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Review

Molecular Biomarkers for Diagnosis & Therapies of Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) has been discovered before the century but scientists still have not found the way to cure the disease. The basic requirement for successful cure requires the early diagnosis of the disease. Presence of Aβ42, total tau protein and phosphorylated tau have been used in the earlier days but these diagnostic markers fail the detection in the initial stages. Hence the need of the hour is to identify the various biomarkers which can be detected in the earlier stages of AD. Impaired cellular signaling is common in all the diseases and identification of particular signaling pathway helps in the identification of biomarker. Important signaling pathways such as Akt, FAS/NO, MAPK, Ca^{2+} are found to be altered in the AD brain. Protein molecules upstream or downstream to these signaling molecules can be potential molecular markers in the diagnosis of AD. This review comprises molecular markers which are crucial in the AD and play a significant role by altering the signaling pathways. These biomarkers will not only help in the understanding of pathobiology of AD but also provide an insight for the researchers working in the direction of AD biomarker discovery and drug discovery.

Keywords: molecular markers; $A\beta_{1-42}$; Tau; amyloid plaque; neurofibrillary tangles; hyper-phosphorylation

1. Introduction

Among many other neurological diseases Alzheimer's disease is the most wide spread and deadliest disorder. The symptoms of this disease start with frequent memory loss but ends with severe immobility, total loss of verbal skill and confusion about past and present. In the current scenario by the time the individual is diagnosed with AD, he is almost into the 2nd or 3rd stage of the disease. According to Alzheimer's Association, AD is the 6th leading cause of death in USA and every 66 seconds someone in the USA develops AD. According to Alzheimer's disease International UK, approximately worldwide 46.8 million people were affected with AD in 2015 and the number will rise by 68% by 2050. The numbers suggest why it is important to study the pathobiology of Alzheimer's disease and come up with molecular marker for early detection and treatment. In order to develop a biomarker it is essential to understand the mechanism behind the development and progression of disease.

2. Mechanism behind the Alzheimer's Disease

Memory loss in an AD is a result of constant neuronal damage caused by development of amyloid plaque and neurofibrillary tangles (NFTs) [1,2]. Amyloid plaques mainly comprises of insoluble Aβ fragments of varying amino acid length [3]. These fragments are generally formed due to the cleavage of amyloid precursor protein abbreviated as APP. APP is a substrate of variety of proteases including α-secretase, β-secretase and γ-secretase which are involved in the pathogenesis of AD [4]. APP is processed by one of the two competing pathways. In a healthy person the pathway which is predominated involves, cleavage of APP by α-secretase and γ-secretase resulting in the formation of soluble fragment [4]. This soluble fragment is generally known as sAPPα. The generated sAPPα fragment has shown to have various neuro-protective effective [5]. The other pathway involves β and γ-secretase cleavage of APP. This pathway results in the release of insoluble Aβ fragments which are capable of forming toxic amyloid plaques [4]. Cathespins, an another class of proteases have also shown β-secretase like activity [6]. APP cleaving by β and γ-secretase also releases amyloid precursor protein intracellular domain (AICD) [7]. This AICD generated due to amyloidgenic pathway can only perform nuclear signaling which may result in the progression of Alzheimer's disease [8]. AICD act as a gene expression regulator, controlling expression of many genes related to AD pathology including NFT [8].

NFT is another important pathological condition related to AD [9]. The most crucial component of these NFTs is hyper-phosphorylated tau (pTau) protein [9]. The hyper-phosphorylation is carried out by variety of protein kinases, some of which are GSK3β, CDK-5, mitogen activated protein kinase (MAPK1) and casein kinase 1 [10]. Tau proteins are usually associated within the neurons and causes many neurological disorders which involve Alzheimer's disease, Pick's disease, progressive supranuclear palsy are some of the taupathies extensively studied [11]. Figure 1 shows the generalized processing of APP and Tau aggregation in normal vs AD brain.

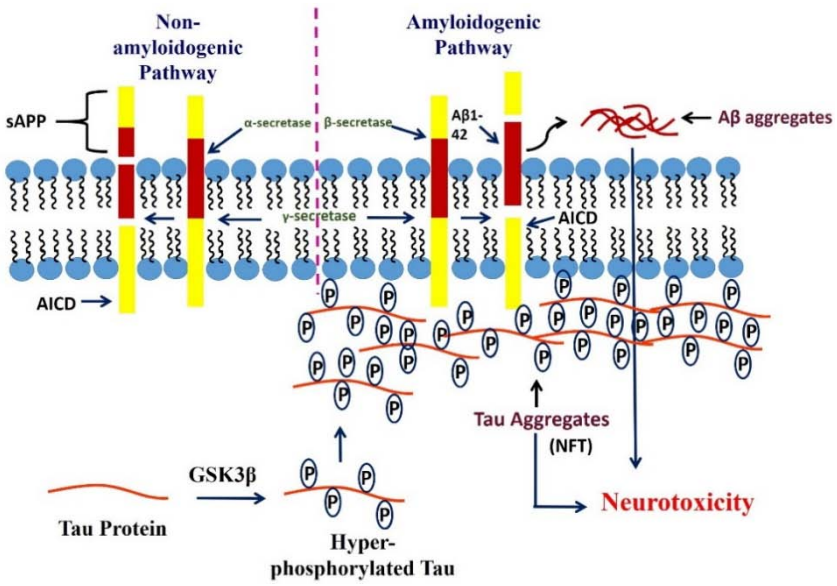

Figure 1. Schematic differentiation between Non-amyloidogenic and Amyloidogenic Pathway Genetic mutations behind Alzheimer's disease.

AD is broadly divided into two types, sporadic and familial AD. Sporadic is further classified into two categories, early onset and late onset AD. Early-onset AD (EOAD) is rarely observed where the people are affected and diagnosed with AD before the age of 65. Late onset AD (LOAD) is very frequent form of dementia affecting in people of age more than 65. Familial type of AD (FAD) is a form where the disease is observed within the family due to mutation and is inherited in the generations. Point mutation at SMT2 gene on chromosome 1 is responsible for AD [12]. Chromosome10 have various loci which were observed to increase the Aβ accumulation in AD patients. Many of the loci were found near Insulin degrading enzyme (IDE) [13]. IDE is capable of degrading Aβ fragments. This suggests that mutation at gene coding for IDE may result in abnormal increase in Aβ accumulation and amyloid formation. A risk locus has been found on chromosome 12 [14]. The SNP is observed near the gene coding for Vitamin D receptor (VDR). Vitamin D deficiency is associated with learning and memory dysfunction in adults [15]. Hence it is possible that alteration in the expression of VDR may lead to memory impairment in AD patients. Evidence exist for locus near to α-1-antichymotrypsin gene present on chromosome 14 can also results in FAD [16,17]. The well-known mutation in the AD is mutation in the gene coding for presenilins (PSEN). Presenilins 1/2 are important component of APP cleaving enzyme γ-secretase. Gene for presenilin 1 is present on the chromosome 14 whereas gene for presenilin 2 is observed on chromosome 1. Several studies show that mutation in presenilin 2 is a relatively rare cause of AD when compared to presenilin 1. Chromosome 19 is also associated with both sporadic and familial AD due to apolipoprotein E gene allele ε4 located at 19q13.2 [18,19]. Apoliprotein E combines with the fats to form lipoprotein, this lipoprotein carries out transfer of cholesterol along the bloodstream. Individual inheriting one or

more than one copy of apoliprotein ε4 allele are said to be more susceptible for AD [20]. Merely exhibiting apo ε4 allele does not necessitates AD, since study exist where apo ε2 and apo ε3 can lead to taupathies observed in AD [21]. Last but not the least, mutation of an APP coding gene on chromosome 21 is considered important for an EOAD [22]. All these mutations are useful for the diagnosis of familial type of AD i.e. early onset Alzheimer's disease. The diagnosis of LOAD is carried out by neuropsychological testing and brain imaging. Brain imaging studies involve techniques using Magnetic resonance imaging (MRI), computerized tomography and positron emission tomography.

3. Molecular Markers Involved in AD

In the early stages of AD, symptoms of the disease coincides with the other neurological dementia. It is important to distinguish the AD from the other diseases in the early stages. Established biomarkers involved in AD are based on the analysis of cerebrospinal fluid (CSF) content for lowered $A\beta_{1\text{-}42}$, increased phosphorylated tau (pTau) and total tau (tTau) [23]. These biomarkers can only be seen in the CAF in later stages of AD. Hence it is crucial to identify early diagnostic markers for AD, since early diagnosis helps in starting the treatment in the earlier stages. These biomarkers can be either CSF based or blood based biomarkers. AD is a complex disease and the actual cause of the disease is unknown. In the initial stage $A\beta_{1\text{-}42}$ and tau proteins act as signaling molecule in AD and they cause the disease progression by impairing cellular signaling. But what causes the production of Aβ and tau is a mystery and faulty cellular signaling can be a potential reason. In this review, we have tried to summarize about these potential biomarkers for the diagnosis of AD. These biomarkers are important since they are related to the production of Aβ fragments, tau filaments and the aggregation of these proteins. There are some molecular markers which are also associated with other neurological disease which can be used along with other AD markers in chip based or micro-array based method for the diagnosis of AD. These molecular markers can be classified under 3 types which are Aβ aggregation related markers, NFT Formation related markers and Ca^{2+} signaling pathway related molecular markers.

4. Aβ1-42 Aggregation Related Molecular Markers

4.1. Human Kallikrein 6

Kallikreins belong to the subfamily of serine proteases which can cleave a vasoactive peptide known as kinin from the kininogen. Human kallikrein 6 is one of the various isoforms of kallikrein and it has several names such as zyme, protease M and neurosin. It is highly expressed in the neurons along with many other cell types [24]. The important function of this enzyme is to degrade the α-synuclein (α-syn) which is released by the neuronal cells and is harmful outside the cell. α-syn forms the protein aggregates and spread in similar fashion as that of amyloid plaque and the disease

condition is known as synucleinopathies [25]. Some of the examples of synucleinopathies are Parkinson's disease (PD), pure autonomic failure, multiple system atrophy and dementia with lewy bodies [26]. Comparison of kallikrein 6 immunolabeling performed on AD, PD and control patient revealed that the enzyme was rarely present in the damaged area of brain and RT-PCR showed decreased m-RNA indicating low gene expression of kallikrain 6 [27] which shows non specificity of kallikrain as a biomarker for AD. Since kallikrain is a serine protease it may possess the Aβ clearance activity and decrease in its expression may be associated with the Aβ accumulation as shown in figure 2. Study conducted by Diamandis et al. 2000 reported first time the increased concentration of kallikrain in CSF of AD patients and proposed the idea of using this enzyme as a biomarker in AD. This suggest that protein and m-RNA level of kallikrein 6 can be done in the brain as well as CSF. The study was conducted on the small group of people and required further confirmation in large sample size [28].

4.2. Hypocretin-1/Orexin A

Hypocretin name was given based on their secretion from hypothalamus. Hypocretins are involved in the formation of neurotransmitter system [29]. These are the important peptide which have the ability to regulate neuroexcitory activity, circadian rhythm and sleep [30]. Hypocretin-1 is widely studied for their role in the neurological disorders which include AD, PD and Huntington's disease (HD). In PD and HD patients there is no significant alteration in CSF levels of hypocretins-1 [31,32]. This may state that hypocretin can be a specific biomarker for AD. In AD patient's CSF increased hypocretin-1 levels in the initial staged were observed by Dauviellers et al. Its expression in CSF is increased along with the tTau and pTau content which causes sleep impairment mainly insomnia. *In vivo* study has shown the effect of sleep impairment on the aggregation of the Aβ. Sleep restriction caused increase in the rate of Aβ accumulation. Sleep enhancement via blockage of orexin receptor inhibited the Aβ accumulation. The protein level were determined in the CSF of the sample [33]. The contradicting results have been shown by Friedman et al. 2007 stating decreased hypocretin-1 level in the early stages of AD. This study was supported by another study conducted by Slats et al. 2012 which showed decreased hypocretin-1 and $A\beta_{42}$ in CSF of AD patient [34,35]. One of the studies has revealed gender specificity for immunoreactivity of hypocretin producing neurons in control group. Female AD patients have shown less orexin producing neurons when compared with the male AD patient [36]. Polymorphism in the gene HCRTR2 expressing hypocretin receptor 2 is also associated with the risk of AD [37]. Overall hypocretin levels in CSF can be used as a potential biomarker, but the various contradicting results along with the gender specificity discourage its exploration in biomarker related aspects.

4.3. Neuroserpins

Some of the proteases have the ability to degrade the Aβ filaments and oligomers, for example plasmin. Plasmin is serine protease which are produced as a non-active form called plasminogen [38]. The conversion of inactive plasminogen to active plasmin is carried out by the tissue plasminogen activator (tPA). Formation of plasmin is inhibited by inactivation of tPA which is carried out by neuroserpins. Neuroserpins are exclusively found in brain tissue and it is involved in morphological maturation of neurons [39]. In AD brain plasmin and tPA both have shown the neuro-protective nature. Plasmin and tPA can both increase APP processing by α-secretase [40]. Increased expression of neuroserpins has been observed in CSF of AD patients [41]. The neuroserpins and tPA both are co-localized with amyloid plaque in AD patient brain [42]. Knocking out of neuroserpins reduced the Aβ aggregation and showed almost normal memory function [43]. Interestingly study performed by Kinghorn et al. 2006 showed that the complex formed by the neuroserpins and Aβ is neuroprotective in nature [39]. Up-regulated neuroserpins expression observed by western blotting is co-related with the increased expression of Thyroid hormone rerceptor-1-β (THR-1β) and RNA-binding protein HuD. Binding of thyroid hormone to THR-1β induces expression of many genes one of which is HuD. The HuD increases the expression of neuroserpins by stabilizing the mRNA. This suggest that thyroid hormone response system is playing an important role in the development and progression of AD [44]. Overall, neuroserpins protein level quantification can be done in CSF of AD patient but further studies have to be done in order to validate the biomarker potential of neuroserpin. Also there is no evidence for the up-regulated expression of neuroserpins in PD and HD in order to understand its specificity as an AD Biomarker.

4.4. Spingosine-1-phosphate (S1P)

S1P is phosphorylated form of spingosine, a membrane lipid. The reaction is catalyzed by enzymes spingosine kinase 1 (SphK1) and spingosine kinase 2 (SphK2) [45]. The expression of SphK1 is decreased by the $A\beta_{42}$ whereas, SphK2 expression is not altered [46]. SphK1 play an important role in the neuroprotection by activation of Insulin-like growth factor (IGF-I) as shown in figure 2. IGF-I is an important factor in AD since it is one of the enzyme having capability to cleave the Aβ peptides [47]. The phosphorylation of spingosine carried out by SphK1 results in the product S1P. S1P is an important signaling and cytoprotective molecule acting via G-protein coupled receptor [45]. It is also important in neuronal development since it is involved in the signaling pathways of neurogenesis and angiogenesis [48]. The link between AD and spingosine starts from the ApoE. Where the secretion of S1P is controlled by the ApoE. The variation in the ApoE gene and reduced expression of SphK1 can result in the loss of S1P [49]. This study was done by performing western blotting on the brain tissue sample of AD patient. It showed region specific expression of S1P. It was expressed in regions of brains which are affected early in the AD this involves hippocampus and temporal lobe. The loss in the S1P and SphK1 is found in the early AD event

which states its importance in its use as a biomarker in AD. However down-regulated S1P is observed in case of PD also [50]. S1P is degraded by the enzyme sphingosine-1-phosphate lyase (SPL). Immunohistochemistry have revealed that in AD brain SPL expression is increased whereas, expression of SphK1 is reduced [49]. Interestingly experiment carried out by Takasugi et al. 2012 reported increased expression of SphK2 in AD patients. Importantly S1P was found to be associated with the activity of β-secretase [51]. Hagen et al. 2011 found that whenever the S1P is produced by the SphK2 it induces the neurodegeneration [52].

4.5. Cystatin C (Cys C)

Cys C also called as γ-trace was first identified in CSF. The Cys C have many biological activities which includes tumor metastasis, cell proliferation, growth and inflammatory response modulation [53]. The decrease in the Cys C is observed in various neurological disease which include ALS and AD [54,55]. Whereas in case of PD, protein is found to be elevated in the serum of a patient [56]. The Cys C levels in the CSF and plasma were found to be lowered in AD patients which was measured by ELISA [57]. The polymorphism within the CST3 gene coding for Cys C have shown increased susceptibility to cause AD. The polymorphism induced AD condition was seen in the LOAD than the EOAD [58]. Earlier in this article we stated presenilin 1 mutation causing AD when compared to presenilin 2. But the recent studies have shown mutation in presenilin 2 caused reduced expression of Cys C [59]. Taking into consideration the neuroprotective activity of Cys C it can be said that, though presenilin 2 mutation is less likely involved in the AD pathogenesis but it may be the crucial component in progression of AD. One of the important function of Cys C is inhibition of cathespin B as shown in figure 2 [60]. Hence decreased Cys C leads to the increased amyloid plaque formation. Binding of Cys C to Aβ also inhibit the amyloid plaque formation [61]. Contradicting to neuroprotection of Cys C by binding to Aβ another study stated that aggregation of Cys C and Aβ in cerebral vessels will lead to the cerebral hemorrhage in the AD patient [62]. Also strong Cys C neuronal immunostaining was seen in the AD patient brain whereas, the staining was absent in the control. Also the neurons showing the immunostaining were most susceptible to the cellular death when compared to the neurons without staining [63]. The role of Cys C in the AD development is controversial also taking into an account it is deregulated in various neurological disease it is important to consider it with multiple AD related markers.

4.6. Calsyntenin (Clstn)

Calsyntenin are transmembrane protein belonging to the cadherins superfamily. These are synaptic proteins expressed in 3 forms Clstn1, 2 and 3. Clstn1 is an adaptor protein which is involved in trafficking and processing of APP [64]. Clstn1 is reduced in the AD brain and it plays crucial role in the increased production of Aβ and hence it makes Clstn1 a potential biomarker for the AD. The quantification of protein was done by immunoblot assay on the frozen tissue of AD brain. APP

within the neurons is transported through motor protein kinesin-1. Clstn1 is protein which bind to this kinesin and it is colocalized with APP in the vesicles [65]. Whether the presence of Clstn1 for the transport of APP within transporting vesicles is important or not is yet to be evaluated. Clstn1 is found to be down-regulated in CSF of both AD and PD [66]. As Clstn1 is down-regulated in AD and PD, for further diagnostics, AD specific marker need to be analyzed simultaneously, most probably by chip based assay. Studies have demonstrated that Clstn1 protects the APP cleavage by BACE1 within the vesicles. Decreased expression of Clstn1 mediated via si-RNA increased the APP processing by BACE1. This suggest the association between the APP and Clstn1 which protects from the toxic Aβ generation [67]. Clearly further study needs to be done in order to establish Clstn1 as a biomarker for early diagnosis of AD. The other calsyntetin, Clstn 3 is also found to be related with the AD. Clstn3 is important neurodevelopmental molecule correlated with the cognition. Clstn 3 is up-regulated in the AD brain and the up-regulation is mainly induced by the Aβ. Quantification of protein was done by immunofluorescence assay on transformed embryonic rat cells. The increased expression of Clstn-3 leads to the death of the neurons. The Clstn 3 surround the amyloid plaque generated [68]. Further studies showed Clstn 3 is substrate for α-secretase and γ-secretase. The C terminal fragment of this proteolysis accumulates surrounding Aβ in mouse brain. This shows the pathological role of the Clstn- 3 in the AD [69]. Clstn 3 have not been explored as a biomarker in the AD diagnostics but future studies will help in proving it as an emerging biomarker.

5. NFT Formation Related Molecular Markers

5.1. Reelin

Reelin is a glycoprotein with the serine protease activity with the molecular weight of 388kD. The gene for this protein is present on the chromosome 7 [70]. The protein is an extracellular secreted matrix protein. It is an important signaling molecule for its crucial role in the neural development, maintenance and plasticity of neural networks [71]. Any alteration in the function of this protein or its relevant receptors, results in the altered memory and its cognition [71]. Because of its neurological importance the protein is widely studied in many neurological disorders and have found to play important role in schizophrenia, autism and in AD [72–74]. One of the signaling pathway of reelin results in the inhibition of the glycogen synthase kinase 3β (GSK-3β) and cyclin dependent kinase 5 (CDK5), both of which have found to play an important role in hyper-phosphorylation of tau. Depletion in the reelin gene expression is noted during early stages of AD murine model and patients without affecting downstream signaling molecules [75]. Another study was conducted on frozen brain tissue sample and change in the concentration of reelin was observed at protein level by western blotting and at m-RNA level by RT-PCR [76]. These molecules involve apolyprotein E receptor 2, very low density lipoprotein receptor and phosphorylated disabled-1 (Dab1). Various studies have reported the neuro-protective effect of reelin against the toxicity caused by the $A\beta_{42}$. *In-vivo* and *in-vitro* studies have showed the potency of reelin to alter

the kinetics of $A\beta_{42}$ aggregation [77]. This effect is mainly due to binding of reelin to soluble $A\beta_{42}$ fragment and also to the fibrils formed due to aggregation. The same study also reported that overexpression of reelin in mice models sufficed the recovery of functional and behavioral conditions [77]. Reelin when it is bound to the Aβ peptide cannot activate the Dab-1. Dab-1 is an important component of Akt signaling pathway which leads to the inhibition of GSK-3β [78] as shown in figure 2. Unavailability of reelin due to its co-localization with Aβ renders Dab-1 inactive for GSK-3β inhibition [79]. This might be the potential cause for the increase tau hyper-phosphorylation. Though having been studied extensively for its role in Alzheimer's disease reelin is not yet explored in biomarker aspect. Studies involving change in concentration of reelin in CSF and blood of AD patients need to be carried out. Association of reelin with the Aβ fragment may provide information depleted reelin protein concentration in the CSF or blood in AD patient.

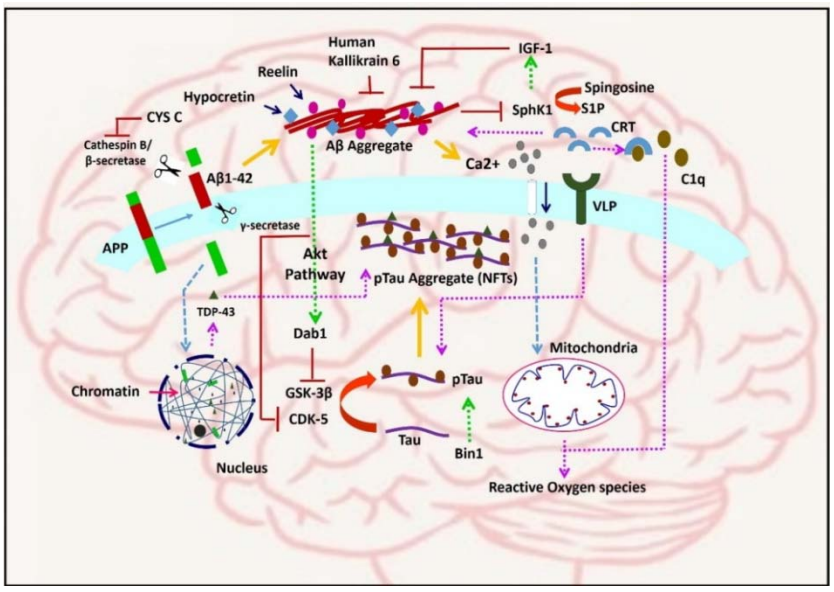

Figure 2. Signaling cascade showing potential biomarkers involved in AD.

5.2. Bridging integrator 1 (BIN1)

BIN 1 also known as amphiphysin 2 or AMPH2. There are around 15 isoforms of BIN1, some of which can be categorized based on their size. It was identified to be one of the Myc interacting protein. Myc gene plays an important role in the progression of cell cycle and tumor development. This lead to the hypothesis of tumor suppressor ability of BIN1 [80]. BIN1 expressions were found to be altered in the AD brain [81]. The large isoform of BIN1 was decreased and smaller isoforms were increased in the AD patient when compared with the age matched control [82]. One of the study revealed that increased BIN1 can lead to AD. BIN1 knockdown have successfully suppressed the neurotoxicity caused by the NFTs as shown in figure 2. The altered DNA methylation pattern was observed at the locus of BIN1 in the AD patients. This DNA methylation has been predicted to have potential in the early onset of AD. The potential use of plasma BIN1 level as a biomarker have been

carried out in AD patient [83]. The study revealed that m-RNA and protein expression levels of BIN1 in the plasma are increased significantly compared to control. Study conducted by Glennon et al. 2013 proposed that BIN1 levels are decreased in sporadic AD and not in the familial AD [84]. Changes in the locus of BIN1 are found to be associated with the PD but change in its expression in case of PD has to be evaluated [85]. Hence convincing evidence about BIN1 as a potential biomarker in AD is still to be found.

5.3. Transactive Response DNA Binding Protein 43 (TDP-43)

As stated in the name TDP 43 is DNA and also RNA binding protein with the approximate molecular mass 43kDa. TDP 43 as it binds to DNA/RNA can bring about transcription repression of genes and also the gene splicing [86]. TDP 43 in its phosphorylated form is associated with many neurological disorders to name a few frontotemporal lobar degeneration (FTLD), ALS and AD [87]. TDP is a nuclear protein but in these diseases it is deposited in cytoplasm of the neuronal cells [88]. Immunofluorescence assay have revealed the presence of these deposits in the brain region similar to the NFTs of AD. It is also found in the association with the NFTs and α-synuclein as shown in figure 2. TDP-43 is observed within diseased neurons in an ubiquitinated form. This ubiquitinated TDP-43 further undergoes proteolytic cleavage. The fragments of this proteolytic cleavage are abundant in the deposits found in the AD [89]. C-terminal fragment generated due to aberrant cleavage of TDP-43 is more abundant in the aggregation rather than complete TDP-43 peptide [90]. TDP-43 is found to be reduced in other neurological diseases including HD and spinocerebellar ataxia 3 [91]. Study performed by Josephs et al have already revealed its importance as a biomarker and therapeutic target in AD [92]. But whether its change in expression can be observed in CSF and blood plasma has to be elucidated.

6. Ca^{2+} Signaling Pathway Related Molecular Markers

6.1. Visinin like protein 1 (VLP 1)

VLP 1 is a protein which belongs to the family of neuronal calcium sensing protein. The protein is known to play an important role in intracellular neuronal signaling. This signaling pathway is mainly driven by the change in concentration of Ca^{2+} ions [93]. The protein is expressed only in the brain tissue and is absent in other peripheral tissue [94]. VLP1 have been associated with the AD. Ca^{2+} levels are altered in the AD patient brain due to the damage caused by accumulation of $A\beta_{42}$. AB_{42} is capable of inducing Ca^{2+} pores on the neurons which leads to the rapid influx of Ca^{2+} ions within neurons. Ca^{2+} may then lead to activation of many mitochondrial as well as endoplasmic reticulum pathway which causes increased oxidative stress [85,86]. One of the hypothesis suggest that the increased concentration of Ca^{2+} may lead to the death of neurons which express VLPs [97]. VLP-1 has been found to be associated with the hyper-phosphorylation of tau protein which further

causes NFTs formation as shown in figure 2. Same study also showed that not only increased expression of VLP-1 is associated with the AD but also the decreased level of calcium buffering protein known as calbinding-D28K is affected [98]. Lee et al. 2008 carried out evaluation of VLP-1 as biomarker in AD. Their result suggest that increased VLP-1 values directly co-relate with the neuronal death as more dead neurons will release more amount of VLP-1. Even though the results of this study sounds positive the sample size of AD patient was less [99]. Also VLP-1 expression was higher in homozygous APOε4 than the homozygous APOε3 and heterozygous APOε3/4 [99]. This increases the complexity in using VLP-1 since genomic aspects interplay with the biomarker level in the blood. Also the VLP-1 is a brain injury biomarker and is implicated in various neurological disorders such as amyotrophic lateral sclerosis (ALS) stroke and schizophrenia [90–92]. One of the study demonstrated VLP-1 and a Chitinase-3-like protein 1 levels increased as the time progressed in Memory cognitive impairment (MCI) patients [103]. VLP-1 protein levels has been significantly detected in the CSF and plasma of AD patient and its level are found to be significantly different from the normal patient [104]. This implies the significant role of VLP-1 in the AD and its potential as a biomarker in its diagnosis.

6.2. Calreticulin (CRT)

Discussing about Ca^{2+} signaling pathway, one important endoplasmic reticulum protein which sequester and store Ca^{2+} in endoplasmic reticulum (ER) is calreticulin. CRT plays an important role in signaling pathways [105]. Because of its important cellular signaling function it is found in various neurological disease which include HD and PD [106]. In ALS mice model the study was performed in order to identify the cause of rapid neuro-degeneration. The outcome of the study stated that CRT is one of the downstream signaling molecule in Fas signaling [107]. Activation of Fas/NO pathway leads to the decreased expression of the CRT followed by rapid neuronal degeneration. Decreased expression of CRT within neurons makes them sensitive to ER related oxidative stress [108]. As discussed earlier excess Ca^{2+} levels may be the reason for the oxidative stress observed in this condition. One important role of CRT is its ability to bind to misfolded proteins and stop their transportation to golgi complex [109]. Importantly *in vitro* studies have shown the ability of CRT to bind $A\beta_{42}$ peptide. The binding was stimulated in the presence of Ca^{2+} ions. This suggests the dual role and altered signaling of Ca^{2+} in AD. Ca^{2+} can increase the binding of $A\beta_{42}$ to CRT but in its increased concentration it results in the oxidative stress and neuronal death via its signaling pathways as shown in figure 2 [109,110]. CRT is a receptor for various other protein molecules one of which is C1q. C1q has been found to increase the reactive oxygen species (ROS) generation [111]. Evaluation of CRT as a biomarker in AD have mentioned its lowered protein and m-RNA expression in AD patient serum [112]. CRT can bind to the $A\beta_{42}$ and C1q and its lower expression suggest that these bindings may be altered in the AD patients which result in the amyloid deposit and also ROS mediated neuronal death [113]. Hence it can be stated that CRT is associated with the early development of AD and the inflammatory cascade is also altered in the AD brain.

6.3. Neurogranin (Ng)

Neurogranin is a small protein first discovered first identified in rat brain and is also named as RC3, BICKS [114]. It is an important protein regulating the synaptic plasticity and signaling within the neurons. Ng regulates the synaptic signaling by controlling the availability of calmodulin an important signalling molecule in Ca^{2+}-Calmodulin pathway [114]. Ng levels have been up-regulated in the AD brain and they have been explored for the early detection of AD and progression of the disease. The study evaluated concentration of the Ng in CSF of AD patient by immunoassay [115]. Vos et al. (2005) showed the presence of C-terminal Ng is increased in CSF but not in the plasma of AD patient. In human Ng is present as peptide of various lengths. Out of these 7 are distinct for the plasma and are not observed in the CSF [116]. Ng peptide 48 to 76 which was absent in plasma was found to be increased in CSF of AD brain when compared with the normal patient [117]. Ng was used in combination with the YKL40 which is also known as chitinase-3 like protein 1 for the evaluation of there accuracy in AD biomarker discovery. The comparison between YLK40/Ng and established biomarker $A\beta_{42}$/pTau provided the evidence for more accurate and sensitive detection by $A\beta_{42}$/pTau than YLK40/Ng [118].

7. Conclusion & Future Direction

Alzheimer's disease is a complex disease involving the alteration of variety of the key components. It is important to identify the early disease specific biomarker so as to better treatment of the disease. Many of the biomarkers have been identify in AD but none of them seems to be efficient in the early diagnostics. The mutations observed within the FAD can be useful for the early diagnosis but the same does not serve the purpose in the LOAD. Hence for LOAD many neuropsychological and brain imaging testing have been adopted. A deep study of AD is required in order to identify the potential biomarkers which will also help in therapeutic targeting of the disease. Overall, Human Kalikrain 6 m-RNA and protein level is found to be altered in the brain tissue as well as in CSF. Hypocretin, Neuroserpins, VLP-1 and Ng showed increase protein concentration in the CSF of the AD patient. Spingosine and reelin showed elevated level of protein in the brain tissue sample of patient suffering from AD, for reelin, even m-RNA level were elevated indicating increased gene expression. Clstn1 protein level was found to be reduced in the brain tissue of patient whereas Clstn3 protein level is increased in AD rat model. Cystacin C protein level was reduced in the CSF and plasma of AD patient. Increased m-RNA and protein level of Bin1 within the plasma is observed by the studies done. TDP43 at protein level is reduced in the AD brain due to its co-localization with the Amyloid. Decreased expression of calreticulin in AD is observed by m-RNA in the serum.

In conclusion these molecular markers can form the basis for studies involving development of biomarker in AD. Use of Chip-based technology or Micro-array technology will be useful in measuring the gene level expression of these markers in the plasma or CSF, this may even help to

distinguish AD from other neurological diseases. Even though many of the markers involved are also markers for other neurological diseases these markers can be used to study therapeutic efficacy of a drug developed against AD.

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Conflict of Interest

All authors declare no conflicts of interest in this paper.

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