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Generation of a novel murine model of Aβ deposition based on the expression of human wild-type amyloid precursor protein gene

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Mouse models of Alzheimer disease (AD) have been generated based on *Amyloid-β Precursor Protein (AβPP)* and the *Presenilin (PSEN)* gene mutations associated with familial AD (FAD). Such models have provided valuable insights into AD pathogenesis and represent an important research tool for the discovery of potential treatments. To model amyloid deposition in AD, we generated a new mouse line based on the presence of two copies of the genomic region encoding human wild-type *AβPP* as well as a mutation (L166P) in the murine *Psen1*. By ~6 months of age, these mice have begun to develop cerebral Aβ pathology with a significant increase in the levels of AβPP C-terminal fragments and Aβ42, as well as increase Aβ42/Aβ40 ratio. Since in the brain and other tissues of these mice, wild-type human *AβPP* mRNA and protein levels are comparable to those of endogenous *AβPP*, this model may allow studies about the role of *AβPP* isoforms in the pathogenesis of AD. This animal model may be suitable to test drugs aimed at inhibiting expression or altering splicing and processing of *AβPP*, without artifacts associated with the presence of mutations in *AβPP* or overexpression due to the use of exogenous promoters. These features of the new model are of critical importance in assessing the success of therapeutic interventions.

Keywords: amyloid, Alzheimer disease, mouse model, neurodegeneration, presenilin

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The neuropatologic hallmarks of Alzheimer disease (AD) are the accumulation of the insoluble 4 kDa amyloid-β (Aβ) peptide in brain parenchyma and vessel walls, the intraneuronal accumulation of neurofibrillary tangles (NFTs) composed of tau paired helical filaments (PHFs), and the extensive neuronal loss.[1] Aβ peptides are generated in the "amyloidogenic pathway" by the proteolysis of the amyloid-β precursor protein (AβPP) by the β-site AβPP-cleaving enzyme 1 (BACE1)[2] and the γ-secretase complex.[3,4] Cleavage by α-secretase (ADAM, a disintegrin and metalloproteinase) and γ-secretase in the "non-amyloidogenic pathway" prevents Aβ generation.[1-4] Missense mutations in *AβPP* located at or near the sites of proteolysis by β- and γ-secretase have been used to develop mouse models of AD.[5] The mouse lines most frequently used express a mutation at the β-secretase cleavage site (Swedish double mutation, AβPP K670N/M671L) or a combination of the Swedish double mutation with a mutation in the γ-secretase site or within the Aβ sequence.[5-9] There are only a few animal models based on the expression of a human *AβPP* sequence without mutations.[10,11] In these models, high levels of expression were obtained by using strong exogenous promoters without significant amyloid deposition. Our laboratory recently reported[12] amyloid deposition in mice expressing the entire wild-type (WT) *AβPP* gene using its endogenous regulatory elements.

Amyloid Deposition in Mice Expressing Human Wild-Type *AβPP* Gene

To further understand the mechanism(s) involved in Aβ generation and deposition associated with *Presenilin 1* (*PSEN1*)

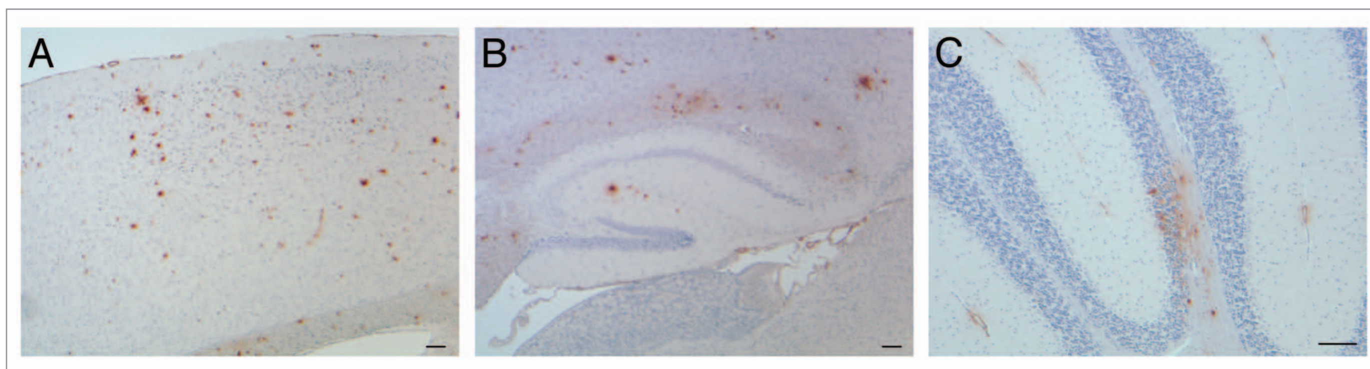

Figure 1. Amyloid deposition in APP YAC x *Psen1-L166P* mice. Sections of a 20-mo-old APP YAC x *Psen1-L166P* (+/+) mouse showing parenchymal amyloid deposition in the cerebral cortex (A), the hippocampus (B) and the cerebellum (C). Amyloid deposition was also observed in cerebral vessels and in pial (leptomeningeal) vessels. Immunohistochemistry using antibody 4G8 was performed as described.[12] Scale bars = μ100.

mutations, we generated a novel knock-in model based on a Leu to Pro mutation at codon 166 in the murine *Psen1*. This mutation was chosen since is one of the most aggressive familial AD (FAD) mutations identified so far, originally found in a patient with onset of AD at 24 years of age.[13] Clinically, the disease in the patient was characterized by progressive ataxia and spasticity soon followed by cognitive deficits. Neuropathologic findings included neuritic and cotton wool plaques, and cerebral amyloid angiopathy (CAA), as well as the presence of NFTs and severe neuronal loss. In addition, pyramidal tract degeneration and severe cerebellar Aβ deposits were present (Boyer et al., manuscript in preparation). The *PSEN1 L166P* mutation leads to a significant increase in Aβ production in cell culture,[14,15] and very severe amyloid deposition in a double transgenic mouse model.[16] However, *Psen1-L166P* knock-in mice do not develop plaque deposits, with mice analyzed up to 24 mo of age. The lack of amyloid deposition in the *Psen1-L166P* knock-in model may be in part due to differences in AβPP processing between mice and humans, and/or the three amino acids difference between the murine and the human Aβ sequence.[17] Plaque deposits can be observed when *Psen1* knock-in models are crossed with FAD mutant AβPP mice, with the *Psen1* mutations causing earlier and more extensive plaque formation.[5,6] As a result, in an attempt to be able to observe Aβ deposition driven by the *Psen1-L166P* mutation without using exogenous promoters or mutant AβPP sequences, we crossed the *Psen1-L166P* knock-in mice with mice carrying the whole human WT *AβPP* gene (without FAD-associated mutations), previously generated using a yeast artificial chromosome (YAC)[17] to produce APP YAC x *Psen1-L166P* mice.[12] Expression of the WT *AβPP* gene in APP YAC transgenic mice do not lead to amyloid deposition.[18] APP YAC transgenic mice were chosen because they express human *AβPP* mRNA and protein at levels comparable to endogenous *AβPP* in the brain and other tissues, with similar relative levels of alternative splice human *AβPP* and murine *Aβpp* products and have all regulatory elements in its chromosomal environment.[18] Double homozygous APP YAC x *Psen1-L166P* mice begin to show Th-S positive amyloid deposition in the cerebral cortex between 5 and 6 months of age. Parenchymal amyloid deposition involved the neocortex, the hippocampus, and the cerebellum (**Fig. 1**). Th-S positive amyloid plaques were surrounded by numerous dystrophic neurites and glial inflammation represented by reactive astrocytes and activated microglia. Diffuse Aβ deposits were detected using antibodies against the Aβ peptide. Immunohistochemical studies also showed the presence of intracellular Aβ deposits. CAA was observed in the cerebrum and cerebellum (**Fig. 1**). Although no obvious neuronal loss was observed, current work in the lab is aimed at assessing neuronal loss in the model as well as cognitive and motor deficits.

Enhanced Production of Aβ42 Peptides Drives Amyloid Deposition

Amyloid deposition was not observed in heterozygous APP YAC x *Psen1-L166P*$^{+/-}$ or in APP YAC x *Psen1-L166P*$^{-/-}$ mice at the oldest age analyzed (24 months). However, replacement of the WT *Psen1* allele led to amyloid deposition in APP YAC x *Psen1-L166P*$^{+/+}$ mice, in agreement with previous work done with *Psen1-M146V* knock-in mice where a reduction of γ-secretase activity rather than an increase in Aβ42 levels was proposed to drive amyloid deposition in the model.[19] As in other animal models, the presence of a *Psen1* knock-in mutation seems to enhance amyloidogenic processing of AβPP.[6-9,20] Analysis of Aβ40 and Aβ42 levels in mouse brain by ELISA showed that in APP YAC x *Psen1-L166P*$^{+/-}$ mice, the replacement of one WT allele for a mutant allele led to a significant increase in Aβ42 levels, a significant reduction in Aβ40 levels and a significant increase in Aβ42/Aβ40 ratios in the neocortex and the hippocampus. The increase in levels of Aβ42 and the decrease in those of Aβ40 were high in APP YAC x *Psen1-L166P*$^{+/+}$ mice, and seemed to correlate with the number of WT *Psen1* alleles replaced by the mutant L166P allele. In comparing with APP YAC x *Psen1-L166P*$^{-/-}$ mice, we did not observe a significant increase in total Aβ (Aβ40 + Aβ42) in APP YAC x *Psen1-L166P*$^{+/-}$ mice but it was significant (between 2 and 2.5 times) in APP YAC x *Psen1-L166P*$^{+/+}$ mice. A detailed biochemical analysis aimed at identifying

and characterizing the Aβ peptide species present in APP YAC x *Psen1-L166P* (+/+) mice, including post-translational modifications such as pyroglutamyl cyclization and N-terminal degradation which produces more hydrophobic Aβ species in humans[8,21] is currently in progress.

γ-Secretase Processing and the *Psen1-L166P* Mutation

In the amyloidogenic pathway, AβPP undergoes successive proteolysis by BACE1 and the γ-secretase complex, while in the non-amyloidogenic pathway by α-secretase and γ-secretase.[1-3] After α- or β-cleavage, the carboxyl terminal fragments (CTFs) of AβPP known as CTFα and CTFβ, respectively, remain membrane-associated and are further cleaved by γ-secretase, a protease complex comprised of presenilin, nicastrin, anterior pharynx defective 1 and PS enhancer 2.[1,4] Levels of CTFs seem to inversely correlate with total γ-secretase activity.[22] Western blot analysis of brain samples showed that while full-length levels of AβPP remained constant, the levels of CTFβ and CTFα were significantly increased in both, cerebral cortex and hippocampus of APP YAC x *Psen1-L166P* (+/+) mice.[12] These data support the view that enhanced amyloid deposition may result from the removal of the WT *Psen1* allele and impaired γ-secretase activity of the *Psen1 L166P* allele.[19] Decreased in γ-secretase activity may affect not only AβPP but it may also alter the processing of other essential substrates.[23] Although we did not detect any significant changes in Psen1 and Notch1 processing or in the expression of the Notch1 target genes *Hes3* and *Hes5* between *Psen1-L166P*$^{-/-}$ and *Psen1-L166P*$^{+/+}$ mice, we observed lack of follicular development in female *Psen1-L166P*$^{+/+}$ mice. We propose that these pathologic changes may be associated with abnormal Notch1 signaling as a consequence of defective γ-secretase activity.[12]

Conclusions

Transgenic mouse models have proven useful for modeling various aspects of Aβ pathology in AD. However, none of these models recapitulates all aspects of AD. Mostly used are transgenic mice expressing mutant AβPP under the control of a strong heterologous promoter, however this feature adds to the complexity of evaluating the pathogenic significance of transgene-induced abnormalities. To circumvent this complexity, we created a mouse model in which the mutated gene is under the control of its own promoter. In order to accomplish this, we used gene targeting to modify the murine *Psen1* gene on a transgenic mouse carrying two copies of the entire human WT *AβPP* gene. The latter possessed all the transcriptional regulatory elements required for proper spatial and temporal expression of *AβPP*. In the new mice (APP YAC x *Psen1-L166P)*, expression of the *Psen1-L166P* mutation at normal levels under its endogenous control mechanism has a significant effect on AβPP processing and Aβ deposition. The APP YAC x *Psen1-L166P* model demonstrates that neither a strong promoter nor mutations in *AβPP* are needed to drive amyloid pathology. This model may be a unique tool, which might be ideal to explore a possible relationship between abnormal expression or splicing of *AβPP* and Aβ deposition. Moreover, it may be particularly suitable for testing therapies aimed at modifying secretase cleavage of AβPP in a cellular environment which is not affected by artifacts due to the presence of amino acid variations in the AβPP sequence. Such a difference may be of critical importance to assess therapeutic strategies for AD.

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