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INSIGHTS

Alzheimer mutant speeds APP transport

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APP^{S198P} segregates with rare familial forms of Alzheimer's disease and resides within exon 5, unlike 27 other mutations that reside in exons 16 or 17. In this issue, Zhang et al. (2021. *J. Exp. Med.* https://doi.org/10.1084/jem.20210313) show that the brains of APP^{S198P} transgenic mice accumulate excess levels of Aβ. In cultured cells, APP^{S198P} undergoes accelerated ER folding, leading to early arrival in late vesicular compartments, thereby enhancing generation of Aβ.

27 mutations within or near the Aβ domain of the human Alzheimer's amyloid precursor protein (APP), all in exons 16 and 17, are associated with familial Aβ proteinopathies, both familial Alzheimer's disease (FAD) and hereditary cerebrovascular hemorrhage with Aβ amyloidosis, Dutch type (HCHWAD; see figure, panel B). At least one third of these mutations alter the aggregation properties of Aβ (Hatami et al., 2017). Elevated cerebrospinal levels of Aβ aggregates and soluble oligomers correlate with the carrier state in presymptomatic human subjects harboring FAD mutant APP genes (Ringman et al., 2012). In this issue of *JEM*, Zhang et al. (2021) report a surprising and unprecedented mutation in the APP ectodomain in exon 5 (see figure, panel A), APP^{S198P}, that accelerates folding in the ER and transport through the Golgi network to late vesicular compartments, including endosomes. Notably, accumulation of aggregates and soluble oligomers of Aβ is enhanced in mouse models expressing this mutation. This finding prompts us to reflect on the current conventional wisdom regarding the molecular pathogenesis of FAD attributable to mutant forms of APP and Aβ and on experience with clinical trials of Aβ-reducing immunotherapies. We would argue that these independent narratives converge to provide fresh support for the "Aβ hypothesis" of AD.

Mutations converting residue Glu^{693} to glutamine or glycine underlie HCHWAD (also known as "Dutch mutant") or Arctic mutant FAD (see figure, panel B), respectively, and their impact is to exaggerate the tendency of these mutant Aβs to form Aβ aggregates and soluble oligomers (Nilsberth et al., 2001; Hatami et al., 2017). The potential significance of this structural perturbation is especially striking in the case of the Arctic FAD mutant APP^{E693G} since clinical AD risk is enhanced by favoring generation and accumulation of Aβ aggregates and soluble oligomers despite the reduction in stoichiometry of total $Aβ^{E22G}$ generated per mole of holoAPP^{E693G} metabolized (Nilsberth et al., 2001). In 2010, our group created a Dutch mutant APP^{E693Q} transgenic mouse model wherein the severity of learning behavior deficits correlated with levels of so$Aβ^{E22Q}$ that accumulated despite an absence of detectable fibrillar $Aβ^{E22Q}$ accumulation even at ages up to 2 yr (Gandy et al., 2010).

Some current trials of anti-Aβ immunotherapies focus on antibodies targeting Aβ aggregates and soluble oligomers (Tolar et al., 2020; Linse et al., 2020; Mintun et al., 2021). Similar to other proteinopathies, Aβ aggregates and soluble oligomers adopt a range of conformational folding states, some of which are toxic while others may be benign (Knight et al., 2016). This biophysical–neuroactive continuum is reminiscent of the "strains" (or conformer subtypes) that prion protein (PrP) aggregates and soluble oligomers may adopt, some toxic and others apparently benign (Condello et al., 2018). In the case of strains of Aβ aggregates and soluble oligomers, there may exist in the brains of living humans at risk for AD a subset of strains of Aβ aggregates and soluble oligomers that are especially potent in catalyzing the prion-like seeding of Aβ aggregates and/or as modulators of tauopathy-related neurotoxicity. Seeding is the term for the property of aggregates and/or soluble oligomers of scrapie-like PrP strains (PrP^{Sc}) to serve as templates that induce physiological

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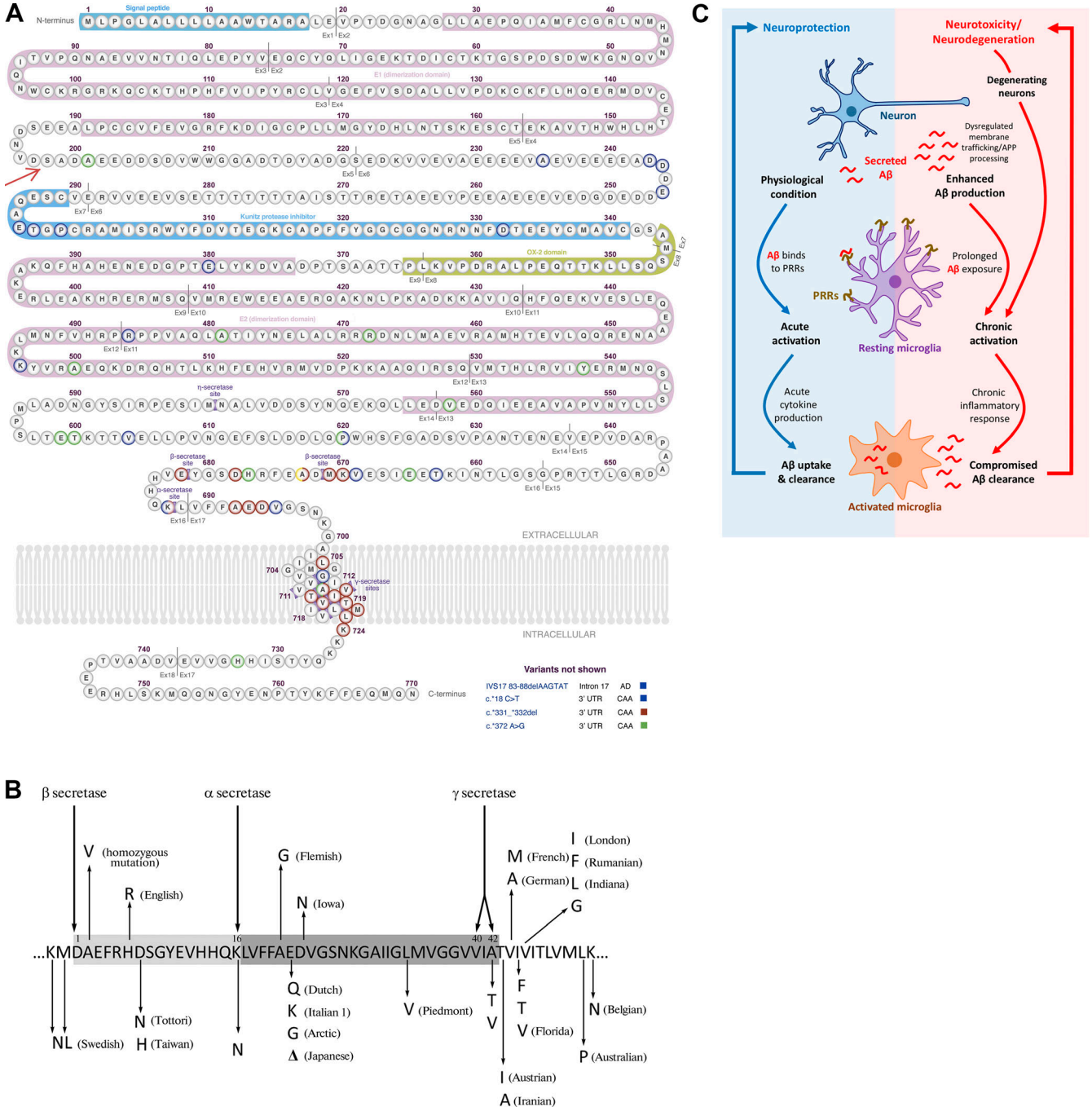

(A) Structure of human Alzheimer's APP with red arrow indicating location of S198P mutation described in Zhang et al. (2021). Panel A is reprinted with permission from Alzforum.org. (B) Structure of the Aβ domain of human APP. α-, β-, and γ-secretase cleavage sites are located near or within the Aβ domain. The positions and amino acid changes associated with 27 pathogenic mutations underlying familial Aβ proteinopathies are indicated by arrows leading from the one-letter code for the wild-type amino acid residue to that for each of the 27 pathogenic variants. In parentheses are the informal names of each (e.g., "Arctic" and "Dutch"). Panel B is reprinted with permission from *Molecular Biology* (Kulikova et al., 2015). (C) Current concepts in AD pathogenesis implicate Aβ proteinopathy, the immune-inflammatory response, and neuronal and synaptic integrity as important events in the initiation and/or progression of AD. Panel C is reprinted with permission from *Biochimica et Biophysica Acta (BBA) – Biomembranes* (Tan and Gleeson, 2019).

PrP molecules to adopt the pathogenic PrP^{Sc} conformation. Donanemab was generated against an N-terminal–truncated, pyroglutamylated Aβ peptide antigen highly prone toward formation of aggregates and soluble oligomers (Nussbaum et al., 2012), raising the possibility that one key to the apparent benefit of donanemab may be its ability to neutralize or clear away these potent, neurotoxic, tauopathy-inducing, and prion-like Aβ aggregates and soluble oligomers. If clearance of N-truncated, pyroglutamylated Aβ is particularly effective in arresting progression of AD, then we require development of a method for determining in the living human brain the levels of not simply the fibrillar amyloid that we can routinely detect now (e.g., with

florbetapir), but we must also develop assays for quantification of the levels of the most detrimental strains of Aβ aggregates and soluble oligomers. As of now, we cannot exclude the possibility that the relatively modest clinical benefit associated with abolition of detectable fibrillar brain amyloid by aducanumab, BAN2401, and donanemab (Linse et al., 2020; Tolar et al., 2020; Mintun et al., 2021) may be attributable to our anti-Aβ passive immunotherapies having left behind important levels of residual undetectable toxic strains of Aβ aggregates and soluble oligomers. Until we can prove that all neurotoxic Aβ strains are depleted, we cannot accept the proposal that "anti-Aβ–oligomer passive immunotherapies are the last call for the amyloid hypothesis of Alzheimer's disease" (Panza et al., 2019).

The subcellular basis for how the APP^{S198P} mutation exerts its effect is also novel. When compared with the synthesis and transport of wild-type APP, newly synthesized APP^{S198P} molecules appear to undergo accelerated folding within the ER as well as highly rapid export to and through the Golgi apparatus and on to the late compartments of the central vacuolar pathway, including the trans-Golgi network and endosomal compartments (Zhang et al., 2021). The rapid passage of APP^{S198P} leads to accumulation of APP and its potentially amyloidogenic C-terminal fragments in these late compartments where β-APP site–cleaving enzyme (BACE1) and γ-secretase complexes are colocalized, thereby enabling generation of Aβ peptides. In addition to the carefully executed kinetic studies of APP^{S198P} movement through the cell, Zhang et al. (2021) also demonstrate that expression of APP^{S198P} associates with enhanced accumulation of Aβ aggregates and soluble oligomers in the brains of amyloid-depositing mice.

There are by now many examples wherein protein mis-sorting emerges as a key theme in the molecular pathogenesis of AD-related genetic variants. One of the first, most robust, and most commonly cited protein-sorting gene variants linked to AD risk is SORL1, a transmembrane protein involved in sorting APP in the identical compartments implicated in the pathogenesis of APP^{S198P}-related AD (Rogaeva et al., 2007). However, unlike APP^{S198P}, AD-related variants of SORL1 do not lead to accelerated anterograde delivery to late compartments, but instead retard retrograde retrieval of wild-type APP from endosomes to the trans-Golgi network. The notion that either accelerated anterograde transport to late compartments or impaired retrograde transport from those same compartments prolongs endosomal residence time of APP and/or its potentially amyloidogenic C-terminal fragments and modulates AD risk provides compelling, converging, and independent evidence for the central role in AD pathogenesis played by Aβ aggregates and soluble oligomers.

In aggregate, the ∼100 genes now associated with AD risk can be grouped into a few classes, with protein-sorting genes, immune-inflammatory genes, and neuronal and/or synaptic genes each being robustly represented. As discovery of the genetic bases for AD may be nearing completion, this new paper by Zhang et al. (2021) joins independent clinical trial data to refocus the unresolved clinico-proteopathic phenomena associated with toxic strains of Aβ aggregates and soluble oligomers. These observations might serve to remind us that Aβ accumulation remains the best documented initiating step on the pathway to AD neuropathology. Still unanswered is whether we can identify safe and affordable methods to modulate brain Aβ levels lifelong, and whether we can demonstrate that preventing or eliminating Aβ accumulation of Aβ aggregates and/or soluble oligomers will reliably produce meaningful clinical benefit.

Yet another challenge is identifying subjects at risk for AD in whom the amyloid accumulation per se is the main driver of cognitive decline, rather than, for example, the immune-inflammatory activities of microglia (see figure, panel C). This challenge is most strikingly illustrated by the linkage of relative risk for clinical AD dementia to an allele of CR1 in which exacerbation of cognitive decline associates with reduced amyloidosis. We have speculated that CR1 variant–related AD may be an example wherein the high-risk CR1 variant acts more at the level of immune-inflammatory events rather than at the level of promoting Aβ accumulation (Gandy et al., 2013). It is important to recognize that, at present, we are unable to specify which immune targets are druggable, nor can we say with certainty when in the course of the illness we might best intervene and the direction of manipulation of activity of each target is more likely to produce therapeutic benefit (Golde, 2019). These are among the hurdles that we must overcome in order to develop personalized precision medicines that provide clinically meaningful benefit in retarding the rate of appearance or progression of cognitive decline in clinical AD.

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