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New Insights on the Role of Residue 673 of APP in Alzheimer's Disease

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Review of Zhang et al.

Alzheimer's disease (AD) is the most common neurodegenerative disorder and its two major pathological hallmarks are neuritic plaques and neurofibrillary tangles (Benilova et al., 2012; Ward et al., 2012). The formation of neuritic plaques through the aggregation of amyloid- β ($A\beta$) oligomers is thought to be foundational to the pathophysiology of AD according to the amyloid cascade hypothesis (Hardy and Selkoe, 2002). Amyloid precursor protein (APP), existing as three major isoforms (APP695, APP751, and APP770), can be processed through two major pathways (Nalivaeva and Turner, 2013) (Fig. 1). In the non-amyloidogenic pathway, APP is first cleaved by α -secretase at the Leu¹⁷ site, resulting in membrane-bound C83 and a secreted soluble APP fragment, s-APP α (Esch et al., 1990). Next, C83 is cleaved by γ -secretase to produce a C-terminal fragment (CTF γ) and P3 (Vardy et al., 2005). In the amyloidogenic pathway, APP is initially cleaved by β -secretase, also known as β -site APP cleaving enzyme 1 (BACE1), which can cleave APP at one of two distinct sites, the major Glu⁶⁸² or the minor Asp⁶⁷² site, which are

commonly referred to as the Glu¹¹ and Asp¹ sites because of their corresponding positions on C99/C89 and $A\beta$. This results in secreted sAPP β and a membrane-bound C-terminal fragment β (CTF β). Cleavage at the Glu¹¹ site forms a C-terminal fragment that is 89 residues in length (C89), which, after γ -secretase cleavage, results in a truncated form of $A\beta$. Cleavage by BACE1 at the Asp¹ residue, however, creates a C-terminal fragment that is 99 residues long (C99), which generates $A\beta$ after cleavage by γ -secretase.

Most cases of AD are considered sporadic, but some cases (<1%) are classified as familial AD (FAD). Some cases of FAD are caused by point mutations in the genes that code for APP (Bekris et al., 2010). A well studied FAD-causing mutation is the dominantly inherited Swedish mutation, APP_{Swe}, which leads to increased C99 levels. Another FAD mutation, found in an Italian family, is inherited recessively and characterized by an alanine to valine missense mutation at residue 673 (A673V) of APP770 (APP_{rlta}). Upon cleavage by BACE1 and γ -secretase, the A673V mutation can be found on C99 and $A\beta$ on the second residue, which is referred to as an A2V mutation (Fig. 1). There is currently limited knowledge about the molecular processes that link these APP mutations to the pathogenic manifestation of AD. A recent study by Zhang et al. (2017) published in the *The Journal of Neuroscience* addressed this question by investigating the role of

residue 673 of APP in BACE1 cleavage site selection and APP processing in the context of amyloidogenesis in AD.

Zhang et al. (2017) investigated whether APP_{rlta} led to increased C99 production that translated into increased levels of $A\beta$. Adeno-associated viruses containing wild-type APP (APP_{WT}) or APP_{rlta} were injected into brains of neonatal mice. An antibody specific for the last 20 residues of the APP C terminus was used to examine the relative levels of C83, C89, and C99. The APP_{rlta} A673V mutation resulted in higher C99 levels and C99/C89 ratios compared with APP_{WT}. This suggests that the A673V mutation shifted the dominant BACE1 cleavage in APP from the Glu¹¹ site to the Asp¹ site, a result consistent with previous research (Kimura et al., 2016). Zhang et al. (2017) then examined the effects of APP_{rlta} and APP_{Swe} *in vitro* using PC12 rat pheochromocytoma cells and a BACE1 stable cell line, 293B2. They showed that APP_{rlta} cleavage generated more C99 fragment than APP_{WT} and APP_{Swe} and therefore had a stronger effect than APP_{Swe} in shifting the preferential β -cleavage site from Glu¹¹ to Asp¹.

Despite APP_{rlta} cleavage producing more C99 than the APP_{Swe} mutation, Zhang et al. (2017) discovered that the APP_{rlta} cleavage pathway produced less $A\beta$ 40 and $A\beta$ 42 than APP_{Swe} (although still more than APP_{WT}). To determine why this is the case, the researchers investigated the fate of C99 after BACE1 cleavage. HEK293 cells ex-

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Figure 1. APP can be cleaved through amyloidogenic or non-amyloidogenic pathways. In the non-amyloidogenic pathway, α -secretase cleaves APP to form sAPP α and CTF83. In the amyloidogenic pathway, APP is cleaved by β -secretase (BACE1) at either the Glu¹¹ site to form CTF89, resulting in a truncated form of A β , or the Asp¹ site to make CTF99, which is cleaved by γ -secretase to form A β (gray). The Ala673 site of APP becomes the Ala² site (red) of A β .

pressing either C99_{WT} or C99_{A2V}, the C99 fragment resulting from cleavage of the APP_{r1ta} mutation, were treated with a γ -secretase inhibitor, a lysosome inhibitor, or a proteasome inhibitor. The γ -secretase inhibitor resulted in an ~184% increase of C99_{WT}, whereas C99_{A2V} only increased by ~53%. In contrast, the proteasome inhibitor resulted in a 25-fold increase in C99_{A2V} compared with only an eightfold increase in C99_{WT}. These findings suggest that the A673V mutation enhances C99 degradation by the proteasome and decreases cleavage by γ -secretase compared with C99_{WT}.

Finally, Zhang et al. (2017) investigated the overall effect of APP residue 673 on BACE1 cleavage. Another mutation at the same residue of APP, A673T, was previously discovered to be protective against AD in the Icelandic population (Jonsson et al., 2012). Knowing that mutation of this site could be either protective or FAD causing, the investigators substituted the hydrophobic alanine on site 673 of APP770 with a range of amino acids including negatively charged aspartic acid, structurally

rigid proline, positively charged arginine, hydrophobic leucine, and polar uncharged glutamine and serine. They then used immunoblotting to examine the CTF cleavage profiles after β -cleavage. These point mutations all reduced C99 levels and increased C89 and C83 levels compared with WT, with the exception of leucine, which shares characteristics with valine and alanine in that they are short-chain alkane residues. In general, these findings imply that cleavage of APP by α -secretase is enhanced by these mutations and that the preferential BACE1 cleavage site is shifted to the Glu682 site of APP.

Compared with the APP_{Swe} mutation, APP_{r1ta} results in less A β generation; however, patients still develop severe FAD pathology. A reason for this discrepancy might be the difference in A β composition: the APP_{r1ta} mutation occurs in the A β peptide (A β _{A2V}), but the APP_{Swe} mutation does not (it produces A β _{WT}). Giaccone et al. (2010) compared these peptides and discovered that A β _{A2V} in a homozygous form had higher aggregation

kinetics and more stable A β assemblies than A β _{WT} (Giaccone et al., 2010). The r1ta mutation is thought to form amyloid aggregates more rapidly, thus triggering the downstream cascade of effects leading to neurotoxicity.

The findings of Zhang et al. (2017) provide insight into the role of APP residue 673 in FAD. They show that a single mutation from alanine to valine can affect BACE1 cleavage site selection, resulting in disproportionately high C99 production but lower A β production relative to APP_{Swe}. Regarding the fate of C99_{A2V} after initial β -cleavage, the investigators revealed that the fragment has reduced γ -secretase cleavage and increased proteasomal degradation. Together, the results presented by Zhang et al. (2017) suggest site 673 of APP as a therapeutic target. Zhang et al. (2017) showed the 673-residue site to be a key determinant between the amyloidogenic and non-amyloidogenic pathways, as demonstrated in the A673V and A673T mutations, respectively. To determine why heterozygous carriers of APP_{r1ta} did

not develop FAD, Di Fede et al. (2009) investigated the interaction between $A\beta_{A2V}$ generated as a result of the APP_{rIta} cleavage pathway and $A\beta_{WT}$. They showed that this interaction in a heterozygous form had a protective effect against $A\beta$ aggregation compared with both $A\beta_{A2V}$ and $A\beta_{WT}$ on their own (Di Fede et al., 2012, 2016). Using this information, Di Fede et al. (2016) developed a synthetic rIta peptide ($A\beta_{1-6_{A2V}}$). The peptide was then tested in mouse and *in vitro* models, where it interacted with $A\beta_{WT}$ and showed beneficial interference with $A\beta$ oligomer production (Di Fede et al., 2016). The methods discussed here and results by Zhang et al. (2017) provide the groundwork for future investigations into how APP mutations have broader implications for AD and for the development of treatment strategies.

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