Molecular diagnosis of Alzheimer’s disease

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Abstract. Since the beginning of time, Alzheimer's disease has been a major issue, which is a neurodegenerative disease which would cause to death. It involves both chronic and acute neurodegenerative. For treating these illnesses, there are few or no potential curative treatment options that are effective. The traditional diagnosis is invasive and people with AD can only treat by drugs, which is for relieve some of the symptoms. Therefore, early diagnosis is considered to be the key factor for prevention and treatment, and it has been gained more attention. Nowadays, it is one of the key topics of current research. Scientists have developed plenty of probes based on different compound, it has an incredible improve when using on the mice, however, it is still a big challenge to validate in human brain.

Keywords: Alzheimer's disease, fluorescence probe, biomarkers of AD, amyloid-β peptides, tau proteins, APOE.

1. Introduction

Alois Alzheimer, the physician who first described the Alzheimer's disease (AD), which is recognised as a progressive and multifaceted neurodegenerative disease with high mortality and high disability. It is a degenerative brain condition caused by a physical illness, and this the most prevalent type of dementia that develops with age, hippocampus atrophy is the neuroimaging hallmark [1]. Memory, language, logic, decision making, visuospatial function, attention, orientation and the loss of synapses and the formation of insoluble amyloid β clumps in the brain (Aβ) and tau proteins are few of the cognitive functions that could be negatively impacted by dementia. The cause of AD is these cells in the brain lose their connections with one another, all of which are interconnected with one another. This is due to the accumulation of proteins, which can lead to the formation of abnormal structures known as "plaques" and "tangles." After a certain amount of time, nerve cells and brain tissue are both lost. Currently, AD is categorised as preclinical, moderate cognitive impairment (also known as MCI), and dementia phases, National Institute on Aging and Alzheimer's Association have issued diagnostic recommendations (NIA-AA) which stated the most recent set of diagnosis guidelines [2]. Even though they have approved some drugs for AD treatment, it relieves only a portion of the symptoms and is more effective if used early on. Diagnostic modalities for AD include It has been demonstrated that magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) chemistry examinations are appropriate for diagnosing dementia: (i) MRI changes in tissue volume are not specific for AD and require much more effort; (ii) There is no evidence that CSF amyloid beta changes as the disease progresses; (iii) Invasive CSF collection [3]. Optical imaging enables safe detection.

However, recent findings suggest that they have good specificity, there are sixteen distinct fluorescent probes with dual functions have been created in recent years for the simultaneous detection of AD biomarkers. As numerous positive characteristics of fluorescence probes, For example, the capacity to detect diseases quickly and with low doses of hazardous probes while still having high sensitivity, efficiency, minimal invasiveness, real-time reaction, and cheap total costs [4]. This review will focus on probes based on several biomarkers and probes for detection those biomarkers.
2. Biomarkers

2.1. Amyloid-β peptides (Aβ)

Aβ peptides, as a biomarker of AD, is characterized by accumulations of its aggregates, however, Apolipoprotein E (APOE) would break this peptide down (See 1.3). In the brain, Aβ peptides can be found in the forms of soluble monomers, oligomers and insoluble aggregates; as the disease advances, these different types of Aβ eventually coalesce into senile plaques. At this time, it is considered that the form of Aβ peptides that is responsible for the majority of the neuronal toxicity is oligomers. It is widely accepted that Aβ peptide deposition is a key beginning component in the development of AD. These peptides originate from the amyloid peptide precursor (APP), which breaks down amyloid peptide into products consisting of 40 amino acids (Aβ40) and 42 amino acids (Aβ42) as a result of the sequential cleavage that is performed by β- and γ-secretases; Aβ40, which is the most frequent version in humans, but the C-terminus contains two extra amino acid residues, which is long form of Aβ42, was found associated with AD [5, 6]. Because it quickly forms fibrils, Aβ42 is associated with increased levels of neuronal damage (Y. Lai). In AD, there is an over accumulation of the Aβ peptides, this can be either an increase in amyloid precursor protein (APP) production or an ineffective removal of Aβ from the brain. The Aβ monomer plays a role in neuroprotection and immunology, but when it is misfolded, Excessive aggregation of oligomers results in both soluble and insoluble plaques within the brain. This can lead to neurological and immunological problems [6]. Lesions that are AD-specific include extracellular amyloid plaques that are formed from aggregates of toxic Aβ and intracellular neurofibrillary tangles that are made up of hyperphosphorylated tau [7]. However, Insolubility of amyloid fibrils and difficulty in purifying plaques and tangles have stymied attempts to characterise their protein composition for many years. This was one of the major causes of the calamity. However, as a result of the discovery of a technique to isolate amyloid plaques [8], The complete amino acid sequence of a 40-amino acid protein that was isolated from AD-affected brain tissue is presented here [9]. The Aβ cascade hypothesis proposes that Aβ deposition is the starting point and leads to a succession of processes, including tau hyperphosphorylation and aggregation, which ultimately culminates in neuronal dysfunction. Aβ deposition can occur anywhere from one to two decades before the symptoms show [3]. The evolution of Aβ plaque formation has grabbed the interest of researchers as a crucial diagnostic for the identification of AD early on.

2.2. Tau/phospho-tau proteins

Tau proteins are common in neurons of the central nervous system (CNS) and known as microtubule-stabilizing proteins which include 50–65 kDa and 352–441 amino acids, respectively [10]. Tau proteins in humans are spliced variants of a single gene; in total, there are six unique variants of tau [11]. It has two different types of tau proteins, total tau (T-tau), and phosphorylated tau (P-tau). Tau is a phosphorylated protein, Tau proteins distort when they are phosphorylated and can no longer effectively stabilise microtubules [11]. According to research in 1986, It has been demonstrated that abnormally hyperphosphorylated tau protein, which has three times the number of phosphorylation sites compared to normal tau, forms tangles. In addition, the surrounding amyloid plaques, neuropil threads and dystrophic neurites contain aggregated hyperphosphorylated tau [7]. In all neurodegenerative diseases which tau is involved, they are all in hyperphosphorylated form, as a result, it causes neuronal malfunction and death [12]. The cytotoxic effects of hyperphosphorylated tau, which is insoluble and has no affinity for microtubules, as well as aggregation of aberrant tau molecules which can be seen in certain cancers[13]. Even in the absence of additional foreign stimuli, such as β-amyloid, the overexpression of nonmutant human tau was shown to result in the development of AD [14], indicating that the quantity of tau or the ratio of isomer may have a significant role in the pathophysiology [15]. Consistent with the review [7], T-tau and P-tau levels in the cerebrospinal fluid (CSF) are linked to an increased risk of cognitive disorders over a clinically relevant time period of 1–2 years is better than Aβ42. Generally, targeting tau protein may prove to
be better therapeutic intervention [1]. Detached phosphorylated tau forms fibrillar deposits which is a prominent clinical characteristic of AD.

2.3. Apolipoprotein E (APOE) gene polymorphic alleles

APOE, which is a protein with highest expression in the liver and brain. APOE gene acts an important role in the brain, which is a principal cholesterol transporter reaching neurons via APOE receptors in the brain [16], which also is a significant genetic risk factor for more common diseases, complex, late-onset AD. Neuronal signalling, glucose metabolism, neuroinflammation, and mitochondrial function are all regulated by APOE [17]. APOE provides instructions for making a protein, which is called apolipoprotein E, which is a protein involved in fat metabolism, and its subtype is associated with AD and cardiovascular disease.

It circulates as a component of lipoprotein particles (when AOPE combines with fats (lipids) in the body to form molecules) [18], APOE has a strong interaction required for catabolism is the low-density lipoprotein receptor (LDLR). The liver and peripheral organ macrophages produce APOE, which has an impact on cholesterol metabolism [4]. Astrocytes manufacture the majority of the APOE found in the brain, which is then transported to neurons through APOE receptors [19].

APOE gene cause a change in the amino acid sequence (Cys/Arg), it’s a protein of 299 amino acids with variations in 112 (Cys/Arg) and 158 (Cys/Arg), E2, E3, and E4 are the three alleles that make up this [20] (figure 1). E3, which is more common that other two alleles, has lower risk of AD that E4, whereas E2 has the lowest risk of AD.

Jiangshowed that the proteolytic breakdown of A can be attributed to the activity of APOE. Clearance of A peptides in the brain is variably regulated by APOE isoforms [21]. APOE4 is not as effective as others, which is the reason why it increased vulnerability to AD [21]. Nevertheless, One-third of Alzheimer's patients are APOE4-negative, while some APOE4 homozygotes do not suffer from the illness at all. As a result of the intricacy of the pathogenic processes that lead to AD, the biological mechanisms behind this connection remain unknown.

![Figure 1](image-url)

**Figure 1.** A graphical depiction of the APOE SNPs and genotypes. The arrows indicate the location of SNPs and the related amino acid changes. Three APOE alleles are the product of two SNPs (rs429358 and rs7412) with high linkage disequilibrium (E2, E3 and E4). A major genetic risk factor for AD is APOE 4. The Apo-E2, -E3, and -E4 isoforms, which are respectively encoded by the 2, 3, and 4 alleles of the APOE gene, vary at amino acid residues 112 and/or 158. SNPs: single nucleotide polymorphisms.
3. Probes

3.1. Probes for the detection of Aβ species

For more accurate detection of Aβ aggregates in AD. Different types of probes based NIR fluorescent have been developed.

Probe 1 (AOI987, Figure 2) [6], when coupled with Aβ plaques, it displayed very minimal affinity and fluorescence quenching, although in vivo imaging of Aβ plaques in the brain with its maximum em wavelength of 670 nm was successful [22]. Researchers in Moore's lab have developed a difluoro boron curcumin probe 2 (Cranad-2) for NIR imaging of Aβ plaques in vivo. (Figure 2), which has em 805nm, a 70-fold rise in intensity and a blueshift from 805nm to 715nm were observed after binding with Aβ plaques, connected with the binding affinity which has Kd of 38.69 × 10⁻⁹ m. Bioimaging indicated that probe 2 was able to cross the blood-brain barrier (BBB) and particularly stain senile plaques in the brain. Probe 3 (DANIR 2c, Figure 2), the moiety of which has N, N-dimethylaminophenyl as its moiety and a moiety of dicyanomethylene linked. Cui et al. disclosed in 2014 the existence of these unique, tiny probes with donor–acceptor design [6]. NIR emitted by solution from this probe measured 665 nm due to its lengthy chain. Probe 3 showed the great affinity 3 of 26.9×10⁻⁹ m when it is binding with Aβ plaques.

Inspired by the above experience, as water solubility and penetration depth are critical for fluorescence imaging of biomolecules in a physiological setting, for bioimaging, a water-soluble and NIR-emitting probe is essential. Cui and colleagues had created more adaptable probes 4 (DANIR 8a–c) and 5 (DANIR 9a–c) with the addition of hydroxyethyl groups to the donor group (Figure 2) [4]. Notably, probe 4 was the most delicate probe with a 629-fold fluorescence increase, a substantial blueshift of 120 nm, an excellent affinity of 14.5–10–⁹ m, and adequate brain biokinetics. A successful BBB penetration was demonstrated in ex vivo using probes 4. Moreover, Quinoline-malononitrile (QM) which is modified by Zhu et al, they used sulfonate to improve the water solubility [23]. Chemists can change the emission wavelength from red to NIR by modifying the substance. A novel fluorescent probe (TM-1) (Figure 3) with the effect of intramolecular charge transfer (ICT) has been created by attaching ANCA to QM when it was adorned with a sulfonate, The ANCA group facilitates access into the hydrophobic location of Aβ species to prevent rotation of the conjugation system, resulting in a substantial increase in fluorescence [24]. TM-1 showed high affinity towards Aβ aggregates [24].

Probe 6, which is based on cyanine. Probe 6 revealed a large bathochromic shift of 59 nm in the presence of Aβ plaques, causing the solution's colour to change from light pink to violet. This was due to electrical structural alignment and intramolecular positioning modifications induced by probe 6 and plaque binding. Concurrently, a 30-fold increase in activate red fluorescence with a Stokes shift of 117 nm and the production of twisted ICT (TICT) complexes were observed. Both the Probe 6 was more likely to connect to Aβ plaques, therefore giving a new diagnostic tool for AD [6].
3.2. Probes for the detection of tau species

In order to detect specifically and distinguish tau and Aβ species, fluorescent probes of a novel class have been produced by Alexander et al, which based on pyrazine, pyrimidine and pyridazine derivatives [22].

Fluorescence microscopy and thiazine red displacement experiment demonstrated that these probes (7a and 7e) bind to tau aggregates with great affinity (Figure 4) [8]. The vivo image of AD mouse model transgenic for 7a accumulation in the brain revealed the presence of aggregates. The fluorescence imaging investigation confirmed the capacity of probe to stain NFT in AD brain tissue slices Pheny, pyridinyl, butadienyl-benzthiazoles and benzthiazoliums (also known as PBBs) derivatives of ThT were synthesized by Maruyama et al (Arora, H). Analyses carried out using fluorescent microscopy on transgenic AD mice revealed that PBB5 (Figure 5) [8] attach in particular to Tau clumps that emit infrared light (685 nm), therefore in vivo optical NIR imaging of tau is therefore achievable; however, PBB5 is limited in its ability to cross the BBB with a faster metabolism in the body. In an effort to solve this problem, PBB3 (Figure 5) [19] is used to analyse tau in vivo by means of two-photon fluorescence scanning microscopy. This probe efficiently traverses the BBB, stains neurofibrillary tangles in the brain, and is suitable for imaging in vivo.
addition, PBB3 recognises Tau fibrils devoid of signals that are less specific when compared to negative controls [18]. The other probe, which is soluble, non-toxic and biocompatible have been developed, it is known as trimethine cyanine dyes. They successfully stain the olfactory tissue's deposits of Tau fibrils, which can be used as non-invasive imaging techniques for Tau fibrils and the detection of Tau pathology [5]. As detailed by Rejc et al., the process of synthesising a sequence of compounds depending on the structure of the parent chemical 2-(1-(6-((2-[18F] fluoroethyl) (methyl)amino)-2-naphthyl) ethylidene) malononitrile [22]. One of the probes has demonstrated excellent specificity for Tau fibrils over Aβ, as well as a strong affinity for Tau aggregates. (Figure 6). Verwilst et al. have developed two BODIPY-based fluorescent probes, which is the purpose of detecting bodily tau tangles, tau 1 and tau 2 (Figure 7)[25]. The crystal structure and docking investigations of the tau hexapeptide fragment (PHF6) with probes tau1 and tau2 showed that the probes fit snugly inside the protein molecules' tunnels. When there are tau tangles present, At 650 nm, the fluorescence intensity increased significantly (6.4-fold for probe tau1 and 9.3-fold for probe tau2), while the fluorescence of Aβ fibrils was not elevated [6]. Due to the fact that probe tau1 has the maximum solubility, following injection into the tail vein, it displayed good BBB penetration and was used to image pyramidal neurons in the hippocampus using fluorescence microscopy to identify tau tangles in vivo [6].

Zhu et al, have constructed another series of pyrazine and quinoxaline probes for the detection of tau tangles. To improve the physiochemical properties of QNNs, π-conjugation links electron-withdrawing pyrazine/quinoxaline with electron-donating N, N'-dimethylamino-containing pyridine benzene thiophene [6]. Among these QNNs, tau3 (Figure 5), it showed the 603 nm yields the highest performance in the long term and had positive BBB penetration while also having outstanding brain dynamics.

In 2017, a brand new investigation was introduced by Yanagisawa et al, tau4 (ShigaX35, Figure 7), for multimodal imaging of tau, a fluorine-19-labeled butadiene derivative is used [2]. Due to the use of 19F, 19F-MRI also has the advantages of high sensitivity, minimal background, and economical. As a result, a helpful method for diagnosing tau pathology in AD is called multimodal imaging agent 115r diagnosing tau pathology.
4. Conclusion

The development of fluorescence probes has brought significant benefits to humans compared with traditional methods. It achieves the aim of early diagnosis with involving in surgery and warrant scientist to study probes in vitro, ex vivo, and in vivo. Fluorescence probes well resolved the problem that those traditional modalities are not widely accepted. Understanding neurodegenerative disorders is indispensable, particularly in the early stages, the diagnoses, and clinical intervention, which requires the correct identification of the knowledge of the formation, accumulation, and development of neuropathological biomarkers. As the disease advances, AD biomarkers are believed to indicate both the neurodegeneration's genesis and several related neural processes, worsen them and creating a complicated pathophysiology. Thus far, this review has concluded three different biomarkers for the diagnosis of AD and probes which based on different compound for the detection of Aβ and tau species, which are the most possible molecular for AD diagnosis and imaging. Furthermore, the merits and demerits have been mentioned in this review, for probes that based on different compound, it requires divergent conditions for the better detection. For instance, the probes that detect Aβ species, increase their selectivity and reduce the number of soluble proteins and other cellular and tissue constituents they bind to are necessary. Although the scientists have done plenty of experiment which involve using probes, it is currently difficult and complex to verify the validity of these probes inside the brain. Early diagnosis is not the only one challenge scientists, but fluorescence probes is also a big provocation in neurodegenerative illnesses.

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