dominant negative mechanism of *PSEN* mutations and/or improving γ-secretase oligomerization may be developed as disease-modifying treatments for FAD.

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