Presenilin-1 mutations and Alzheimer's disease

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Mutations in the *PSEN1* gene, encoding presenilin-1 (PS1), are the most common cause of familial Alzheimer's disease (FAD). PS1 functions as the catalytic subunit of γ-secretase, an intramembranous protease that cleaves a variety of type 1 transmembrane proteins, notably including the amyloid precursor protein (APP) and Notch. Following prior cleavage by β-secretase, processing of APP by γ-secretase generates β-amyloid (Aβ) peptides of varying lengths. Whereas Aβ40 accounts for ∼90% of Aβ production, the minor Aβ42 product is more hydrophobic and is thought to nucleate Aβ aggregation, leading to amyloid plaque deposition in the AD brain. In PNAS, Sun et al. (1) used an in vitro system to evaluate the effects of 138 pathogenic mutations in *PSEN1* on the production of Aβ40 and Aβ42, and their findings provide valuable perspectives on pathogenic mechanisms in AD. The authors' systematic analysis of mutations affecting all PS1 residues altered in FAD provides an unprecedented perspective on the impact of *PSEN1* mutations on Aβ production and γ-secretase activity.

The mechanism by which *PSEN1* mutations lead to neurodegeneration and dementia in FAD remains hotly debated. Two distinct but not mutually exclusive hypotheses have been proposed to explain how *PSEN1* mutations cause FAD (2, 3). The amyloid hypothesis proposed that *PSEN1* mutations initiate disease pathogenesis by increasing production of Aβ42 (2). This view was based on initial studies in which small numbers of clinical *PSEN1* mutations were found to increase levels of Aβ42 in plasma of FAD patients, transfected cells, and transgenic mice, leading to the notion that *PSEN1* mutations triggered FAD pathogenesis by enhancing APP processing and inducing excessive production of Aβ42 (2, 4–6). As inconsistencies with this model subsequently emerged, revision of the amyloid hypothesis shifted the focus to relative rather than absolute increases in Aβ42 production, and the ability to increase the Aβ42/Aβ40 ratio has been widely considered an essential and invariant property of *PSEN1*-bearing pathogenic mutations (7). The presenilin hypothesis offers an alternative view of disease pathogenesis, proposing that *PSEN1* mutations cause a loss of essential presenilin functions in the brain, which in turn triggers neurodegeneration and dementia in FAD (3). This proposal was prompted by earlier genetic findings showing that presenilin is essential for learning and memory, as well as neuronal survival during aging in the adult mouse cerebral cortex (8–10), and was further supported by more recent reports demonstrating that *PSEN1* mutations typically cause loss of PS1 function (11, 12) and that severe *PSEN1* mutations abolished γ-secretase activities and Aβ production in mouse brains (13, 14).

Loss of γ-Secretase Activity by *PSEN1* Mutations

In the new study by Sun et al. (1), the authors leverage their expertise in purification and structural analysis of γ-secretase to perform a systematic analysis of the impact of the full spectrum of clinical *PSEN1* mutations on Aβ production. In an impressive tour de force, the authors purified γ-secretase complexes containing each of the 138 distinct FAD-causing mutations to homogeneity, and then analyzed the effects of these mutations on generation of Aβ40 and Aβ42 from an APP-derived substrate using an established in vitro assay system. These 138 mutations represent each of the 121 PS1 residues affected by FAD-causing mutations, including several examples of residues targeted by multiple mutations in FAD. Remarkably, 90% of the analyzed mutations impaired γ-secretase–dependent APP processing, as reflected by decreased production of Aβ40 and Aβ42. Still more strikingly, detectable production of Aβ40 and Aβ42 was fully abolished by 30% (42 of 138) of the mutations tested, including the previously characterized functionally null mutations L435F and C410Y (13, 14). Contrary to the view that increased Aβ42 production is an essential feature of pathogenic *PSEN1* mutations (2), 75% (104 of 138) of the mutations analyzed decreased production of both Aβ42 and Aβ40 (1). Among these 104 mutations, 67 mutations caused severe reduction (greater than 95%) of Aβ40 production, whereas 14 mutations resulted in

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severe reduction of Aβ42 production. These results demonstrate conclusively that pathogenic *PSEN1* mutations characteristically suppress rather than enhance γ-secretase activity and Aβ production, with a sizable proportion of mutations severely impairing these functions.

Notably, analysis of the purified γ-secretase complexes indicated that pathogenic mutations do not interfere with stable PS1 expression or γ-secretase complex assembly, suggesting that mutations primarily affect the catalytic activity of γ-secretase. Although there was no obvious pattern of correlation between the severity of a mutation's effect on Aβ production and its position in the primary protein structure, future studies mapping pathogenic *PSEN1* mutations to secondary and tertiary structural domains may reveal important structure–function relationships. As a first step in this direction, Sun et al. (1) found that many of the *PSEN1* mutations that severely compromised γ-secretase activity (including L435F and C410Y) affect residues that map near the active site in their recently reported 3D structure of the complex (1, 15), consistent with direct "orthosteric" effects of these mutations on catalytic function. The remaining mutations that do not map near the active site but nevertheless severely impair Aβ production may interfere with functional interactions of PS1 with other γ-secretase subunits or with the APP substrate.

Evidence Against an Increased Aβ42/Aβ40 Ratio as an Essential Pathogenic Feature in FAD

Surprisingly, Sun et al. (1) found that pathogenic *PSEN1* mutations did not uniformly increase the Aβ42/Aβ40 ratio. Production of Aβ40 and Aβ42 was abolished by 42 mutations tested, precluding calculation of the Aβ42/Aβ40 ratio. Furthermore, 13 of the remaining 96 mutations decreased the Aβ42/Aβ40 ratio, whereas 83 mutations increased the Aβ42/Aβ40 ratio. These findings argue against the Aβ42/Aβ40 ratio as a key determinant of the pathogenicity of *PSEN1* mutations, in contradiction with the central premise of the amyloid hypothesis. Sun et al. next examined the relationship between the Aβ42/Aβ40 ratio and the age of disease onset associated with individual mutations and found no significant correlation, further arguing against the pathogenicity of a heightened Aβ42/Aβ40 ratio. However, it should be noted that age of onset is an imperfect proxy for the pathogenicity of individual mutations; for example, the age of onset ascribed to a given FAD mutation is often based on small numbers of affected individuals and can vary substantially based on *APOE* genotype and possibly other modifier effects (16, 17). In contrast, mean ages of onset derived from large numbers of mutations identified in *PSEN1* and *APP* might offer insight into the relative pathogenicity of mutations in each gene; a recent meta-analysis of 1,300 individuals with *PSEN* or *APP* mutations demonstrated that *PSEN1* mutations cause FAD with a significantly earlier mean age of onset than *APP* mutations (18).

It has been proposed that *PSEN1* mutations increase the Aβ42/Aβ40 ratio by impeding the sequential cleavage of longer Aβ peptides by γ-secretase, thereby enhancing relative production of Aβ42 (19). However, this model is incompatible with the frequent occurrence (~30%) of pathogenic mutations that completely abolish Aβ42 production. Rather, *PSEN1* mutations appear to elevate the Aβ42/Aβ40 ratio primarily by decreasing levels of Aβ40. For the mutations analyzed by Sun et al. (1), production of Aβ40 was typically decreased more than that of Aβ42, and complete loss of Aβ40 production precluded assessment of the ratio for ~30% of mutations. Recent studies in *Psen1* knockin mice demonstrated that heterozygosity for two such mutations, C410Y or L435F, markedly impairs overall Aβ production but increases the Aβ42/Aβ40 ratio because of a greater reduction in Aβ40 than Aβ42 (13). An interesting implication of these findings is that the Aβ deposited in the brains of FAD patients heterozygous for such functionally inactivating *PSEN1* mutations is presumably produced by PS1 expressed from the normal *PSEN1* allele. As these findings underscore, analysis of the functional impact of *PSEN1* mutations in a heterozygous context (corresponding to the genotype of FAD patients) may provide a more complete picture of the operative pathogenic mechanisms. Indeed, the dominant inheritance, missense nature, and loss-of-function effects of pathogenic *PSEN1* mutations are most consistent with a dominant-negative disease mechanism, and studies in cell culture have shown that mutant PS1 can exert dominant-negative effects on coexpressed wild-type PS1 (12). In addition, whereas the results of Sun et al. (1) reveal that age of FAD onset does not correlate with either relative or total Aβ production, it would be informative to assess correlations of disease severity with other measures of PS1 function and γ-secretase activity as potential biomarkers. Notch processing activity represents one commonly used measure, but identification and assessment of physiological PS1 substrates that support neuronal survival in the adult brain will ultimately be important, because Notch mediates presenilin function in the developing but not the adult brain (20).

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Efforts to develop disease-modifying therapies for AD have been heavily focused on the amyloid hypothesis, but repeated failures in late-stage clinical trials of experimental agents based on this hypothesis heighten the urgency to explore alternative disease mechanisms. The study by Sun et al. (1) provides the most comprehensive assessment to date of the impact of FAD mutations on γ-secretase activity and Aβ production, and their results point to loss of γ-secretase activity as the primary molecular defect imposed by pathogenic *PSEN1* mutations. Thus, therapeutic strategies aimed at restoring γ-secretase activity offer a valid and complementary approach to develop disease-modifying treatments for FAD.

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