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More than a FAD: The In Vivo Effects of Disease-Linked *Presenilin-1* Mutations

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Mutations in presenilins are linked to familial autosomal dominant Alzheimer's disease. In this issue of *Neuron*, Xia et al. (2015) show that a disease-linked mutation leads to loss of γ-secretase function, cognitive decline, and neurodegeneration when knocked into the mouse genome.

More than a century has passed since Alois Alzheimer described a peculiar pathology in the brain of a woman with a progressive dementia (Alzheimer, 1907). Since then, tens of thousands of papers have been published on the topic of Alzheimer's disease (AD)—a PubMed search for ''Alzheimer's disease'' conducted in February 2015 yielded 104,935 citations—yet the cause of the disease remains unknown. Clues to the etiology of AD have come from genetics and from the identification of the proteins that compose the characteristic lesions of AD. In 1984, Glenner and Wong identified the β-amyloid peptide (Aβ) as the major protein component of the senile plaques described by Alzheimer (Glenner and Wong, 1984). Three years later, four independent groups identified a gene on human chromosome 21 that encodes the Aβ sequence as part of a larger protein, now called the amyloid precursor protein (APP) (reviewed in Ashe and Zahs, 2010). Subsequently, it was shown that Aβ is generated from APP by sequential cleavage by β- and γ-secretases. β-secretase first removes the majority of the extracellular portion of the protein, releasing sAPPβ and leaving the APP C-terminal fragment (CTF). Then γ-secretase first cuts the CTF within the membrane at the ε cleavage site, producing the APP intracellular domain (AICD) and then cuts the remaining intracellular fragment at a γ site to generate Aβ. Gamma cleavage can produce Aβ peptides of various lengths, possibly via sequential removal of amino acid residues from the C-terminal of Aβ (Morishima-Kawashima, 2014).

Although most cases of Alzheimer's disease arise sporadically, some cases (<10%) show an autosomal dominant pattern of inheritance. All known mutations causing familial autosomal dominant Alzheimer's disease (FAD) occur in the APP gene or in genes encoding one of the presenilin proteins, which form the catalytic sub-unit of the γ-secretase enzyme complex. FAD-linked APP mutations cause an over-all increase in levels of Aβ or increase the ratio of the more aggregation-prone, 42-amino acid form of Aβ (Aβ42) relative to the 40-amino acid form (Aβ40) (Alzforum, 2015). This is important, because it has been shown that the relative ratio of Aβ42 to Aβ40 is a strong determinant of the toxicity of Aβ assemblies (Kuperstein et al., 2010). FAD-linked presenilin mutations also consistently lead to an increase in Aβ42:Aβ40 (reviewed in De Strooper, 2007). In addition, there is a wealth of experimental evidence, derived from studies in vitro and in *C. elegans*, showing that these presenilin mutations result in (partial) loss of γ-secretase function (De Strooper, 2007; Shen and Kelleher, 2007). It has been hypothesized that the mutations increase Aβ42:Aβ40 by causing incomplete digestion of the Aβ peptide at the γ site (De Strooper, 2007).

Based on the genetic findings, two competing hypotheses have been put forward to explain the etiology of AD. The Amyloid Cascade Hypothesis posits that AD is triggered by abnormal accumulation of toxic Aβ species (Hardy and Higgins, 1992). The Presenilin Hypothesis posits that partial loss of presenilin function underlies memory impairment and neurodegeneration in AD (Shen and Kelleher, 2007). Under the latter theory, a change in the Aβ42:40 ratio may arise secondarily to loss of presenilin function, but this is not the key pathogenic trigger for AD. How APP mutations cause FAD under the Presenilin Hypothesis is not immediately obvious, although it has been suggested that the Aβ42 peptide might itself partially inhibit presenilin function (Shen and Kelleher, 2007).

The Amyloid Cascade and Presenilin hypotheses lead to very different strategies for developing Alzheimer's therapeutics. The Amyloid Cascade Hypothesis would support interventions aimed at promoting clearance of Aβ (e.g., anti-Aβ immunotherapy) or reducing its generation (e.g., β- or γ-secretase inhibitors). The Presenilin Hypothesis would encourage intervention to restore presenilin activity, perhaps even through activation of γ-secretase. Although γ-secretase inhibitors are currently out of favor following a disastrous Phase III trial, debate continues over whether they might provide therapeutic benefits in AD (De Strooper, 2014). In this context, a critical question is how FAD-linked mutations in presenilins affect γ-secretase function in vivo.

In this issue of *Neuron*, Shen, Kelleher, and colleagues (Xia et al., 2015) used a knockin (KI) strategy to assess the in vivo effects of two FAD-linked mutations in presenilin-1 (PS1), L435F and C410Y,

that they had previously shown virtually eliminate γ-secretase function in vitro (Heilig et al., 2013). They first showed that these FAD-related mutations abolished the function of PS1 in vivo, thus inactivating gamma-secretase. Gamma-secretase products (Notch intracellular domain and APP intracellular domain) were absent from the brains of mice homozygous for the mutations (KI/KI), while substrates of γ-secretase (APP- and N-cadherin- C-terminal fragments) accumulated, similar to what is observed in the brains of PS null mice. No Aβ was produced by extracts from embryonic KI/KI brains in an assay of de novo Aβ generation. Using extracts of KI/wild-type (WT) brains, de novo generation of Aβ was reduced to ~50% of that by wild-type extracts, with levels of Aβ40 and Aβ42 equally affected by the mutations. Paradoxically, although the mutations did not alter the ratio of Aβ42:Aβ40 generated by γ-secretase activity, the ratio of the steady-state level of Aβ42 to Aβ40 did increase. This surprising finding suggests the intriguing possibility that presenilin mutations could somehow influence the aggregation and/or clearance of Aβ. KI/KI mice showed the same perinatal lethality and neurodevelopmental abnormalities observed in PS1 null mice (Shen et al., 1997).

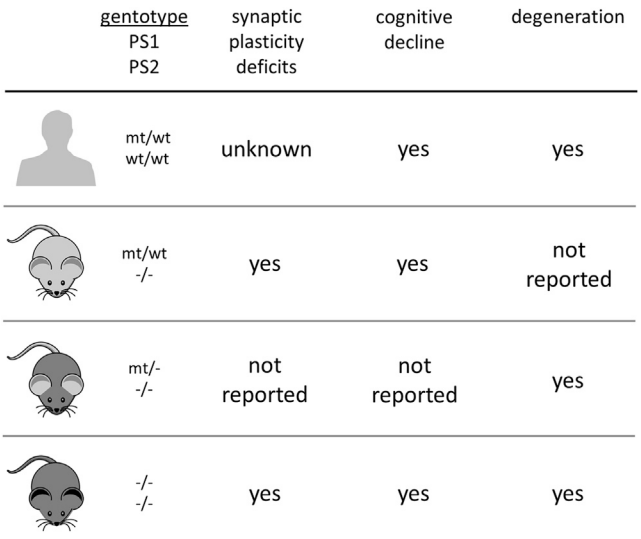

Figure 1. Summary of the Neurological Effects of the FAD-Linked L435F Mutation in Presenilin-1

Effects of presenilin deletion (Saura et al., 2004) are shown in the fourth row for comparison. "mt," naturally occurring mutation in humans or L435F mutation introduced into the genomic *Psen1* locus in mice.

Xia et al. (2015) then went on to assess the neurological consequences of the L435F mutation (Figure 1). Because they had previously observed that loss of PS1 function results in a compensatory upregulation of presenilin-2 (PS2), they studied synaptic and memory function in KI/WT mice on a PS2 null background. Compared to littermates with two WT PS1 alleles, the KI/WT mice exhibited deficits in hippocampal-dependent memory and in hippocampal synaptic plasticity.

The Shen lab had previously shown that loss of presenilin function in the adult mouse brain caused progressive cognitive decline, neurodegeneration, and gliosis, all characteristics of AD (Saura et al., 2004). Because genetic ablation of presenilins results in perinatal lethality, they devised a clever strategy to study the effects of loss of presenilin in the adult brain. In a PS2 null background, mice with floxed PS1 alleles were crossed with mice expressing Cre-recombinase under the control of the calcium-calmodulin kinase II promoter. In such mice, PS1 levels declined in forebrain neurons beginning at ~3 weeks of age. In the current study, Xia et al. (2015) isolated the effects of the mutant L435F-PS1 by using the same strategy to eliminate expression of wild-type PS1 in the adult forebrain, on a PS2 null background. Compared to littermates expressing either one or two wild-type PS1 alleles, mice expressing one copy of L435F-PS1 showed a decrease in cortical volume, a decrease in neuron number in the cortex, and an increase in astrogliosis. These results show that, in the absence of PS2, L435F-PS1 cannot support aging neurons.

Like most good science, this study raises as many questions as it answers. First, is γ-secretase function necessary to support aging neurons? If γ-secretase function is critical, which substrate is involved—APP or one of the many other targets of γ-secretase (Wakabayashi and De Strooper, 2008)? If the substrate is APP, is the critical event the loss of Aβ or AICD acting as trophic factors or the accumulation of toxic APP CTF's? Finally, is the neurodegeneration seen by Xia et al. (2015) necessarily a consequence of the loss of γ-secretase function or might PSs have functions independent of γ-secretase (Wakabayashi and De Strooper, 2008)? FAD is not associated with mutations in other γ-secretase subunits, suggesting that it might not be the loss of γ-secretase function that is responsible for the pathogenicity of PS mutations.

We also must ask to what degree the results of the experiments of Xia et al. (2015) can be extrapolated to the human disease. In order to see the neurodegenerative phenotype resulting from knocking in the L435F PS1 mutation, the authors eliminated all wild-type PS alleles (PS1 and PS2). Human carriers of FAD-linked PS1 mutations have one intact copy of PS1 in addition to two copies of PS2. However, such "genetic exaggeration" is routinely done in modeling human diseases in mice; for example, APP transgenic mice frequently overexpress several-fold human APP containing one or more FAD mutations. It is possible that such genetic exaggeration represents an acceleration of phenomena that in humans result from the accumulation of small insults over years or decades, but this is hard to validate in humans.

Based on their data, Xia et al. (2015) provide a model in which PS1 mutations that inhibit γ-secretase function act via two mechanisms, which then converge to cause AD: (1) synaptic dysfunction leads to neurodegeneration independently of Aβ; (2) Aβ deposits contribute to AD via an undefined mechanism. (We would suggest that soluble Aβ assemblies rather than amyloid plaques are the pathogenic entities.) Further experimentation is needed to test the validity of this model, and to determine whether both of these mechanisms are necessary or whether either is sufficient to trigger AD. However, it is not clear how such experiments could be accomplished using current animals models, which require

genetic exaggeration to recapitulate the key features of AD.

Human clinical trials may provide a laboratory to test theories about the etiology of AD. Two large-scale prevention trials are currently underway to test the effects of anti-amyloid immunotherapy in people with FAD. One trial will enroll subjects with either APP or PS mutations, while the second trial will focus on a large Columbian kindred with a mutation in PS1. If the trials succeed, they will provide strong support for the Amyloid Cascade Hypothesis. However, if they fail, what can one conclude? Pharmacokinetic considerations aside, the most likely explanations are that: (1) the target (i.e., Aβ) was correct, but that the timing of intervention and/or the antibody were wrong, or (2) Aβ was the wrong target. If the trials fail to produce the expected results, the findings in Xia et al. (2015) may provide an early clue as to why.

REFERENCES

Alzforum. (2015). http://www.alzforum.org/mutations.

Alzheimer, A. (1907). Allgemeine Zeitschrift für Psychiatrie und Psychisch-Gerichtliche Medizin *64*, 146–148.

Ashe, K.H., and Zahs, K.R. (2010). Neuron *66*, 631–645.

De Strooper, B. (2007). EMBO Rep. *8*, 141–146.

De Strooper, B. (2014). Cell *159*, 721–726.

Glenner, G.G., and Wong, C.W. (1984). Biochem. Biophys. Res. Commun. *122*, 1131–1135.

Hardy, J.A., and Higgins, G.A. (1992). Science *256*, 184–185.

Heilig, E.A., Gutti, U., Tai, T., Shen, J., and Kelleher, R.J., 3rd. (2013). J. Neurosci. *33*, 11606–11617.

Kuperstein, I., Broersen, K., Benilova, I., Rozenski, J., Jonckheere, W., Debulpaep, M., Vandersteen, A., Segers-Nolten, I., Van Der Werf, K., Subramaniam, V., et al. (2010). EMBO J. *29*, 3408–3420.

Morishima-Kawashima, M. (2014). Front. Physiol. *5*, 463.

Saura, C.A., Choi, S.Y., Beglopoulos, V., Malkani, S., Zhang, D., Shankaranarayana Rao, B.S., Chattarji, S., Kelleher, R.J., 3rd, Kandel, E.R., Duff, K., et al. (2004). Neuron *42*, 23–36.

Shen, J., and Kelleher, R.J., 3rd. (2007). Proc. Natl. Acad. Sci. USA *104*, 403–409.

Shen, J., Bronson, R.T., Chen, D.F., Xia, W., Selkoe, D.J., and Tonegawa, S. (1997). Cell *89*, 629–639.

Wakabayashi, T., and De Strooper, B. (2008). Physiology (Bethesda) *23*, 194–204.

Xia, D., Watanabe, H., Wu, B., Lee, S.H., Li, Y., Tsvetkov, E., Bolshakov, V.Y., Shen, J., and Kelleher, R.J., III. (2015). Neuron *85*, this issue, 967–981.

Short Circuiting the Circadian System with a New Generation of Precision Tools

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Circadian behavior in mammals is coordinated by neurons within the suprachiasmatic nucleus (SCN). In this issue, Lee et al. (2015) and Mieda et al. (2015) applied state-of-the-art genetic tools to dissect the microcircuits within the SCN generating circadian rhythmic behavior.

One of the fundamental goals of neuroscience is to link specific brain regions to specific functions. While in many cases this goal has proven elusive, an overwhelming body of evidence shows that the suprachiasmatic nuclei (SCN) of the anterior hypothalamus are the site of the master circadian pacemaker in mammals. The SCN functions to synchronize a network of circadian oscillations throughout the body; the resulting circadian rhythms have a profound impact on our health and wellbeing. In addition to the identification of the SCN as a key region regulating circadian activity, at the cellular level, we currently have a relatively firm understanding of the transcriptional/translational feedback loops that are responsible for generation of these molecular oscillations. However, major gaps remain in understanding circadian regulation at the intermediate level of analysis, including the roles of specific cell-types within the SCN. Two exciting back-to-back studies in this issue have applied state-of-the-art genetics tools to analyze the SCN and make headway in understanding its circuitry and its role in circadian rhythmic behavior (Lee et al., 2015; Mieda et al., 2015).

Some of the challenges in studying the function of the SCN and its subpopulations lie in its structure. Anatomical studies generally support the division of the SCN into at least two subdivisions including a dorsal (shell) region and a ventral (core) region (Figure 1; top). At the cellular/synaptic however, the SCN can be likened to a tightly packed ball, composed of GABAergic neurons whose synaptic connections form more of a plexus rather than an ordered structure like the hippocampus, cortex, or cerebellum. Furthermore, an influential study using fully isolated SCN neurons found