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Molecular Investigation of Alzheimer's Disease: Aromatic-Aromatic Interactions

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ABSTRACT

Alzheimer's disease is a neurodegenerative disorder characterized by cognitive decline and memory loss, resulting from a protein called amyloid beta. This review describes how Aβ forms, how computer models help scientists study it, and how clinicians detect its accumulation. Aβ proteins can misfold and stick together, forming fibrils and plaques that interfere with neuron function. These structures are held together by forces such as hydrogen bonds and aromatic–aromatic interactions, especially between the amino acids phenylalanine, tyrosine, and tryptophan. Computational simulations, such as molecular dynamics, have highlighted how such interactions take place and are used by researchers in designing drugs that can block them. Detection tools for Aβ buildup in the brain include CSF tests and PET scans. Newer and less invasive approaches, like blood-based biomarkers, make early detection and better treatments possible.

INTRODUCTION

Recent studies show that 1 in 9 people aged 65 and older has Alzheimer's disease (AD), a neurodegenerative condition that affects memory and thinking. Around 7 million people in the United States suffer from it and this number is expected to increase to 13 million by 2050[1]. There's no current cure but scientists have identified that one of the leading causes of Alzheimer's is genetic mutation of APP (Amyloid Precursor Protein) gene that affects the production and accumulation of amyloid beta (Aβ)[10]. Amyloid beta is a polypeptide (PP) that's composed of 38-43 amino acids and forms a cross-beta sheet where molecules are grouped into fibrils[20]. Fibrillation is primarily formed by the three aromatic amino acids: phenylalanine (Phe/F), tyrosine (Tyr/Y), and tryptophan (Trp/W)[6]. When amyloid beta proteins build up excessively, it creates plaques which disrupt neuronal communication and function, and potentially lead to apoptosis.

This article is a narrative review on the mechanisms of amyloid-β aggregation in AD, with a focus on the contribution of aromatic-aromatic (π–π) interactions. The central question is to what extent aromatic interactions contribute to Aβ aggregation relative to other forces, and how informative are they for

computational modeling and diagnosis? The paper is limited to Aβ pathology in humans and computational studies published between 2000 and 2024.

METHODS (LITERATURE SEARCH)

A focused literature search was conducted using PubMed, Google Scholar, and Web of Science. Keywords included were amyloid-β aggregation, aromatic-aromatic interactions, π–π stacking, molecular dynamics simulations of Aβ, and Alzheimer's disease biomarkers. Peer-reviewed studies and review articles published between 2000 and 2024 were primarily targeted. Articles were selected based on its relevance and methodological clarity. Studies that included tau pathology or unrelated neurotransmitter systems were disregarded.

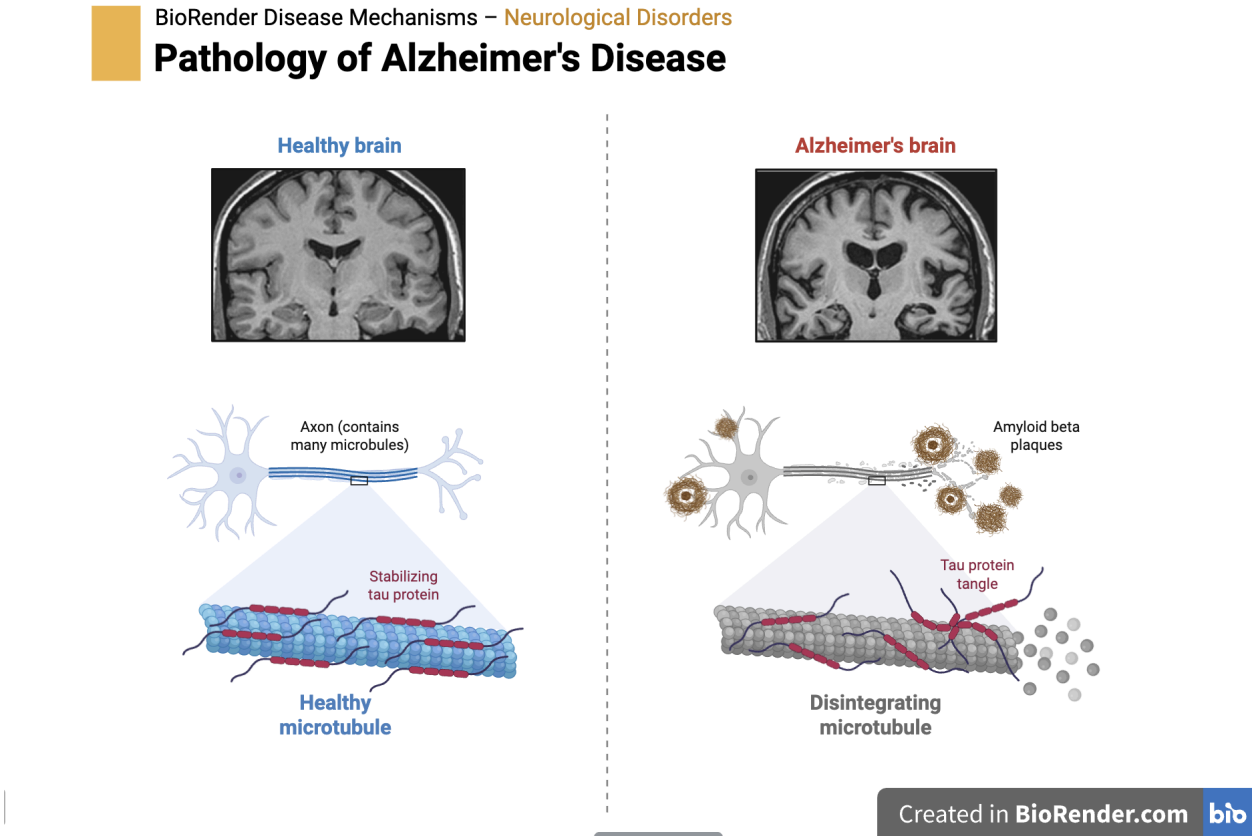

Figure 1: These are the key pathological differences between a healthy brain and an Alzheimer's brain, primarily focusing on brain atrophy and protein aggregation. The healthy brain has intact microtubules stabilized by tau proteins, while the Alzheimer's brain shows significant shrinkage, the accumulation of extracellular amyloid beta plaques, and intracellular tau protein tangles, leading to microtubule disintegration and neuronal dysfunction.

Formation of amyloid fibrils

Amyloid fibrils are highly ordered, β-sheet protein aggregates that form through a double strand process. This involves nucleation, elongation, pH, mutation, and higher-order assembly. The main feature of amyloid fibrils is their cross-β architecture, where β-strands form perpendicular to the fibril axis, making hydrogen-bonded networks that have great stability[11].

Fibril formation generally begins with the misfolding of a soluble monomeric protein into a β-sheet–prone conformation. In Aβ, this involves a transition from a mostly random coil to one that exposes hydrophobic and aggregation-prone regions[5]. The conformational shift is because of mutations, post-translational modifications, environmental factors, or interactions with lipid membranes. Primary nucleation, the first aggregation stage, involves the assembly of a small oligomeric nucleus that is the template for further growth[13].

Once a nucleus forms, fibril elongation continues through the addition of monomers to the fibril ends. It's driven by non-covalent interactions, including hydrogen bonding along the β-strand axis, hydrophobic packing between side chains, and aromatic–aromatic stacking in certain sequences such as Phe-rich regions[9]. Protofilaments are created, which are linear assemblies that intertwine to form mature fibrils with defined morphologies.

Next are secondary processes, like fragmentation of existing fibrils or surface-catalyzed nucleation on fibril surfaces, that greatly accelerate amyloid proliferation[24]. Fragmentation generates multiple new fibril ends, each serving as an elongation site, while surface nucleation uses soluble monomers to β-sheet templates without requiring full fibril breakage. In AD, amyloid fibrils of Aβ further form into dense extracellular deposits known as senile plaques. Plaques can incorporate various biomolecules, including metal ions, potentially influencing both stability and neurotoxicity.

Self-assembly refers to the spontaneous organization of molecular components into ordered structures without external help and is driven by specific intermolecular forces. Amyloid fibrillation is a specialized form of self-assembly where the resulting structure adopts the cross-β motif. Not all self-assembled protein aggregates are amyloid fibrils. Some may form amorphous aggregates or non-amyloid filaments.

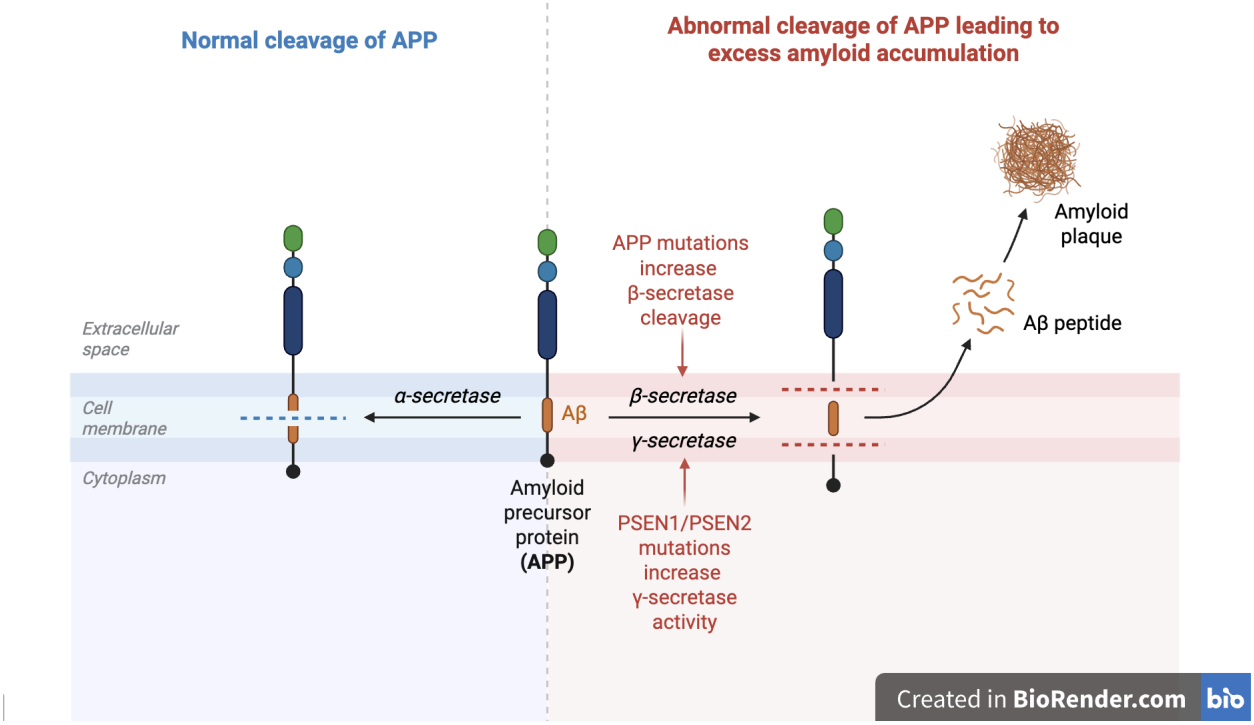

Figure 2: The two main pathways for the cleavage of APP are the normal pathway and the abnormal, amyloidogenic pathway. It includes the difference in secretase activity and cleavage, where gene mutations increased it in the abnormal pathway.

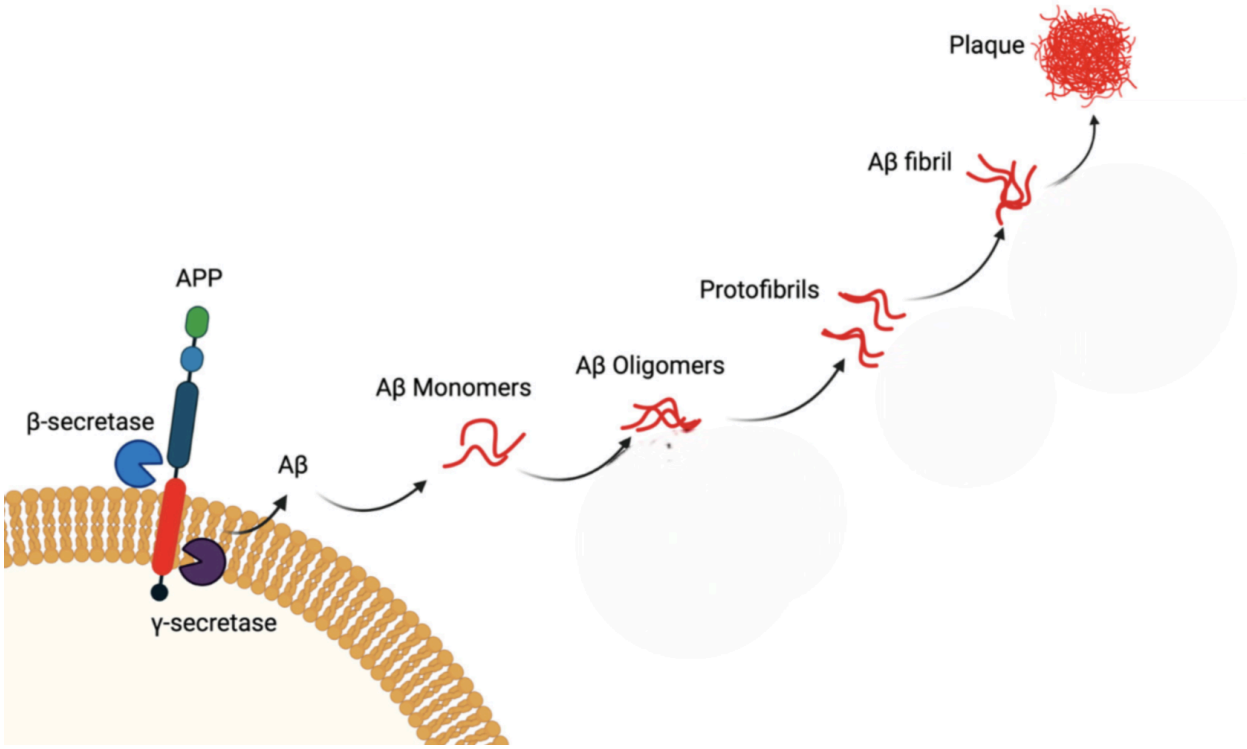

Figure 3. Targets of monoclonal anti-Aβ agents currently in phase III clinical trials. Adapted from Vogt et al., *International Journal of Molecular Sciences* (2023), CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). Changes: label removed.

Mechanism of amyloid beta

Aggregation of Aβ peptides is done by a dense array of noncovalent interactions that stabilize β-sheet-rich fibrillar structures[15]. Among these interactions are π-π interactions, which contributes to peptide self-assembly. Aβ contains several aromatic amino acids (Phe, Tyr, and Trp) that mediate stacking interactions between their π-electron systems, facilitating ordered alignment of β-strands. These π-π interactions are facilitated by quadrupole-induced attraction between adjacent aromatic ring delocalized electron clouds to create a noncovalent stabilizing network that enhances close packing of peptide backbones and overall fibril cohesion.

Two sequence regions are most critical in Aβ aggregation: the central hydrophobic cluster (residues 17-21) and the C-terminal (residues 30-42). Each region allows fibril growth and nucleation but places the Phe-Phe pair at residues 19 and 20 in a uniquely central role. π–π stacking between PHE19 and PHE20 of neighboring peptides directs parallel β-sheet structure development, which stabilizes protofilament interactions into mature fibrils. This configuration also drives hydrophobic collapse, where tightly clustered nonpolar side chains push water out to create a stabilizing aggregate structure. In combination with inter-strand hydrogen bonding along the β-sheet axis, aromatic stacking creates the cross-β spine structure characteristic of amyloid fibrils.

These aromatic interactions also account for amyloid fibril polymorphism, which are small changes in aromatic side-chain orientation that give rise to fibril morphologies with varying mechanical properties and toxicity of aggregates[21]. Such structural heterogeneity has been postulated to be a cause of the heterogeneous course of disease seen in Alzheimer's patients. In addition, aromatic-aromatic interactions lie at the heart of oligomeric intermediate formation, dynamic aggregates that predate mature fibrils but display increased neurotoxicity[4]. The small assemblies are able to embed hydrophobic aromatic residues into neuronal membranes, forming pores that interfere with ion homeostasis and cause oxidative stress, eventually resulting in synaptic dysfunction.

Aromatic residues also contribute to chemical reactivity of Aβ. Surface-exposed π-systems on fibril surfaces can chelate metal ions such as Cu^{2+} and Zn^{2+} to create reactive oxygen species that kill neurons and enhance oxidative stress[3]. Dense π-stacking networks also render fibrils highly resistant to proteolytic degradation, and this is the reason for their durability within neural tissue in the face of active clearance.

While aromatic interactions are important, they act alongside hydrophobic effects, electrostatic interactions, hydrogen bonding, membrane interactions, and impaired clearance mechanisms. Evidence for π-π interactions is strongest in computational and in vitro studies, though their role in vivo is not as well understood.

In concert, amyloid-β fibrillogenesis is regulated by the balance between aromatic stacking, hydrophobic packing, and hydrogen bonding. All of these interactions not only determine fibril shape and thermodynamic stability but also regulate the generation of toxic oligomeric intermediates that lead to neurodegeneration. Determination of how specific residues, such as PHE19 and PHE20, participate in these interactions is the foundation for rational drug design. Targeting π-π stacking motifs is a potentially useful therapeutic strategy, as blocking these aromatic interactions can inhibit fibrillization and diminish the downstream neurotoxic consequences that define Alzheimer's disease pathology[17].

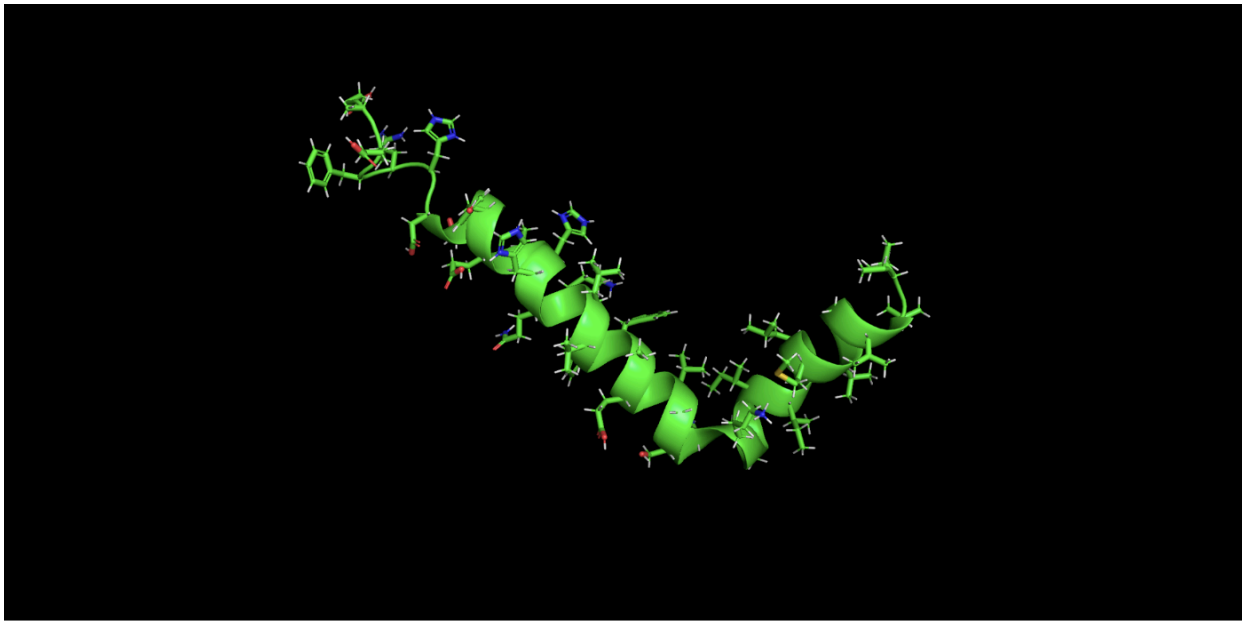

Figure 4: PYmol model of Aβ-43

Computational analysis of amyloid beta

Computer simulations have provided insightful information on molecular interactions and dynamic processes that cause amyloid-β (Aβ) misfolding and aggregation. Computational methods such as molecular dynamics (MD) simulations, quantum mechanical calculations, and coarse-grained modeling have enlightened the energetic and structural fundamentals of fibril formation[8]. These approaches have demonstrated that β-sheet–dominant structures arise from the cooperative action of hydrogen bonding, hydrophobic packing, van der Waals forces, and aromatic stacking, with each contributing to the stability of growing aggregates.

Simulations identify two critical sequence regions: the central hydrophobic cluster and the C-terminal region[19]. These hotspots facilitate intermolecular β-sheet formation to initiate aggregation. Calculations also show that early oligomeric intermediates (small, metastable clusters) are critical nucleation centers of the fibrillization process[2]. Oligomer stability is based on noncovalent interactions of hydrophobic and aromatic residues, providing a structural explanation for their known neurotoxicity.

Among the aromatic residues, PHE is particularly critical. PHE19 and PHE20 in the central hydrophobic cluster participate in tight π–π interactions that cause peptide stacking and β-strand registration between neighboring monomers. This interaction is a molecular glue that permits protofibril assembly and determines fibril morphology. Free-energy analysis and computational mutagenesis have shown that substitution of PHE19 or PHE20 by non-aromatic residues significantly reduces aggregation rates and destabilizes fibrils, supporting their contributory role rather than exclusive control over aggregation[7].

Besides static pictures, MD simulations reveal the dynamic nature of Aβ aggregates: how monomers switch between random coil and β-sheet–rich structures, and how solvent exposure, ionic strength, and temperature modulate aggregation rates. Coarse-grained modeling completes the story by extrapolating long-timescale processes, like fibril twisting and polymorphic variation. These models agree with the idea that subtle variations in aromatic ring orientation can generate fibril morphologies with different toxicities, consistent with experimental descriptions of Alzheimer's clinical heterogeneity.

Computational chemistry likewise completes the cycle between mechanism comprehension and therapeutic discovery. Molecular screening and in silico docking were central to the identification of peptides and small molecules to perturb π–π stacking at selective locations such as PHE19 and PHE20[16]. Certain candidate inhibitors have been designed to insert into aromatic interfaces, blocking fibril extension and inhibiting β-sheet stacking. Quantum mechanical calculations further aid in quantifying interaction energies, atom-level validation of binding affinities, and inhibitor specificity.

Reconciliation of computational and experimental results presents a balanced molecular model for Aβ aggregation and toxicity. Through specification of the particular contribution of aromatic residues and energetic landscape regulating fibril assembly, computational studies not only advance knowledge of Alzheimer's disease pathogenesis but also offer rational grounds for the development of targeted anti-amyloid therapies.

Molecular diagnosis of amyloid fibril

Molecular diagnosis of amyloid fibrils is an essential aspect of detection and management of AD, given that Aβ aggregation forms an early and most typical pathological feature of the disease. Accurate molecular diagnosis relies on biomarkers to identify amyloid deposition and the secondary effects that follow, therefore enabling clinicians to identify disease processes even in preclinical conditions. One of the most commonly used diagnostic techniques is positron emission tomography (PET) which scans using fibrillar Aβ plaque-selective radiolabeled probes. PET scanning provides a noninvasive method of visualization in brain regions and has been found essential to distinguish AD from other dementias[12]. At the same time, cerebrospinal fluid (CSF) analysis remains a prominent molecular diagnostic tool for AD. Decreased concentrations of soluble Aβ42 in CSF and altered Aβ42 to Aβ40 ratios have been positively associated with amyloid plaque deposition, reflecting segregation of Aβ into insoluble fibrils. Both CSF biomarkers and PET imaging are the best for diagnosis of amyloid pathology in AD but because of their invasive and expensive nature, studies have aimed at the development of less burdensome diagnostic technologies[14].

The development of new biomarker technologies has enabled improved promise for the AD diagnosis, notably with the creation of blood-based biomarkers. Advances in ultrasensitive technology such as single-molecule array (Simoa) platforms have allowed the measurement of Aβ species in plasma that are as accurate as CSF and PET measures. Aβ42 to amyloid-40 plasma ratios have been shown to significantly correlate with amyloid PET positivity, which supports the potential of plasma biomarkers as less invasive indicators of brain amyloid pathology[23]. Beyond plasma, novel technologies such as seed amplification assays take advantage of misfolded amyloid protein ability to self-replicate, thus small amounts of Aβ aggregates can be identified[18]. These technologies could potentially detect at presymptomatic ages, with a therapeutic window for treatment before extensive neuronal damage. While not widely approved in everyday clinical practice, these emerging tools signal the next breakthrough in Alzheimer's molecular diagnosis.

Regulatory approvals have facilitated the transition of amyloid biomarkers into the clinic, especially on their use in AD diagnosis. The U.S. Food and Drug Administration (FDA) has approved three amyloid PET tracers: florbetapir (Amyvid), florbetaben (Neuraceq), and flutemetamol (Vizamyl)[22]. These are made for clinical application for imaging amyloid plaques in patients being considered for AD. The medications permit clinicians to detect amyloid disease with high sensitivity and specificity, providing critical evidence when cognitive testing and structural imaging are nondiagnostic. In addition, CSF measurements of Aβ42, as for tau and phosphorylated tau, have progressively standardized and are being used more widely in clinical practice, though FDA approval for CSF-based tests is slower than for imaging agents.

FDA-approved biomarkers are rapidly improving Alzheimer's studies and treatment by permitting earlier and more accurate diagnosis, improved patient selection for clinical trials, and tracking of disease progression or drug action. Despite ongoing challenges, including cost, restricted availability, and the need for greater standardization, the integration of FDA-approved amyloid PET tracers with emerging blood-based biomarkers is establishing a new model for Alzheimer's diagnosis. With the evolution of molecular detection of amyloid disease, these technologies provide a foundation for early and precise diagnosis while also a pathway to improved therapeutic outcomes in a disease requiring earlier intervention.

LIMITATIONS

This review is limited by its narrative design and does not represent a systematic or comprehensive analysis of the literature. Emphasis on aromatic-aromatic interactions may underrepresent other important mechanisms such as membrane-mediated aggregation. Additionally, some studies discussed rely on simplified experimental or computational systems that may not fully reflect in human neuropathology.

CONCLUSION

Amyloid beta plays a significant role in the development of Alzheimer's disease. Understanding how it forms, behaves, and can be detected is essential for improving diagnosis and treatment. The formation of Aβ fibrils is caused by misfolding and strong molecular interactions, one such being aromatic–aromatic forces that allow proteins to stack and form plaques. Computational studies support these findings by showing how amino acids, like PHE, contribute to aggregation and by helping scientists to potentially block these interactions. Diagnostic tools, including PET scans, CSF testing, and newer blood-based biomarkers, make it possible to detect amyloid buildup earlier. These scientific and technological advances highlight the importance of targeting Aβ formation for future therapies. Continued research allows for earlier detection, better treatment options, and a deeper understanding of Alzheimer's disease.

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