

New Structural View on How Amyloid Beta Production Hints Alzheimer's Disease Pathology

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Abstract

The accumulation of amyloid beta (A β) peptide faulty isoform has been found to cause Alzheimer's disease (AD) as it is the major component of amyloid senile plaques in the extracellular matrix of brains of AD patients. A β 42 (only two amino acid longer than A β 40) tends to aggregate, forming neurotoxic oligomers and fibrils. A lot of studies were focused on A β 42, but all failed explain why the production balance of A β 42 and A β 40 leans towards A β 42 in neurons of Alzheimer's patients. A recent study, utilizing NMR technology, was able to illustrate the molecular mechanism of abnormal formation of A β 42. A mutant of the amyloid precursor protein found in familial AD, V44M, was shown to alter the structure of its transmembrane region, leading to the exposure of residue T48. The exposure of T48 leads to the shift of cleavage by γ -secretase from residue L49 to residue T48, thus producing the A β 42 isoform. In this highlight, we explain the work in detail and discuss its implications on potential treatment of AD.

Keywords: amyloid beta peptide, amyloid precursor protein Alzheimer's disease, transmembrane protein

Alzheimer's disease (AD) is the most notorious cause of dementia. AD is a neurodegenerative disorder in which patients gradually lose their abilities to remember and to think. The painful progression of the disease eventually leads to death. Millions of people are diagnosed with AD worldwide. Sadly there are currently no effective treatments for this disease.

Several hypotheses have been proposed for the pathogenesis of AD. The most convincing is the amyloid cascade hypothesis: the abnormal production and aggregation of the amyloid beta peptide (A β)'s "bad" isoform—A β 42. A β 40 and A β 42 are the most common forms of A β , composed of 40 and 42 amino acid residues, respectively. Both tend to aggregate into oligomers and fibrils in the extracellular matrix. A β 42 aggregates faster than A β 40 and is more toxic for neurons, directly causing AD [1-2]. The amyloid cascade hypothesis has also been supported by the fact that A β 42 forms neurotoxic oligomers and fibrils *in vivo* [3]. Interestingly, A β 40 protects A β 42 from

aggregation [2, 4-5]. Therefore, the A β 42/A β 40 ratio is the key in the pathogenesis of AD. The molecular mechanism of A β production and why a higher ratio of A β 42/A β 40 is produced in Alzheimer's patients is the question to answer for understanding AD and developing treatments.

In a recent issue of *Nature Communication*, Chen and his colleagues made significant progress to answer this question[6]. They focused their study on familial AD (FAD), a form of Alzheimer's disease that is caused by genetic mutations. Mutations V44M and V44A were used for their study. Both mutations are within the transmembrane region of the amyloid precursor protein. There are totally 12 FAD mutations discovered in the transmembrane region of the precursor protein (APP). V44M was chosen first since it has the most helical content[7].

The maturation of A β involves two sequential proteolytic cleavages of its precursor protein, APP, by β -secretase and γ -secretase, respectively

[8]. It has been shown that the γ -secretase cleavage site determines the species of A β isoforms [9]. If γ -secretase cleaves after residue T48 of APP this leads to an increase in A β 42. Otherwise, if cleavage is at L49, less A β 42 will be produced.

To illustrate why the precursor would form two distinct amyloid beta peptides, Chen and his colleagues utilized solution NMR to determine the high resolution structures of the transmembrane region of APP (APPTM) wild type (WT) and FAD mutant V44M for the first time. The APPTM samples were reconstituted in DPC micelles, a well-known membrane mimic system [10-11]. Through comparing ^{15}N - ^1H HSQC spectra (fingerprints of structure given protein) of V44M and WT APPTM, they were able to map residues responsible for the changes caused by the V44M mutation. Interestingly, they found that T48 has the largest chemical shift change among all residues. This finding strongly indicates that the V44M affects the local structure of APP around T48 and possibly shifts γ -secretase cleavage from L49 to T48.

To further investigate the V44M mutants, Chen et al. determined the structures of WT and V44M APPTM. The overall structures of the two are very similar: two APPTM molecules form a dimer through a GXXXA motif [6]. The structures are consistent with recent evidence showing that APPTM functions as a dimer [12-14]. More interestingly, in V44M, the pattern of dimeric interaction between two subunits has a noticeable change around residue 44–50 compared to WT. In the WT protein, I45 and L49, interact whereas in the V44M mutant T43 and V50 interact. The overall crossing angles between the two APPTM molecules are also different: 20 degrees in WT and up to 30 degrees in V44M. Therefore, the V44M dimer is more unwound towards the C-terminus than the WT. With these findings, it becomes obvious that the structural change induced by V44M mutation leads to the exposure of T48 and to its cleavage instead of L49 cleavage.

A more direct evidence of this hypothesis is through hydrogen-deuterium (HD) exchange experiment of WT and V44M. During the experiments, H₂O in APPTM NMR sample was freeze-dried. The dry power was then dissolved in 100% D₂O. The exchange rate of amide proton to deuterium of all residues was measured. The exchange rate is a direct indicator of water exposure of a particular residue [15-17]. V44M has a larger exchange rate than the WT in the residues T48–V50. The effect is obvious for residue T48. Such results further suggest that V44M destabilizes the C-terminal half of the helix. In addition, another FAD mutant, V44A, was also tested, because it has the same mutation site as the V44M. V44A mutation also resulted in greater exchange rates of the residues T40–V50. Similarly, T48 showed the highest increase.

In conclusion, Chen and his colleagues hypothesized that the different APP maturation products, A β 42 and A β 40, are due to the structural changes induced by mutations in the APPTM region. The structural changes of the mutants expose T48 instead of L49, resulting in the formation of the A β 42 isoform instead of the A β 40 isoform.

Through a combination of biochemical and biophysical tests, Chen et al. provided solid evidence supporting their hypothesis and came up with a model that could explain the unknown mechanism for A β 42 production (Figure 1). Another contribution of this research is the first structure of the dimeric APPTM WT and V44M mutant at atomic resolution. With both structures, it is now feasible to design inhibitors that can block the T48 site and expose the L49 site to alter γ -secretase accessibility. In addition, correlating the structural changes in APPTM FAD mutants with shifted γ -secretase specificity can be a promising starting point to understand the interaction between γ -secretase and APPTM. γ -secretase modulators can be designed and studied based on this interaction. Hopefully, the treatment of AD can be realized in near future.

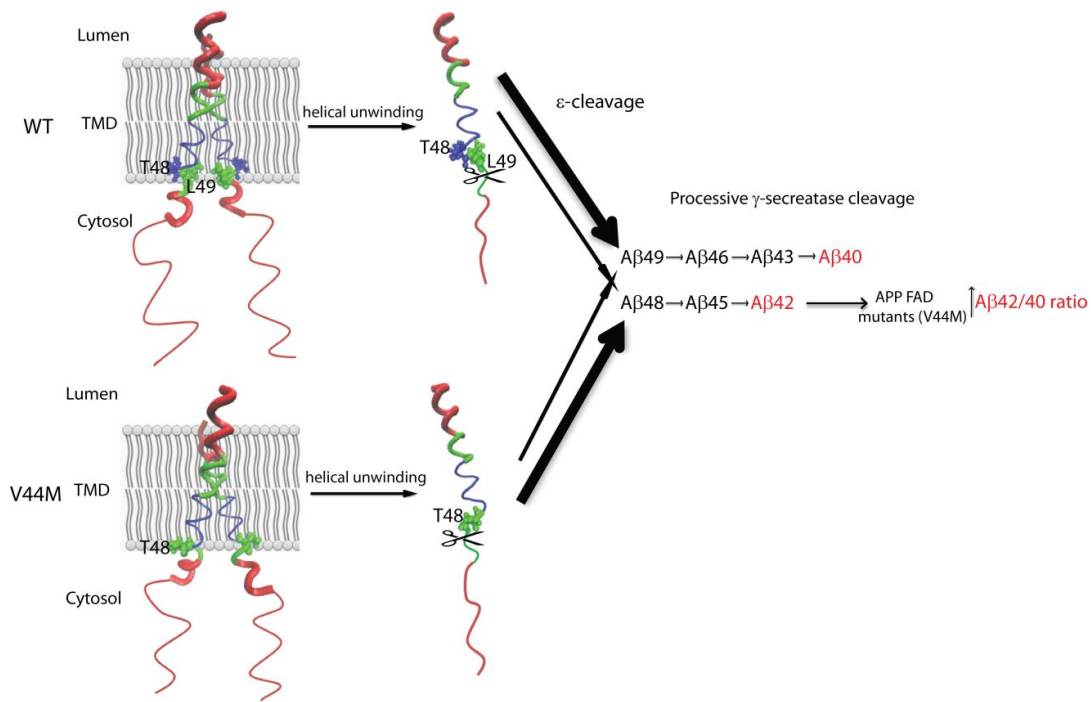


Figure 1. Structural basis of V44M mutant-induced familial Alzheimer's disease. (A) Dimer structure of APPTM wild type (WT) and V44M mutant are presented in the cartoon. Transmembrane regions are colored in green and solvent exposed regions are colored in red. In the APPTM WT (upper panel), residue L49, which is located at the membrane-cytosol interface, is represented as dots and colored in green. Residue T48 is shown as dots and colored in blue. In the APPTM V44M mutant (bottom panel), residue T48, which is now located at the membrane-cytosol interface, is represented as dots and colored in green. (B) APPTM WT and V44M are cleaved at L49 and T48, respectively. The subsequent cleavage by γ -secretase is shown. The thick line indicates a higher probability to undergo pointed process, whereas thin line indicates a lower probability.

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