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Structural Impacts of Familial Alzheimer's Disease (FAD) Mutations on Amyloid Precursor Protein (APP) Cleavage by γ-secretase Subunits APH1A and PSEN1

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Abstract. This paper surrounds the question of how Familial Alzheimer's Disease (FAD) mutations in the C99 fragment of the amyloid precursor protein (APP) affects its structure, as well as the γ-secretase subunits PSEN1 and APH1A. These interactions are critical for determining the production of amyloid-β (Aβ) peptides in the β-processing pathway, particularly the ratio of Aβ42 to Aβ40, which is implicated in Alzheimer's pathology. While many FAD mutations in APP are known to increase Aβ42 production, the precise structural mechanism by which these mutations disrupt C99's interaction with γ-secretase, and how that disruption leads to altered cleavage patterns, has not been well characterized. Previous studies have identified the correlation between mutations and disease progression, but few have explored the structural and binding changes at a molecular level using predictive modeling. From Alpha Fold-2 and RMSD analysis in PyMOL, several mutations like V715A, T714I, or V717G, were found to significantly alter the structure of C99, which subsequently also changed the way it bound to PSEN1 or APH1A. Conversely, other mutations like V717I or I716V were found to have minimal structural impact. These results suggest that the destabilization of C99 and its altered binding may prevent γ-secretase from completing the necessary sequential cuts, which provides an explanation for the increased Aβ42:Aβ40 ratios in patients who have these mutations.

Keywords: Familial Alzheimer's Disease, Amyloid-beta/C99 structures, mutational structural analysis, γ-secretase (APH1A, PSEN1), Alpha Fold-2

1. Introduction

Alzheimer's disease is typically characterized by the accumulation of amyloid beta (Aβ) plaques in the brain. While these plaques have historically been categorized as neuropathogenic, recent research has found it to be an antimicrobial peptide against herpes simplex virus type-1 (HSV-1). Aβ binds to HSV-1 glycoprotein B (gB), which is thought to neutralize HSV-1's viral activity; however, this activity also causes increased plaque formation and neuroinflammation [1].

Aβ starts off in the form of amyloid precursor protein (APP), which is 770 amino acids long, containing an extracellular domain, a transmembrane region, and an intracellular domain. There are two pathways by which APP can be cut, by the amyloidogenic pathway with BACE-1, or the non-

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amyloidogenic pathway with α-secretase [2]. There are two enzymes involved in this cleavage, β secretase, named BACE-1, and γ secretase, which contains multiple subunits: PS, APH-1, nicastrin, and presenilin enhancer 2 [3]. After BACE1 cleaves the protein at the β site, the APP becomes C99, which contains the last 99 residues of APP. This is the preferred processing pathway, as the β' site produces C89 instead, which does not get further processed [4]. The PSEN1 subunit of γ-secretase is the catalytic site, while the APH1A subunit is mainly responsible for maintaining the stability of the interactions between C99 and γ-secretase. PSEN1 cuts the C99 sequentially — the first cut is at 49/48, the second at 45/46, and another at 43/42. After this cut, the Aβ 42 may dissociate from the protease, or the protease may continue to make another cut at 40. Aβ-42 is more prone to oligomerization, in comparison to Aβ-40. There exists an even shorter version, Aβ-38 which may have the opposite effect of Aβ 40 and 42, where it may counteract 42 and reduce the formation of plaque deposit [3].

Mutations in the amyloid precursor protein have been found to increase the likelihood of developing Alzheimer's disease. More specifically, mutations in the region of what will be Aβ (40/42) also tend to increase the chance of developing cerebral amyloid angiopathy, which is a condition where these amyloid plaques form on the blood vessel walls, which may cause hemorrhagic stroke. These mutated Aβ proteins have a higher tendency to clump together and oligomerize to form plaques in comparison to the wild type. Mutations near the γ-secretase cut site were also studied, specifically: 1716T, I716V, T714A, T714I, V715A, V715M, V717F, V717G, V717I, V717L. These mutations, especially the ones near positions 716 and 717, increase the production of Aβ 42 as they destabilize the enzyme-substrate complex [5].

In this study, Alpha Fold-2, and online software for modeling proteins, was used in order to study the effects on Familial Alzheimer's Mutations (FAD mutations) on the stability of the interactions between the C99 region and the PSEN1 and APH1A subunits of γ secretase; specifically, here, it was used to create a structure of the proteins. Alpha Fold-2 is able to use previously experimentally determined structural models with similar sequences in order to predict an inputted sequence.

2. Methods

To obtain the images in the figures, the online platform Alpha Fold-2 was used. The protein sequences came from online databases, namely Uniprot's human DNA sequences. The proteins for the C99 region of the amyloid precursor protein (APP), as well as the PSEN1 and APH1A subunits of γ-secretase, were modeled. Experimentally determined structures from the Protein Data Bank (PDB) were used for comparison with the wild-type proteins predicted by Alpha Fold2. Mutated and wild-type structures were also analyzed in PyMOL, and differences were quantified using root mean square deviation (RMSD) values.

3. Results

Each C99 mutation was modeled using Alpha Fold-2, both within the Aβ sequence as well as at the γ-secretase cut site, to determine a structure to use as a control. This control was compared to experimentally determined structures.

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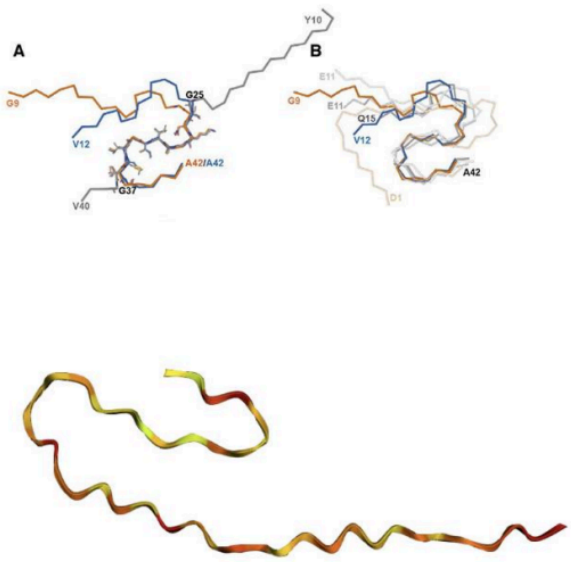

Figure 1. The image on top shows the experimentally determined structure of Aβ-42 using cryo-electron microscopy (cryo-EM). Type 1 Aβ-42 protofilaments are colored in orange, while type 2 Aβ-42 protofilaments are colored in blue [6]. The image on the bottom shows the simulated structure of Aβ-42 determined by Alpha Fold-2

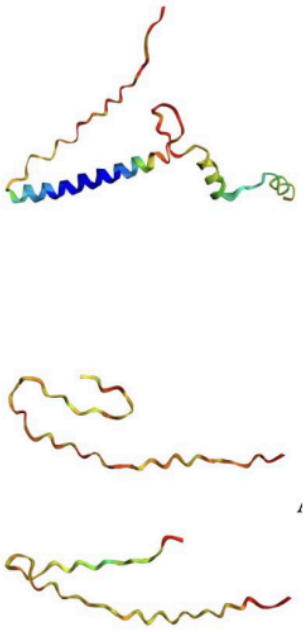

Figure 2. Simulated structures of C99 WT, Aβ-42, and Aβ-40 (in order from top to bottom) using Alpha Fold-2

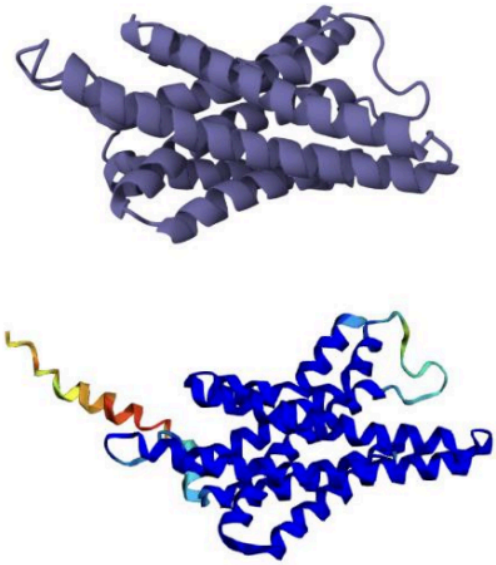

Figure 3. Comparison of APH1A by cryo-EM on top and Alpha Fold-2 on the bottom [7]

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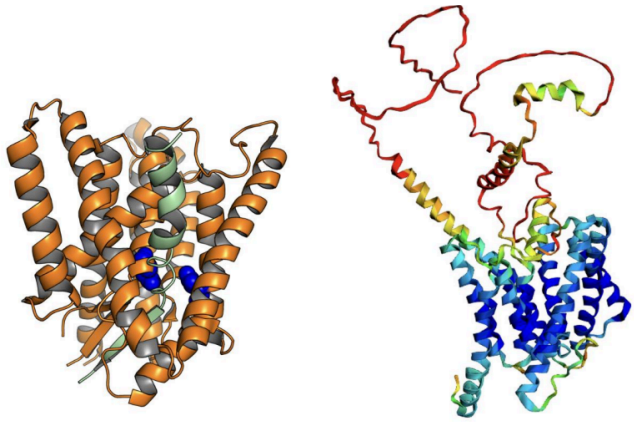

Figure 4. Depicts the PSEN1 subunit in orange to the left, determined by cryo-EM [8]. The right side shows the wild type PSEN1 subunit simulated by Alpha Fold-2

3.1. Control: wild types

The control structures included the wild types of Aβ-42, Aβ-40, C99, APH1A (Anterior Pharynx Defective 1 Homolog A), and PSEN1 (Presenilin 1). As shown in figures 1, 3, and 4, the structures determined by Alpha Fold-2 were compared to experimentally determined structures.

Analyzing the 3-D structure, the Aβ was largely loose and unstructured, determined by the pLDDT coloring. The structure is mostly yellow and orange, with areas of red, which all suggest low confidence in the predicted structure. However, despite the low confidence, it matches with the experimentally determined structure more or less. The structure looks like it is made up of β-sheets rather than α-helices.

The C99 region mutations were also modeled independently. For the D694N mutation, previous findings were corroborated: although cleavage was not affected, the Aβ42 fragment appeared more prone to aggregation. This was supported by the presence of more defined α-helices and β-pleated sheets, suggesting increased structural stability in the Aβ region.

3.2. Specific mutations

Modeling the full C99 region revealed three distinct α-helices, which are connected by largely unstructured segments. Figure 6 shows the point mutations, which induced a change in local folding and subsequently structure. Using PyMOL to superimpose the mutant structure and the wild-type C99, there was a significant deviation in the RMSD (root mean square deviation) values, which confirms the structural instability of the mutated proteins.

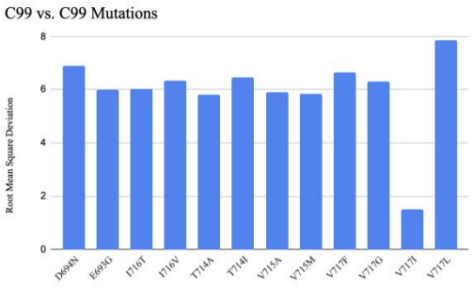

Figure 5. RMSD values between C99 WT structure and its mutants

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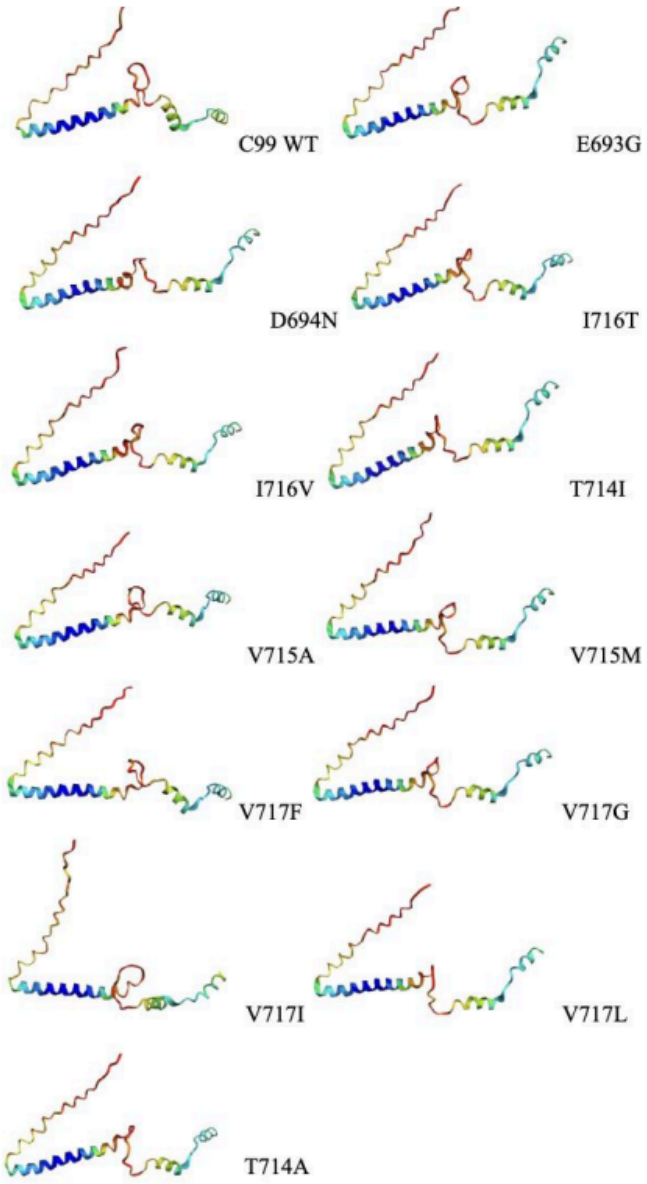

Figure 6. Comparison of C99 WT to mutated structures with Alpha Fold-2. The major difference is in the region colored red, where many of the mutations occur

3.3. C99 mutations and the PSEN1 subunit

Having determined the structures of C99 and its mutants, the next step was to model the interaction between C99 and the PSEN1 subunit of γ-secretase. Figure 7 shows that in the interaction containing the wild-type, only the Aβ-containing segment of C99 made contact with PSEN1. This is logically accurate, as PSEN1, along with PSEN2, are the catalytically active subunits of γ-secretase [3]. This suggests an altered binding orientation that could disrupt normal cleavage and favor premature release of Aβ42.

The V715A was especially disruptive: the mutation caused the C99 to bind to an allosteric site instead of the correct site on PSEN1. This explains the impaired trimming of the residues, especially those longer than Aβ-45 [9].

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On the other hand, several mutations like V717G, I716V, T714A, and T714I did not result in significant changes in its interactions with PSEN1. RMSD supported this visual analysis, with all the values being under 1Å – typically, 2 or 3Å would be needed to indicate a significant difference. Additionally, comparing this data to the numbers from simply modeling the mutated C99 regions, the data suggests that the PSEN1 subunit might even play a role in stabilizing the protein in order to cleave it effectively.

In the V717L, V717F, and V717I mutations, the second set of α-helices in the C99 fragment was observed to interact with the PSEN1 subunit of γ-secretase. These interactions occurred at two distinct sites: the primary cleavage site and a region within the transmembrane domain containing the α-helices. Structural modeling suggested a bending between the Aβ region and the transmembrane domain, which may account for the additional interaction site.

When modeled alone, the C99 region showed moderate structural confidence, with a mixture of red and other confidence colors in Alpha Fold-2 predictions. However, when bound to the γ-secretase complex, the C99 model appeared uniformly red, indicating a drop in Alpha Fold's predicted structural confidence despite minimal visible conformational changes.

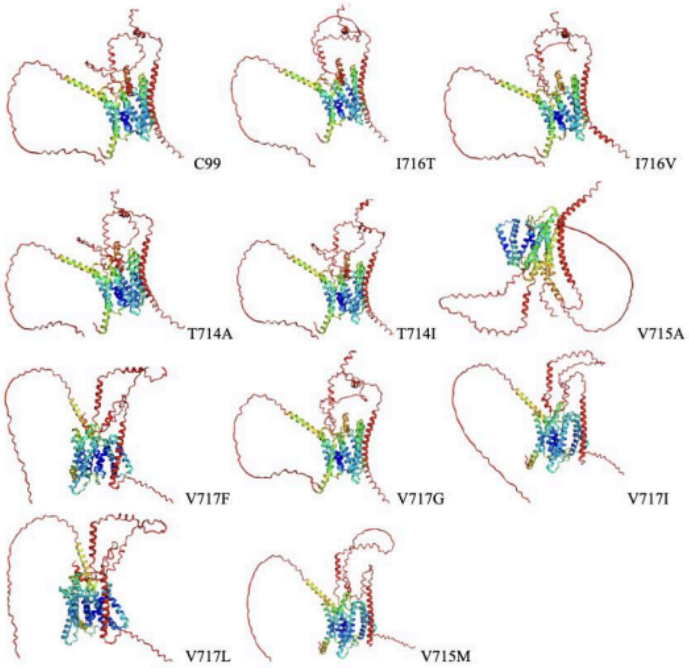

Figure 7. PSEN 1 subunit of gamma secretase bound to C99 on the right, modeled by Alpha Fold-2

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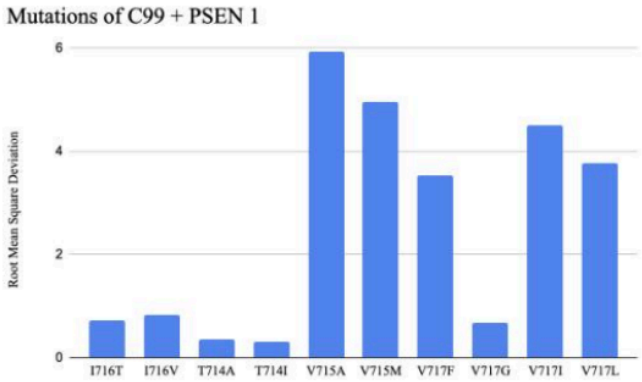

Figure 8. Depicts the RMSD values of each mutant complex (C99 and PSEN1) from the wild type

3.4. C99 mutations and APH1A

The APH1A unit of γ-secretase plays an important role in stabilizing the interaction between C99 and the secretase during the cleavage process. Alpha Fold-2 modeling shows that the site of C99 that binds to APH1A corresponds directly to the region where many APP mutations occur — specifically, the 710s.

For APH1A itself, the modeled structure was consistently dark or light blue, suggesting high confidence and subsequently rigidity across the protein. The only red part is where C99 binds, suggesting that this interaction requires some flexibility.

Despite this, the consistency from structure to structure with the exception of T714I, V715A, and V715M, determined by PyMOL and RMSD data, highlights its role in effectively stabilizing the C99 region of APP. Surprisingly, some mutations that seemed to have a visible difference, such as V717L, only altered the RMSD value minimally.

The mutations that had significant changes were T714A, T714I, V715A, V715M, and V717G. As shown in Figure 9, these mutations caused the transmembrane domain (TMD) of C99 to fold back into the APH1A site, which likely reduces the stability of the interaction. Additionally, the “tail” of the TMD, which in wild type and other models extends past APH1A, often folded back in these mutants, which may also play a role in reducing the stability of the interaction between γ-secretase and C99. While the rest of the mutations only involved the TMD and past that folded, the V717G C99 had the Aβ region fold in as well – this was unexpected, as the mutation shouldn’t have affected a region that far down the protein. It is also possible that the altered binding of C99 at APH1A goes on to affect the way C99 interacts with other subunits of γ secretase.

Finally, for mutations V717F, V717I, and I716T, the structural changes were minimal, which align with the chemical similarities between the amino acid substitutions: all involved nonpolar side chains.

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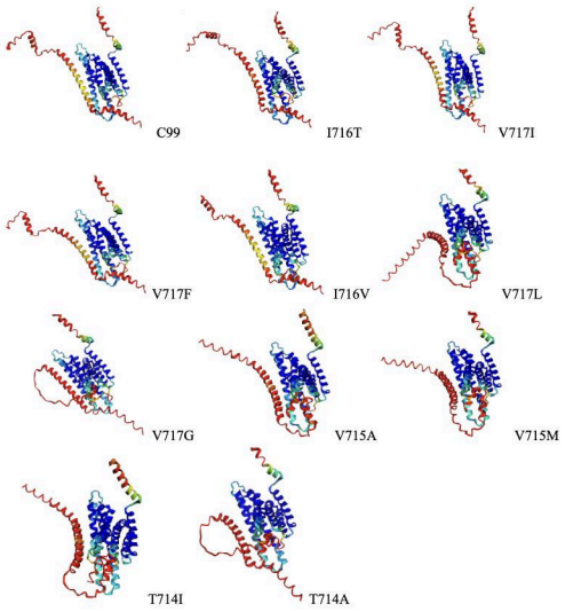

Figure 9. The protein on the left (mostly colored red) represents C99, while the larger, dark blue protein on the right represents APH1A. Modeled by Alpha Fold-2

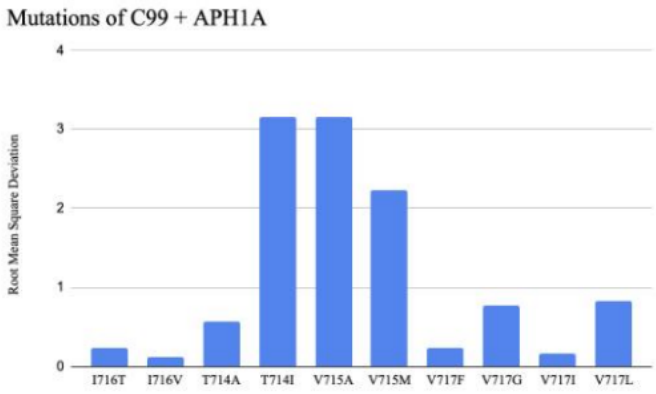

Figure 10. The RMSD values for C99 and APH1A interactions

4. Discussion & conclusion

In the Aβ wild type, the structure, especially that of Aβ-42 was seen to be made up of mostly beta-pleated sheets. The structure of β-sheets makes them more susceptible to oligomerization, contributing to its pathogenic properties, as well as its anti-microbial properties [10]. Additionally, as shown in results, the models of Aβ-40 and 42 were lacking in confidence. A plausible explanation may be because of its relatively flexible structure at its side chains, even when it is in plaque formation [11]. This may interfere with Alpha Fold-2's ability to predict one specific configuration, resulting in the red coloring.

In the Aβ-42 region, three mutations were analyzed. D694N (Aspartic Acid to Asparagine): Aspartic Acid is negatively charged amino acid with a carboxyl group, while asparagine is neutrally charged, but still retains polar properties. This may affect the aggregative properties of Aβ 42 – the negative charge in aspartic acid may play a role in reducing the interactions between Aβ, while the

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change to a neutrally charged residue reduces the repellent forces, and makes the Aβ more prone to oligomerization. The next mutation within the Aβ region is E693G (Glutamic Acid to Glycine), and E693Q (Glutamic Acid to Glutamine). These all have the same effect as D694N, especially as E693Q removes a negative residue.

It can also be assumed that the way in which V715A increases the ratio of Aβ-42:Aβ-40 isn't caused by the ineffective cleavage from PSEN1, but due to some other mechanism, which calls for future investigation into the reasons behind the pathogenic properties of this mutation. The additional interaction between C99 and PSEN1 in these V717 mutations may alter the positioning of the substrate within the active site, which will disrupt the cleavage process. It's possible that the structural changes induced by these mutations hinder the proper alignment of C99, which lead to the increased production of Aβ42 as the fragment gets released earlier. The presence of the red coloring on the model may indicate increased structural flexibility when associated with PSEN1.

For APH1A, the more stable the interaction, the more likely γ-secretase will complete the set of sequential cuts and produce Aβ40. The mutations examined seem to interfere with this stability in two main ways: by disrupting the folding of C99 around the APH1A binding site and by altering the flexibility of the TMD region. The V717G mutation stood out for its unexpected impact on the Aβ region, suggesting that some mutations may cause long-distance structural changes that extend beyond the immediate site of substitution. This could explain why even seemingly minor mutations have disproportionate effects on APP processing and Aβ peptide ratios. My results also align with what's known about the well studied V717I (London) mutation: it loosens the C99–γ-secretase interaction and increases Aβ42 without affecting Aβ40. AlphaFold-2 modeling offers a plausible explanation by showing how this mutation, though structurally subtle, disrupts binding with both PSEN1 and APH1A, leading to less efficient cleavage and increased pathogenic Aβ42 production.

This study demonstrates the ways in which various mutations of the genes involved in familial Alzheimer's disease can affect an individual's likelihood of getting the disease. These results corroborate past research, while also offering new insights into the mutations that may not have been modeled before, like T714I, or V715A. Analysis using Alpha Fold-2 showed that the mutations affected the conformation C99, which subsequently affected the ways in which they interacted with the PSEN-1 and APH1A subunits of γ-secretase. The binding differences affected the ways in which C99 was cleaved to become amyloid-β, with more mutations than not, increasing the likelihood of producing amyloid-β 42; due to the altered structure, the interaction between C99 and γ secretase destabilized.

It is also important to mention the limitations of this study. Because this study relies only on online software, Alpha Fold-2 is unable to account for factors like post-translational modifications, membrane environments, or conformational changes over time. As a result, the differences in binding or folding may not be fully reflective of the complex's behavior in vivo. Additionally, the two γ-secretase subunits modeled were in isolation. The full complex involves two more subunits, nicastrin and PEN2 (presenilin enhancer-2), whose effects have not been accounted for in this paper. These structural predictions by Alpha Fold-2 can be validated by experimental trials.

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