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Title: An Insight into the Molecular and Therapeutic Targets of Amyloid Plaques in Alzheimer's Disease and an Update on the Prospects of Drugs in Research

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Abstract:

Alzheimer's disease (AD) is characterized by progressive loss of cognition and a gradual decrease in memory. Though AD is considered the most persistent form of dementia and a global concern, no complete cure or agents that can completely halt the progression of AD have been found. In the past years, considerable advancements in the understanding of cellular and molecular changes associated with AD has been investigated and numerous pharmacological targets have been recognized to enable drug development for the condition. Amyloid-beta ($A\beta$) plaques and neurofibrillary tangles (NFT) are the major attributes of AD. Symptomatic relief is the only possible treatment available at present and a disease modifying drug is of utmost importance. Development of drugs that can inhibit different targets responsible for the formation of plaques is a potential area in AD research. This review is not a complete list of all possible targets for AD but serves to highlight the targets related to amyloid pathology and pathway concerned with the formation of amyloid fragments. This shall serve as a prospect in identification of amyloid plaque inhibitors and pave the strategies for newer drug treatments. Nevertheless, substantial research is done in this area but to bridge, the clinical difficulty remains a concern.

Keywords: Dementia, Alzheimer's Disease, Amyloid Precursor Protein, Secretases, Amyloid Plaques.

Highlights:

- Amyloid plaques are a major pathological hallmark of AD.
- Targets of amyloid plaque formation.
- Strategies to inhibit amyloid plaque formation.

1. Introduction:

AD is a progressive neuronal disorder that develops with age and leads to cognitive decline and thinking skills. Alzheimer's disease (AD) has been a global concern for years, comprising 60-80% of dementia cases (Ashraf et al., 2016). The Alzheimer's Association estimated that 10 million around the globe are affected by AD and the number is on the rise. India is ranked third highest in the caseload related to dementia and particularly in AD after China and the US. According to statistics, the population of the elderly in India will reach around 300 million, accounting for almost 20% of the total population of the country. The expected rise of AD patients in the world by 2050 would be from 1.6 million cases in 2015 to 4.6 million cases by 2050 (Edwards, 2019; Madav et al., 2019; Rizzi et al., 2014; Soto et al., 2007). Pathologically AD is characterized by intracellular neurofibrillary tangles and extracellular amyloid plaques which are first seen in the brain areas of the cortex and the hippocampus. This induces neuronal injury resulting in neuronal death and successively damaging the cholinergic transmission leading to loss of memory and cognition along with neurotransmitter abnormalities (Benny & Thomas, 2019; Chaudhary et al., 2018; Gopalakrishnan et al., 2019; Shankarappa et al., 2012).

Over the years, advances in the field of pathogenesis have inspired researchers to identify novel therapies concentrated more towards the pathological aspects of the disease. Although the exact root cause of AD remains unclear, several factors have now been known and studied that could play a major role in the progression of the disease (Madav et al., 2019). The various established hypothesis for pathogenesis of AD has been included in **Fig.1**. With ample shreds of evidence of the research, the amyloid hypothesis remains the most studied area of AD pathology and preferred target for drug development. The proteolytic processing of amyloid precursor protein (APP) is said to be the central dogma of this hypothesis. APP is a transmembrane protein located on chromosome 21 in humans and is expressed in brains at high levels and is metabolized rapidly. APP is mainly synthesized in the endoplasmic reticulum and then it moves to the Golgi-network where it plays a role in neural growth and repair. The proteolysis of APP occurs by amyloidogenic (pathogenic) or the non-amyloidogenic (non-pathogenic) pathway. The amyloidogenic pathway releases insoluble amyloid peptides and the aggregation of these peptides leads to plaque formation (Du et al., 2018; Edwards, 2019; Serrano-Pozo et al., 2011; Zhang et al., 2011).

Fig 1: Established hypothesis of AD

2. Aβ: The Miracle Worker

Aβ is a peptide generated throughout life, while the formation of amyloid plaques is a neuropathological hallmark of AD stimulated by synaptic activity. The generation of smaller Aβ peptide is not toxic but aggregated Aβ fibrils is a pathological condition of AD (Soto et al., 2007). Although early studies have suggested that large accumulation of Aβ is the cause of plaque formation and neuronal cell toxicity but, now they have come to the conclusion that even the small insoluble Aβ may be more toxic (Coman & Nemeş, 2017). In a normal brain, an enzyme called α-secretase acts on APP and cleaves into secreted APPα (SAPPα) and an 83 amino acid membrane bound C-terminal fragment called CTF83. Alternatively, in an AD brain, an enzyme called β-secretase acts on APP and cleaves it into secreted APPβ (SAPPβ) and a 99 amino acid long, membrane bound C-terminal fragment called CTF99. In a normal brain CTF83 is further cleaved by γ-secretase complex and leads to generation of APP intracellular domain (AICD) fragments which translocates to the nucleus and regulate the proteins of neuroprotective pathways. Whereas in AD, γ-secretase cleaves CTF99

fragment into A β 40-42 peptides leading to the formation of amyloid (A β) fragments. Soluble A β fragments get dissolved whereas under certain conditions insoluble A β fragments gets aggregated leading to plaque formation and disruption in cell communication (Chaudhary et al., 2018; Du et al., 2018; Edwards, 2019; Octave, 1995). In humans, the estimated physiological rate of production of A β is 7.6% per hour and clearance rate is 8.3% per hour and proteolytic degradation is the major route of clearance. The targets particularly related to the formation of amyloid plaques have been mentioned below. Few targets directly contribute to amyloid plaques by cleaving APP directly (BACE-1, γ -secretase) whereas the others play their roles indirectly by over expression of APOE, DYRK1, NLRP-3, CK-1, CDK-5, TREM-2 and MMP as mentioned in **Table.1**.

Table 1: Targets directly contributing to amyloid plaque formation

Target	Source	Functions	Reference
BACE-1	BACE1 is predominantly expressed in brain and is richly expressed by neurons.	Synaptic functions, synaptic transmission Amyloid plaque formation	(Coimbra et al., 2018; Das & Yan, 2017; Evin & Hince, 2013; Laird et al., 2005; Vassar & Kandalepas, 2011)
Y-secretase- PSEN1s	Located on chromosome 14 in humans	Play an important role in generation of amyloid beta (A β) from amyloid precursor protein (app)	(Basi et al., 2010; Kounnas et al., 2010; Panza et al., 2010; Wolfe, 2012)
Cdk5	Part of the paired helical filament (PHF), an integral part of neurofibrillary tangles	-Neuron development, neuronal survival, phosphorylation of cytoskeletal proteins and synaptic plasticity.	(Dhavan & Tsai, 2001; Huber & O'Day, 2012; Shah & Lahiri, 2014; Shukla et al., 2012)

DYRK1A	A gene, located on chromosome 21	-Implementation of amyloid plaques and over-expression of tau proteins, biological markers of Alzheimer's disease.	(Ferrer et al., 2005; Pathak et al., 2018; Stotani et al., 2016; E. J. Yang et al., 2001, p. 1)
APOE	Primarily produced by the liver and macrophages, Also in microglial cells, & lesser extent in stressed neurons.	-Regulate lipid metabolism in the brain by mediating the uptake of lipoproteins; -It modulates the clearance of amyloid- β .	(Adalbert et al., 2007; Boehm-Cagan & Michaelson, 2014; C.-C. Liu et al., 2017; Morris et al., 2010; Safieh et al., 2019)
CK-1	Nucleus, Cytoplasm and mitotic spindle	- Neuronal processes, including dopamine signalling in striatum, circadian rhythm, brain	(Adler et al., 2019; Chon et al., 2015;

		receptor signalling, and AD. - Important regulator of APP processing. Tau hyperphosphorylation and A β aggregation.	Sundaram et al., 2019; Yasojima et al., 2000)
NLRP-3	Epithelial structures including the lining of the small intestine and stomach.	Induces apoptosis -Immune system regulation -Formation of plaques	(Heneka et al., 2013, 2013; Song et al., 2017; S.-J. Yang et al., 2018; Y. Yang et al., 2019)
MMP	Expressed in different organs such as brain, lung, heart, colon	Plays an important role as inflammatory components in the pathogenesis of AD A β plaque formation	(Baranger et al., 2016; Fragkouli et al., 2014; Yong et al., 2007; Yoshiyama et al., 2000)
Neprilysin	Neprilysin (NEP) is the dominant A β peptide-		

	degrading enzyme in the brain	Major A β peptide-degrading enzymes in the brain Over-expression of NEP leads to reduction of amyloid load	(El-Amouri et al., 2008; S.-M. Huang et al., 2006; Schiering et al., 2016; Vodovar et al., 2015)
Dopamine 2 receptor	Widely expressed in the human brain, with highest levels in the basal ganglia, accumbens, ventral tegmental area, and substantia nigra.	Amyloid plaque formation	(Ambrée et al., 2009; Kemppainen et al., 2003; Martorana & Koch, 2014; Nobili et al., 2017)
TREM 2	Triggering receptor expressed on myeloid cells 2 also known as TREM-2 is a protein	Modulates inflammatory signalling Also show anti-inflammatory properties A β plaque formation	(Gratuze et al., 2018; Karanfilian et al., 2020; Tang et al.,

	Soluble TREM2 has been detected in human cerebrospinal fluid (CSF)		2019; Zheng et al., 2018)
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2.1 Beta-secretase 1 (BACE-1):

BACE-1 was identified in the year 1999 and belongs to the class of aspartyl proteases with disulfide bond arrangement and consists of 501 amino acid sequence. It is predominantly found in the regions of the brain and is said to be the major determinant of amyloidosis in the CNS ((Evin & Hince, 2013). BACE-1 is a key enzyme that regulates the beta-secretase activity on APP. Mutation of APP leads to the increased access of the enzyme towards β -secretase cleavage site and augments A β production (Vassar & Kandalepas, 2011) . Deposition of A β peptides leads to the formation of large amyloid plaques in the brain and can distort the neuronal synapses. Lower A β levels can be achieved by decreasing BACE1 which may also reverse microglial activation and abnormality in the neuronal functions ((Coimbra et al., 2018; Yan et al., 1999) Accumulation of amyloid aggregates in the synapses affects synaptic plasticity leading to decline in memory. Studies have shown that BACE-1 deficient mice exhibited cognitive impairments significantly. For this reason, inhibition of BACE-1 is logically viewed as beneficial to AD patients ((Laird et al., 2005) .

A study published in the journal of experimental medicine has shown that reduction in A β deposition was seen on deletion of BACE-1 in adult mice providing us with genetic evidence((Hu et al., 2018). Pharmacological inhibition of BACE-1 may slow down the amyloid pathology, but to take advantage of this, treatment should be initiated before the widespread of A β deposition. Thereby, it might serve to regulate BACE-1 inhibition to a level that will keep the formation of new plaques at bay and delays the progression to AD((Blume et al., 2018; Das & Yan, 2017) .

BACE-1 is considered as a potential target to treat AD, as it is a prime enzyme involved in the amyloid cascade as shown in **Fig 2**. Intense efforts are being made to find a drug that can inhibit BACE1 but, because it plays important role in the various physiological processes, the challenge is to develop selective and specific BACE1 inhibitors (Cole & Vassar, 2007; Rajendran et al., 2008).

Over 20 years, studies are done to design and develop a BACE-1 inhibitor with good selectivity, bioavailability and blood-brain barrier (BBB) permeability but clinical trials indicate a high failure rate of many lead drug candidates. Many BACE-1 inhibitors have been suspended due to adverse

effects, inability to reduce symptoms associated with AD even though plaque formation is found to be reduced. Nevertheless, BACE-1 inhibition is still considered the best of the targets to stop AD, as it is the neck of the disease.

Verubecestat (MK-8931) is the first BACE1 inhibitor (a small molecule) from Merck pharmaceutical Company that had a good bioavailability and BBB permeability. On administration of verubecestat in preclinical animals it reduced the A β oligomers in CSF and the brain, no adverse effects was seen. Phase-1 clinical trials were also shown to be well tolerated and reduction in the plaque concentration in the CSF was also seen. In phase -2 clinical trials, the focus was on testing for cognition property of the drug. Verubecestat did not exhibit any improvement in the cognitive capacity of AD participants and the clinical trial was terminated in 2018 February. Side effects like rashes, change in colour, insomnia, weight loss was reported by the participants(Egan et al., 2018; Kennedy et al., 2016; Scott et al., 2016; Zwan et al., 2017).

Lanabecestat an oral BACE-1 inhibitor developed by AstraZeneca pharmaceutical Company and was tested first on mice, guinea pigs and dogs prior to clinical trials in their primary cortical neurons. The half life of 9h meant that it has a prolonged reduction of A β oligomers. In 2014, in collaboration with Eli Lilly, Phase I Studies begun, the results were satisfactory with a good metabolic and tolerability profile in mild AD patients. Similar to the Merck's verubecestat, lanabecestat also decreased the level of CSF A β in the group that was treated. In phase 2 clinical trials, that was conducted in over 1400 participants, potency and safety was analysed by using amyloid positron emission tomography(PET) scans , levels of A β in CSF. The expected outcome of the study was improvement in the cognition and decrease in the A β levels in CSF. Unfortunately, the trials had to be discontinued as the lanabecestat treatment did not meet the primary endpoints (Cebers et al., 2017; Eketjäll et al., 2016; Sakamoto et al., 2017).

Atabecestat (JNJ-54861911) is another small-molecular BACE1 inhibitor developed by Shionogi pharmaceutical company. This has entered phase I clinical trial in collaboration with Janssen pharmaceuticals. Administration of atabecestat, 5–150 mg for a period of 14 days has shown a considerable reduction of A β in CSF and plasma. In another trial termed EARLY (NCT02569398) was begun in 2015. Participants that are at risk of developing AD or suffering with mild Ad were given drug for 4.5 years with continuous monitoring of cognitive functions. Unfortunately, Janssen had to announce the termination of this trial as observations indicated a rise in the liver enzymes in few patients. This suggested that there might be liver toxicity associated with it and the drug was discontinued on 17 may 2018 (Lahiri et al., 2014; Timmers et al., 2018).

Elenbecestat (E2609), was developed first by Eisai pharmaceutical company in December 2014, a small molecule BACE-1 inhibitor. After early trials a ninth Phase-1 clinical trial was conducted in 2016 which was satisfactory at 50mg dose in 16 healthy volunteers. In October, 2016 a program

called MISSION was started to explore the outcomes in the change of amyloid PET and hippocampal volumes. In 2018 Eisai planned for phase -2 clinical trials as they found it to be effective in reducing the brain amyloid level and positive cognitive effects. However, in 2019 BIOGEN/Eisai has announced the termination of MISSION trials due to unfavourable risk-benefit ratio and cognitive improvement was also not significant. However, the only relief was no hepatotoxicity was found, which has been a feature in clinical trials of other drugs(Imbimbo & Watling, 2019; Panza et al., 2010).

A new small molecule BACE-1 inhibitor CNP520 (umibecestat) from Novartis pharmaceutical company is being developed. In preclinical models CNP520 has shown to reduce amyloid beta reduction, penetrate the BBB and a good selectivity towards the target. No phase-1 clinical trial results have been out but phase -2 trials was conducted with Amgen which is aimed at delaying the progression or onset of AD. In July 2019, the research had a premature end citing a cognitive decline and weight loss in the treatment groups. Researchers continued to monitor the cognition capacity and the level of amyloid in brain after the treatment was stopped, they found the cognitive decline and volume loss in the brain was reversible(Neumann et al., 2018). Regardless of the failures in clinical trials, discovering a BACE-1 inhibitor should not be stopped. Inhibition of BACE-1 along with other targets could be potential strategy and is likely to be more effective in AD. Nevertheless, a breakthrough in the drug discovery for BACE-1 inhibition has happened on June7,2021. The first FDA approved drug for AD treatment is aducanumab developed by Biogen and Eisai under accelerated approval pathway(Schneider, 2020).

Fig 2:
Schematic

representation of BACE-1 enzyme cleaving APP. Three main proteases— α -, β -, and γ -secretases—are involved in APP processing through the amyloidogenic pathway. In particular, BACE1 serves as the β -secretase enzyme by cleaving the transmembrane APP that represents the rate-limiting step for A β production.

2.2 Gamma secretase – Presenilin 1:

It is an integral intermembrane protein with many subunits forming a protease complex and consists of four individual proteins- PSEN1, PSEN2, APH1, and Nicastrin. γ -secretase and its components are generally synthesized in the Endoplasmic reticulum, but for maturation, it requires the coordination of ER-Golgi circuit(Basi et al., 2010; Wolfe, 2014).

Presenilin mutations were first seen in connection with familial AD, and further research established that PSEN1 along with structurally close PSEN2 are the catalytic components of γ -secretase which is involved in the cleavage of APP at varying lengths. In AD, APP cleavage by γ -secretase happens at CTF99 fragment (a fragment of APP after cleaved by BACE-1 enzyme) of BACE-1 leading to the formation of amyloid plaques. The hypothetical reason behind the action of γ -secretase after α -or β -cleavage is purposed to the large ectodomain of the substrate that hinders binding to the γ -secretase. Being the final complex in APP processing, γ -secretase might be contributing to the levels of the A β and Notch signalling pathway as shown in **Fig 3**. Notch receptor is responsible for cell differentiation

events in all multicellular organisms, in embryogenesis and adulthood(Basi et al., 2010; Bavishi et al., 2015).

Inhibition of γ -secretase presents an obvious door to block the production of A β (De Strooper, 2007; Wolfe, 2014). The first reported γ -secretase inhibitors (GSIs) were peptide aldehyde type calpain and proteasome inhibitors. Despite their less potency and lack of selectivity, these are said to be the answers for γ -secretase inhibitors (Wolfe, 2012).

The complete inhibition of γ -secretase seemed a good approach. However, it was found that, it has an extensive biological role and cleaves multiple proteins to yield physiologically essential products. The total inhibition of γ -secretase has resulted in severe adverse effects in *in vivo*. This has been evident from the clinical trial conducted by Eli Lilly for the γ -secretase inhibitor called semagacestat. Patients experienced severe side effects which lead to the discontinuation of the clinical trial in 2010(Kounnas et al., 2010; Schiering et al., 2016).

A substantial amount of research is being done for the discovery and development of small-molecule inhibitors for presenilin-containing γ -secretase complex as a potential therapy for AD (Kounnas et al., 2010).

The future focus will be: Firstly, to develop an inhibitor that can reduce the A β levels without involving in the Notch signalling pathway and secondly, to discover γ -secretase modulators which can shift the A β 1-42 to less pathogenic forms. Studies say that pharmacological inhibition of γ -secretase is a well-documented target for lowering plasma A β peptide and has a greater impact on the pathology of AD (De Strooper et al., 2012; Wolfe, 2014) .

PF-3084014, γ -secretase inhibitor was in development in Pfizer but has to be abandoned due to the lack of data on its effect on cognition and the level of amyloid plaques in animal models of AD (Panza et al., 2010).

GDI-953 a potent γ -secretase inhibitor developed by Wyeth has shown to inhibit A β production at nanomolar concentrations in preclinical animals but in phase 2 clinical trials it did not show much effect on the AD patients. The dose administered was not sufficient enough to clear the plaques or improve the cognition capacity of AD patients(Martone et al., 2009) .

Studies suggest that E-2012, a novel γ -secretase inhibitor reduces A β by modulating γ -secretase without interfering with the notch processing. This drug was developed by Eisai in collaboration with Torrey-Pines Therapeutics. In 2006, the drug candidate entered into clinical phase-1 but was terminated because of the side effects observed in preclinical animals after 13 weeks. The drug is now again back and the clinical trial is under process and is in phase-1(Panza et al., 2010).

FRM-36143 is another novel γ -secretase inhibitor that reverses the PSEN mutations without interfering with the notch signalling pathway in the preclinical studies. Thus, FRM-36143 is a potential candidate for further research to take into clinical sector(Blain et al., 2016).

As γ -secretases play multiple roles, targeting PSEN1 mutations specifically will be the best way to minimise and prevent the disturbances in the physiological functions in the body.

Fig 3: The initial cleavage results in forming a soluble APP derivative and carboxy-terminal fragment (CTF-99) which acts as a substrate for γ -secretase. Mutations in presenilin, which is a subunit of γ -secretase leads to the formation of amyloid plaque formation and deposition.

2.3 CDK 5:

It's been two decades since cdk5 has been discovered. Cdk5 belongs to the family of cyclin-dependent kinases which is necessary for brain development during embryogenesis and is essential for various neuronal functions related to cognition and memory in adults. CDK5 is mainly found to be active in post-mitotic neurons (Dhavan & Tsai, 2001).

In a normal brain, the activity of CDK5 is highly regulated by specific-cyclin related molecules p35 and p39. Among the two, p35 is the most studied activator protein of cdk5. Regulated Cdk5 maintains

synaptic plasticity, the survival of neurons, and cognition. When the activity of Cdk5 is deregulated, it promotes oxidative stress and mitochondrial dysfunction (Shukla et al., 2012; W.-Y. Wang et al., 2015; Wilkaniec et al., 2018) .

Whenever there is stress, there is an increase in the level of calcium in the cytoplasm, leading to proteolytic cleavage by calpain. Calpain belongs to the family of calcium dependent cysteine proteases that are involved in cell development, cell motility, apoptosis, cognition and cell differentiation. Calpain has two forms associated with it m-calpain and μ -calpain. These require only milli molar and micro molar concentrations of calcium for their activation. Calpain cleaves p35 and p39 resulting in p25 and p29 as cleaved fragments. This p25 can activate Cdk5 leading to a higher and active Cdk5/p25 stable complex. This causes further A β formation, mitochondrial dysfunction, and other pathological events leading to degenerated neurons and apoptosis as represented in **Fig 4** (Cruz & Tsai, 2004; F. Liu et al., 2003) . In AD conditions, plaques are formed due to the deposition of A β aggregates extracellularly. Over expression of Cdk5 promotes further A β deposition resulting in neurotoxicity, activation of kinases and further neurofibrillary tangles (NFT) formation (F. Liu et al., 2003; Reinhardt et al., 2019; Taniguchi et al., 2001).

A study published that upon neuronal toxicity by glutamate or by A β , neurons showed intensified CDK5 activity but on treatment with known CDK5 inhibitors, cells have shown a reduction in hyperactive CDK5. This strongly indicates that hyperactivation of Cdk5 may be involved with phosphorylation of APP leading to excessive A β formation (Cruz & Tsai, 2004; Fischer et al., 2002; Shah & Lahiri, 2014)). An increase in CDK5 activity of p25 is observed in the post-mortem brains of AD patients making it an apparent choice to be considered as a therapeutic target for AD.

In research related to Cdk5 inhibition, two inhibitors have been tested: Cdk5 inhibitor (Roscovitine) (Huber & O'Day, 2012; Menn et al., 2010) and calpain inhibitor (MDL28170) for AD. Roscovitine is in clinical trials phase 2 for the treatment of small lung and nasopharyngeal cancer. Various research studies have established that Cdk5 deregulation is related with melanoma and neurodegenerative diseases suggesting that roscovitine can be further investigated for AD. Proofs of (R) stereoisomer of Roscovitine exhibiting neuroprotective activity in *in vitro* and *in vivo* is widely accepted (Dhavan & Tsai, 2001; Khalil et al., 2015; Shah & Lahiri, 2014) .

Fig 4: Accumulated amyloid plaques will induce activation of Cdk5 by calpain leading to the conversion from p35 to p25. The Cdk5-p25 complex triggers neuronal death.

Indeed, number of studies done on AD mouse models suggest that inhibition of Cdk5 activity genetically or pharmacologically will prevent synaptic dysfunction and neuronal death (Seo et al., 2017). Even so, trials with Cdk5 inhibitors in the clinical sector have not been promising due to side effects or off-target events (Cicenas et al., 2015) .

AK275 is the first exogenous calpain inhibitors and was mostly preferred because of its selectivity, membrane permeability, solubility, and in vitro efficiency. AK275 is considered to be a neuroprotectant for the treatment of focal brain ischemia indicating a possibility of potential drug for AD(Yildiz-Unal et al., 2015). There are other inhibitors that researchers are working on to improve their stability, potency and efficiency with improved pharmacological profiles(Bartus et al., 1994; Wilkaniec et al., 2016).

In a recent study on mouse models of AD, anti-diabetic drugs troglitazone and pioglitazone belonging to the class of thiazolidinedones (TZD) has shown to inhibit Cdk5 kinase activity and reduce synaptic deficits(Cho et al., 2013) . However, the use of TZDs is more than usually related to edema, weight gain, and heart failure (Rizos et al., 2009) which raises a concern.

In another study on *APP/PS1* mice in which Cdk5 was hyperactivated in the hippocampus, an anti-diabetic drug metformin was administered. The reports showed that on chronic administration of metformin, cleavage of p35 into p25 was prevented and in turn inhibited Cdk5 which was assessed

by a combination of pharmacological and molecular techniques. Not only that, the reports have shown that on chronic administration of metformin, it also restored impaired synaptic plasticity and improved cognitive function in mice (Y. Wang et al., 2020). Collectively, this data suggests a captivating note that anti-diabetic drugs can be of use as drugs to treat neurodegenerative diseases. In the end, it may be mentioned that drug candidates that can reduce the hyperactivity of Cdk5 may be regarded as a highly potential candidate to decrease the production of A β (Fischer et al., 2002).

2.4 Dual-specificity tyrosine-regulated kinases (DYRK 1A):

DYRK 1A is a dual specific tyrosine-phosphorylated regulated kinase enzyme that belongs to the member of the DYRK family and is one among 5 protein kinases that direct signals in the development of the nervous system (Ferrer et al., 2005; E. J. Yang et al., 2001). DYRK1A is extensively found in the cerebellum, olfactory bulb region, hippocampus and is also present in the Down syndrome region on chromosome 21 and controls brain growth through neuronal proliferation and neurogenesis. DYRK1A is involved in the formation of amyloid plaques and NFT's which has been evident from the reports of higher concentration of DYRK1A in the brains of AD patients (Souchet et al., 2019; Stotani et al., 2016). Although DYRK1 plays a role in the formation of plaques, it is essential for the brain during embryonic development in the early stages, where DYRK1 is involved in signalling pathways related to cell growth and proliferation, separation of cells into mature neurons, and as well as in the formation of dendritic spines which are important for the transmission of impulses. However, in a mature brain, DYRK1A can become aggressive and may initiate AD pathology (Galceran et al., 2003). The dysfunction of DYRK1A is a prominent feature of Down Syndrome and patients with this disorder are highly susceptible to developing AD early in life (Velazquez et al., 2019).

When DYRK1 comes across APP, it binds an aggregate of oxygen and phosphorus atoms leading to phosphorylation. When the phosphorylation of these proteins is more, it leads to harmful effects in the brain. This hyperphosphorylation of APP leads to the formation of amyloid plaques as shown in **Fig 5**. Experimental evidence shows that over expression of DYRK1A contributes to enhanced β -amyloidosis and neurofibrillary degeneration. Research studies have pointed out that over expression of DYRK1A has been shown to increase the chances of proteolytic cleavage of APP and hence an increased production of A β peptides. Besides, DYRK1A also phosphorylates Presenilin 1 (PS 1), and thereby increase γ -secretase activity causing more A β production (Souchet et al., 2019; E. J. Yang et al., 2001).

Fig 5: DYRK1 comes across APP and binds an aggregate of oxygen and phosphorus atoms leading to phosphorylation. The phosphorylation of DYRK1 in turn hyperphosphorylates APP and further leading to amyloid plaques

Drug repurposing study of CX-4945, a CK-2 inhibitor that is under clinical trials for various cancers is found to inhibit DYRK1A related pathology of AD in mouse models (Chon et al., 2015). A study has shown that inhibition of DYRK1A in chronic condition has reversed memory problems in 3x-Tg AD mice. The results obtained were found to be associated with A β and tau pathology. It showed that inhibition of DYRK1A has reduced the amyloid plaques by inhibiting APP cleavage and also reduced insoluble tau phosphorylation (Branca et al., 2017; Pathak et al., 2018).

This suggests that drugs that can inhibit DYRK1A could be a possible therapeutic choice for AD. Knowledge of the mechanisms initiated by DYRK1A over expression will get us closer to comprehend AD and develop effective therapies.

Number of DYRK1A inhibitors has been tested in *in vitro* assays but only few have shown the potential to be clinical drug candidate. Harmine is an extensively studied DYRK1A inhibitor in

preclinical research but because of its selectivity towards kinases, it is not suitable for clinical development (Melchior et al., 2019).

ManRos therapeutics are developing small molecule DYRK1A inhibitor named as Leucettine L41 based on the analog of leucettamine B derived from marine sponges (Burgy et al., 2013).

Samumed is developing a DYRK1A inhibitor named as SM07883 which has shown to protect against hyperphosphorylation of tau and is currently under test in clinical phase-1 (Melchior B, 2019).

KVN93, a molecule that has considerably reduced DYRK1A kinase activity in an *in vitro* study, is speculated to be a potential novel DYRK1A inhibitor that can improve cognitive deficits and regulate A β pathology (Lee et al., 2020).

A current study by using natural product epigallocatechin -3- gallate has shown inhibition of excess DYRK1A activity and promoted improved cognitive functions. However, these drugs being non-selective to the target and possessing numerous off-target reactions has raised questions on their utility further (Feki & Hibaoui, 2018) .

Preclinical research reports shows that DYRK1A inhibitors are effective in slowing down the onset of AD in people suffering with Down's syndrome. Despite many efforts on developing DYRK1A selective inhibitors, only few of them have crossed preclinical stage and their clinical manifestation remains to be tested further. The safety parameters are the primary concern for DYRK1A inhibitors and this depends on their kinase selectivity and their potency towards the target.

2.5 Apolipoprotein E (APOE):

APOE is a gene present on chromosome 19 and makes a protein ApolipoproteinE that helps in the metabolism of fats in the body (C.-C. Liu et al., 2013; Yamazaki et al., 2019). ApoE has two regions - an N-terminal which is the receptor binding region and a C-terminal domain which is a fat binding region. APOE gene of humans is classified into three polymorphic alleles (E2, E3, and E4) and they have a prevalence rate of 8.4%, 77.9%, and 13.7%, around the world. But, the frequency of the E4 allele is remarkably more up to 40%, in AD patients. APOE consists of 299 amino acids with a molecular weight of 34 kDa. The number of amino acid residues differentiates the three Apo-E isoforms (Morris et al., 2010). A 1996 research study by *in vitro* experiments states that APOE is involved in amyloid formation and the ApoE4 allele is considered to be the driving factor than E2 or E3 isoform (Adalbert et al., 2007). ApoE is mainly found in liver and the brain and helps in transporting lipid among cells or tissues by binding with lipoprotein via ApoE4 receptor in liver. ApoE4 slows down the rate of transportation of cholesterol from blood which may lead to coronary heart disease. In brain, APOE is mainly secreted in the astrocytes. Various experiments have indicated that APOE isoforms have the ability to efflux cholesterol. ApoE2 and ApoE3 have a better efficiency

than ApoE4 in reducing the cholesterol level. Cholesterol is needed for different functions of neuron which includes synaptic plasticity and maintaining dendritic spine density. The less ability of ApoE4 in lipid metabolism impacts the condition of a neuron leading to neuronal death as seen in **Fig 6**. Carriers of ApoE4 tend to have enhanced pathology related to AD before clinical symptoms become evident. ApoE4 also presents pathogenesis of AD by independent A β mechanisms which include change in the synaptic plasticity, lipid homeostasis, and neuronal inflammation. The exact pathology between APOE isoforms and A β metabolism is still not clear. Current studies have found out that ApoE4 could alter the production, aggregation and clearance of amyloid (Namba & Ikeda, 1991; Safieh et al., 2019). Adding to that ApoE4 carriers have lesser levels of cerebrospinal fluid (CSF) A β 42 and show higher binding on PiB and PET than do noncarriers (C.-C. Liu et al., 2013). Moreover, An Article published in (2019) mentions that ApoE4 is believed to enhance A β production by affecting the activity of γ -secretase (Adalbert et al., 2007; C.-C. Liu et al., 2017).

ApoE2 is considered to be a protective allele against AD. Studies report that the present ApoE2 could delay the AD by an average of 7.5-8.5 years. Scientists have developed Adeno-associated virus (AAV) type of particles that was able to reduce the amyloid load and bring back the cholesterol to normal levels in transgenic mice. A study has shown that RXR agonist (regulation retinoid X receptor) ligand bexarotene reversed both brain pathologies and cognitive dysfunctions that are driven by ApoE4 and also induced compensation for the lipid deficiency of ApoE4. This suggests that RXR agonist may be a way to counteract the pathological effects of apoE4 leading to AD (Boehm-Cagan & Michaelson, 2014).

Fig 6: ApoE is produced mainly by astrocytes and

microglia. In the extracellular space the lipidated ApoE binds to the Aβ₄₂ and influences the further formation of amyloid beta leading to more aggregation and finally neuronal death.

A recent *in-silico* study reported that Epicatechin-gallate has a binding affinity towards ApoE₄ and can be a potential lead compound to inhibit the progression of AD (Bano et al., 2019). Thus, elucidation of the pathogenic link between ApoE₄ and memory functions is a considerable challenge. Inhibitors selectively targeting ApoE₄ or increasing the expression of ApoE₂ could bring assistance in the way of drug development for AD. Another strategy that can be implemented is to edit the gene from E₄ variant to E₃ variant.

2.6 Casein kinase 1 (CK1):

Exactly two decades ago, the regulation of casein kinase in AD has been known. Casein Kinase 1 belongs to the family of protein kinases which are mainly involved in maintaining circadian rhythms. Until now seven different isoforms of CK1 have been characterized out of which CK1-δ and CK1ε are expressed in the brain (Rodrigues & Silva, 2017).

CK1-δ and CK1ε play an important role in maintaining homeostasis of the circadian rhythm. In specific genetic ablation, CK1-δ changes, the period of rhythm and CK1ε may result in arrhythmicity. This is one of the reasons subjects with AD go to sleep later than the rest of the population. Disruption of the clock by CK1 hyperactivity and mutations in the period gene (PER) may be a causative factor in the pathogenesis that underlies AD as described in **Fig 8**. An in-vitro study has demonstrated that

CK1 ϵ can phosphorylate the A β precursor APP and both β and γ secretases that can cleave APP (Chauhan et al., 1993; Yasojima et al., 2000).

An observation from a study suggests that there is an involvement of CK1 activity in the regulation of APP cleavage (Flajolet et al., 2007). A higher association of CK1 is seen in neurodegenerative lesions in the final stages of neuronal death in the brain which is same with AD. The key enzymes that are involved in the cleavage of APP (β -secretase and γ secretase) are also related with CK1 as targets to inhibit plaque formation. Brains of AD patients have thirty times more CK1 than that of a normal human brain (Adler et al., 2019; Chauhan et al., 1993).

A research study (2019) done on APP-PS1 mice has shown that inhibition of CK1 improves cognitive function and also reduces the load of amyloid in the brain. Another study has reported that A β stimulates casein kinases along with other protein kinases which may then initiate tau hyperphosphorylation leading to neurofibrillary tangles and ultimately neuronal death (Manakadan et al., 2015; Sundaram et al., 2019). Further characterization and identifying new inhibitors of CK1 that can regulate AD regulated circadian shifts can be focussed as it is essential for the clearance of A β .

A small molecule inhibitor -PF-670462- of CK1 δ/ϵ is found to alter rhythmicity, improved cognitive functions and reduced the amyloid load in APP-PS1 mouse (Sundaram et al., 2019).

PF-05251749, a Casein kinase 1 delta and epsilon (CK1 δ and ϵ) inhibitor initially developed by Pfizer Company for AD was tested for phase-I safety and tolerability following preclinical studies considering circadian rhythm as a major parameter for assessment. No further development was updated since October 2018, however Biogen has acquired the molecule for further studies in AD population in January 2020 (Benn & Dawson, 2020). Future research should concentrate on early intervention to reduce CK1 ϵ/δ by understanding the prophylactic potential.

Fig 7: CK1 hyperactivity leads to phosphorylation of APP and circadian disturbances. Mutations in the period gene (PER) because of the rhythmic disturbances of sleep leads to formation and aggregation of amyloid plaques.

2.7 Nod-like receptor protein 3 (NLRP-3):

NLRP3 is an intracellular oligomeric multi-protein complex that is being extensively studied for neurodegenerative and immune system-related studies (Song et al., 2017). NLRP-3 is also called an inflammasome, that belongs to the family of NLR (Nod-like Receptor) and is present in astrocytes and microglia in the CNS, and elicits an immune response against damaged signals. The NLRP-3 protein complex consists of the sensor protein NLRP3, the adaptor protein, a caspase activating and recruiting domain, and pro-caspase-1. Assembly of this complex and activation of this leads to cleavage of procaspase-1 to active caspase-1. The activated caspase-1 in turn cleaves and activates proinflammatory cytokines. In AD, the aggregation of misfolded proteins or amyloid plaques triggers NLRP-3 that can act as a platform for the activation of caspase-1 that initiates an inflammatory response which triggers the pro-inflammatory cytokines IL-1 β and IL-18 that is released into the extracellular space. These promote various inflammatory and immune diseases and mediate neuronal death (Halle et al., 2008; S.-J. Yang et al., 2018; Y. Yang et al., 2019) Tan et al., 2013). The inflammasome constituents such as NLRP1, NLRP3, ASC, Caspase-1 along with the downstream

effectors like IL-1 β and IL-18 are upregulated at both mRNA and protein levels in AD patients. **Fig 8** describes the pathway of amyloid plaque formation by NLRP3.

A study conducted in the year 2013 has assessed the amount of cleaved caspase-1 in AD patients and found that the levels are more compared to that of the control. The same case was mirrored in aged APP/PS1 transgenic mice (J. Yin et al., 2018).

A study of a rationally designed NLRP-3 inflammasome inhibitor (JC-124) in Transgenic CRND8 APP mice showed that on treatment with (JC-124), the levels of A β deposition decreased in the brains of CRND8 mice along with reduced cleavage of APP (Heneka et al., 2013). This suggests that discovering agents that can control the activation of NLRP3 might be a potential target to tackle neuroinflammation and plaque formation.

Since NLRP3 inflammasome activation is a multistep process, inhibition of the activation of NLRP3 can be done by different approaches: subduing molecules that initiate the NLRP3 complex formation, suppressing the upstream signals by inhibiting the NLRP3 directly or indirectly depending on the molecule targeted and inhibiting caspase-1 cleavage. Number of molecules have shown inhibition of NLRP activation and is said to have been validated into animal models. An *in vivo* study describes a potent small molecule inhibitor MCC950 has blocked NLRP3 activation selectively at nanomolar concentration (Coll et al., 2015). A recent study on MCC950 also demonstrated that it can block nigericin-induced activation of NLRP3 inflammasome by inhibiting efflux of chloride that acts an upstream pathway for NLRP3 activation (Zahid et al., 2019). A study finds that cystic fibrosis transmembrane conductance regulator (CFTR) channel inhibitor-C172 can also inhibit NLRP-3 activation. Another report on C172-CY09 confirms that it selectively binds to NLRP-3 inflammasome but not to any other and inhibits its ATPase activity. ATPase activity of NLRP3 is important for the activation of NLRP3 and its oligomerisation (Jiang et al., 2017).

Most predominantly, CY-09 showed an inhibitory effect on NLRP3 and with good pharmacokinetic properties in terms of tolerability, bioavailability in a mouse model related to NLRP3 related disease. CY-09 is the first compound identified that was specifically able to inhibit NLRP3 both in *in vitro* and *in vivo*. However, additional studies needs to be performed for its selectivity towards NLRP3 (Jiang et al., 2017; Lamkanfi & Dixit, 2017).

OLT1177 which was identified as a candidate for the treatment of degenerative arthritis, has successfully cleared phase I clinical trial, and now is in phase 2 clinical trial for the treatment of acute gouty arthritis. Marchetti et al. has described that OLT1177 possess anti-inflammatory action and can inhibit NLRP3 inflammasome activation. Like CY-09, OLT1177 also directly binds to NLRP3 and inhibits ATPase activity (Marchetti et al., 2018; Toldo et al., 2019).

Till date, so many molecules have been identified and studies in mouse models as NLRP3 inflammasome inhibitors but only few were identified to be of clinical value. However, a new study

identified the anti-allergic drug, Tranilast can be used to treat inflammatory diseases. Huang and colleagues identified Tranilast can inhibit NLRP3 specifically. Like the other specific inhibitors Tranilast does not interfere with the signalling pathways of NLRP3 inflammasome. Tranilast can also inhibit NLRP3 inflammasome activation via an ATPase-independent manner. Further, *in vivo* studies showed that Tranilast has a preventive effect on mouse models related to NLRP-3 related diseases. The safety profile needs to be investigated further(Feng et al., 2020; Y. Huang et al., 2018) .

Fig 8: Amyloid beta deposits will activate the NLRP-3 inflammasome and in turn increases the tau

hyperphosphorylation. This causes the degeneration of neurons and AD pathology.

A widely used OTC herbal medicine for the treatment of inflammatory diseases *Rabdosia Rubescens* has an active constituent oridonin which is reported to exhibit anti-inflammatory effects (Kuo L, 2014). Previous reports have mentioned that oridonin can suppress inflammatory mediators and exhibited therapeutic effects on neuroinflammation. Studies suggest that oridonin can specifically inhibit NLRP3 inflammasome activation(Kuo et al., 2014; S. Wang et al., 2019; W.-Y. Wang et al., 2015; Y. Yang et al., 2019). These drugs are used in clinical practices for different disease but studies need to be further done for the therapeutic potential of these agents in NLRP-3 inhibition associated with AD.

2.8 Matrix metalloproteinases (MMP):

Matrix metalloproteinases are also known as matrixins or matrix metalloproteinases and are calcium-dependent proteases belonging to the metzincin superfamily. MMP family consists of other 28 MMPs which are enzymes capable of degrading and processing many proteins in the extracellular matrix. MMPs are further divided into six main subgroups. Out of which gelatinase type (MMP-2,9) and membrane-type MMPs(MMP 1,2,3,4,5,6) are considered to be having a role in AD and other neurological diseases (X.-X. Wang et al., 2014; K.-J. Yin et al., 2006). MMPs play an important role in cytokine inactivation responsible for inflammation, and physiological processes such as neurogenesis and angiogenesis. MMPs are generally expressed in neurons but are also found in astrocytes and microglia. Astrocytes have been recently considered as mediators in the degradation of A β . Evidence suggests that MMP-2, MMP-3, and MMP-9 are important players in AD compared to the other MMPs (Brkic et al., 2015; Duits et al., 2015; K.-J. Yin et al., 2006; Yoshiyama et al., 2000).

MMP-2 is the major MMP that is directly linked to A β in the brain and the dysfunction of this enzyme exerts influence on the processing of A β (1-40/42) in *in-vitro* and in *in vivo* as deletion of MMP-2 leads to a greater A β accumulation rather than MMP-9 knockout. The proteolytic activity of MMP-2 and MMP-9 in clinically diagnosed AD patients showed higher activity of MMP-9 but not MMP-2 when compared with healthy brains suggesting that MMP-2 may have a protective role in AD(K.-J. Yin et al., 2006). Expression of MMP-9 is seen in cytoplasm, NFT, senile plaques, and in the regions of the hippocampus and cerebral cortex in AD patients. A post-mortem report of human frontal and parietal cortical tissues obtained from clinically diagnosed AD patients has shown a higher activity of MMP-9 rather than MMP-2 when compared with healthy brain samples. A recent study also has found out that astrocytes present near the amyloid plaques have shown an increased expression of MMP-9 in aged APP/PS1 mice(Brkic et al., 2015; Fragkouli et al., 2014). From these reports, a conclusive evidence can be drawn that MMP-9 is over expressed and is found to be higher in the plasma of people with AD. As AD is a complex disorder with many agents playing a role, targeting more than one MMP with pan-specific inhibitors or combination of MMP with another target of AD can also be explored (Lorenzl et al., 2003).

Minocycline, an anti-inflammatory drug that is used for multiple sclerosis and huntington's disease was tested for its effect on AD. It showed good tolerability and pharmacokinetic profile in *in vitro* as well as *in vivo*. However, during a randomised clinical trial conducted in AD patients, it did not show any improvement in the cognitive capacity nor did it slow down the progression of AD. The negativity is explained because of the poor dose that has been tested in animals. This can be further explored for AD by altering the doses in the preclinical studies and thereby testing in humans considering that

minocycline has a good penetration of BBB(Howard et al., 2020; Rosenberg et al., 2015; Yong et al., 2007).

MT-5 MMP is a protease that has come into limelight as a potential target related to amyloid plaques in AD. A study in 5xFAD mouse model of AD has shown that MT5-MMP deficiency has reduced amyloid levels in the cortex and the hippocampus suggesting that inhibitors targeting MMP-5 specifically can be explored for AD (Baranger et al., 2016).

ZHAWOC7726, a TIMP peptidomimetic has a good selectivity towards MMP-9, MMP-12, MMP-13 in the therapy of anti-cancer treatment. This molecule could be further studied as it is acting on different MMP's associated with AD(Gall et al., 2019).

2.9 Neprilysin (NEP):

Neprilysin, also called membrane metalloendopeptidase (MME) is an enzyme encoded by the MME gene in humans and cleaves peptides at the amino side of hydrophobic residues(Vodovar et al., 2015). It is found in a variety of tissues but more prominently is expressed in the kidneys and to a lesser amount in the brain and is an enzyme that controls A β degradation. NEP has a higher affinity towards the A β than other neuropeptides. **Fig 9** shows the role of neprilysin in degradation of amyloid plaques(Marr & Hafez, 2014; Webster et al., 2014).

During the early stages of AD, NEP is inactivated and down-regulated. Early studies suggest that maintaining NEP levels in the brain could be a potential therapeutic strategy to slow the progression of AD(El-Amouri et al., 2008; S.-M. Huang et al., 2006; Madani et al., 2006). The importance of NEP in amyloid regulation was demonstrated by using NEP knockout experiments. Elevated levels of A β species were seen in the hippocampus and brainstem when mice deficient for the NEP2 gene was used. In NEP2 knockout mice cross-bred with APP Tg mice, an increase in the level of A β was found (El-Amouri et al., 2008; Yasojima et al., 2000).

The discovery of endopeptidases such as NEP and NEP2 has opened up doors for viral-mediated gene therapy. While NEP has been a prominent target, NEP2 also can be considered selectively for A β clearance. A study has reported that the destruction of NEP has increased the levels of A β in the brain of mice(S.-M. Huang et al., 2006; Madani et al., 2006; Maruyama et al., 2005).

Taking into account that NEP expression and activity are reduced in AD, an idea of increasing the NEP upregulation might be beneficial. Saito et.al. has reported that somatostatin, a neuropeptide, and a NEP substrate can upregulate NEP activity by regulatory feedback cycle. Activation of somatostatin receptor 4 - subtype increases the activity of NEP in the cortical regions suggesting that somatostatin can be considered as an interesting target to increase the NEP level (Bavishi et al., 2015; Eckman & Eckman, 2005). Hence, effective strategies and studying the pathways to increase the levels of NEP

expression and activity may give us new opportunities for therapeutic interventions in AD as NEP inhibition has been a successful therapeutic story in heart failure with less ejection fraction(Pavo et al., 2020) .

Fig 9: NEP is present in the pre-synaptic and the post-synaptic region of the neuronal cells and cleaves the neuropeptide substrates ($A\beta$) leading to accumulation of the degraded $A\beta$ and promoting the pathology of AD.

2.10 Dopamine 2 receptor: (D2R)

Dopamine is a neurotransmitter and is generally expressed in the limbic system and the cortex that is related to mood and emotional stability(Ambrée et al., 2009; Kemppainen et al., 2003). Dopamine generally acts through five different receptors(D1,D2,D3,D4,D5). Cortical functions are influenced by the number of dopamine (D2) receptors available (Lidow MS, 1989). AD is associated with

deficits in several neurotransmitters but cholinergic and dopamine systems are the most investigated in neuronal research. Existing data suggests the levels of dopamine were found to be higher in AD patients than in control (Martorana & Koch, 2014; Reeves et al., 2017).

Dopamine (DA) containing neurons are mainly located in the midbrain. Dopamine plays an important role in synaptic transmission. Being a neuromodulator, there are chances of dopamine affecting the neurotransmitter release and membrane excitability of the pre- and the postsynaptic cells (Nobili et al., 2017). The evidence says that dopamine can modulate the activity of cholinergic release from the neurons located in the basal forebrain. The dysfunction of dopamine is being seen as a new player in the pathogenesis of AD (M. Liu et al., 2016).

A 1986 report has shown that administration of levodopa has restored cholinergic neurotransmission (Rinne et al., 1986). Taken altogether, we can say that dopamine regulates synaptic plasticity and also has the ability to maintain cognitive functions. Maintaining dopamine levels may reduce plaque formation by increasing synaptic plasticity and reducing oxidative stress (Cross et al., 1981; Martorana & Koch, 2014; Pan et al., 2019).

In a recent study, an article published in the Journal of Alzheimer's Disease, the researchers were trying to understand the link between the ventral tegmental area (VTA) and other parts of the brain. They analyzed the subjects by using 3 Tesla MRI technology. The reports indicated that, there is a relationship between size and the functionality of VTA, the size of the hippocampus and learning ability. The smaller size of VTA indicated that only a less amount of dopamine goes to the hippocampus and reduced memory performance. They concluded that this might be the reason for AD patients to have memory loss suggesting agents that can increase the dopamine levels in the body can reduce plaque formation and progression of AD (Pan et al., 2019). Thus, maintaining the dopamine levels in the brain can at least delay the progression of AD by stabilizing the signalling pathway and reducing the neuronal degradation.

2.11 Triggering receptor expressed on myeloid cells 2 (TREM 2):

It is a protein encoded by the TREM2 gene. In the CNS, TREM2 is expressed mainly in microglia and is involved in the production of proinflammatory cytokines and controls neuronal inflammatory events as depicted in **Fig 10** (Gratuze et al., 2018; Karanfilian et al., 2020). Being a transmembrane receptor, the involvement of immune and inflammatory pathways supports the fact that it has a role in AD. TREM2 is necessary for maintaining the microglial progression to a fully mature disease-associated microglia (DAM). Expression of TREM2 is associated with plaque formation. Microglia responds to A β accumulation and transforms into disease-associated microglia (Jay et al., 2017).

In a study reported, microglia have been shown to surround and limit A β plaques in the AD brain in the absence of microglial scavenging receptors and resulted in decreased clearance of A β by microglia (Carmona et al., 2018; Jay et al., 2017; Zheng et al., 2018). So far 46 variants of TREM-2 have been worked upon about AD. A rare genetic variant p. Arg47His (rs 75932628) is said to have an increased risk of developing AD in the European and American populations. But, the association of the same has not been prominent in the Asian countries suggesting that TREM-2 may play a population-specific role (Jay et al., 2017).

Several *in vitro* and *in vivo* studies have been done concerning TREM-2 and amyloid burden. *In vitro* studies have shown that TREM-2 is strongly involved in A β 40 and A β 42 uptake by the microglial cells and plaque formation. *In vivo* studies conducted on different mouse models suggest that an age-dependent effect on A β deposition is seen (Cheng et al., 2016).

Finally, the data from the study says that TREM-2 may result in greater A β deposition in the early stages of the disease and then becomes beneficial in the end stages by interfering inflammatory signalling and maintaining the ability of microglia to recover the injured neuron (Carmona et al., 2018). A full understanding of the dual role of TREM-2 in neurodegenerative disease, especially in AD is important. Addressing questions on whether altered phagocytosis of TREM-2 is the reason for the accumulation of amyloid plaques or is it the interaction of TREM-2 with other targets of AD can be a viable therapeutic strategy.

PY134 developed by Pionyr's for specifically targeting TREM-2 in cancer is in phase-1 clinical trial. As the molecule is able to bind to TREM-2 specifically, further studies can be done to explore its efficacy in AD (Tang et al., 2019).

Fig 10: TREM2 allows the activation of microglia, and promote clusters of microglia around Aβ leading to decreased phagocytic responses and increased levels of stress mediators. This build-up of microglia around Aβ results in reduced clearance and accumulation of Aβ , ultimately leading to degeneration of neuronal cells.

Activating a particular enzyme or target is difficult than inhibiting it. A study suggests that Alektor antibody (AL002) has the capacity to bind and activate TREM-2. Mouse version of the antibody, AL002a binds and activates TREM2. In cell culture, AL002a treatment increased phosphorylation of Syk, a downstream effector of TREM2 signalling. Another study has also reported that a similar monoclonal antibody developed in Germany is said to activate TREM-2 signalling by boosting the Phospho-syk signalling. Anti-TREM2 antibodies could perhaps serve as biomarkers, as well as therapeutic agents(S. Wang et al., 2020).

3. Recent developments:

June 7, 2021 can be termed as a historic day in the therapeutic sector of AD. Aducanumab, a monoclonal antibody approved by the FDA as a drug of choice to treat AD. The drug is branded as aduhelm and is licensed by Biogen and Eisai pharmaceutical companies under collaborative development. The FDA has approved this therapy under the accelerated approval pathway to benefit the patients with serious neurodegenerative diseases following the priority review. Aduhelm works by targeting the amyloid beta in the brain and reduces the amount of amyloid plaques, potentially slowing down the neurodegeneration and progression of the disease (Schneider, 2020; Sevigny et al., 2016).

4. Conclusion:

Several studies support the amyloid theory that A β is the starter to a complex number of pathological events in the brain concerning AD. A β starts to emerge long before the clinical symptoms appear physically. The deposition of A β not only causes hindrance to the signalling system but also triggers cerebral deficits, calcium homeostasis and cognition problems. Unfortunately, the research on amyloid plaques as a target for AD has been on a downward graph because of the failure of drugs in clinical trials. Even so, the re-launch of aducanumab as an anti-amyloid drug has stirred up this theory once again. The difficulty in pacing up with the molecular knowledge is still evident as we still depend heavily on the classical pathology. However, studies are done on a faster pace to identify the exact pathology behind AD suggesting that it is feasible to provide new targets for drug discovery and development directed towards the A β accumulation. In this article, we have listed down the targets that play various roles in plaque formation which could be helpful in designing effective drugs for AD.

The best possible way to eliminate A β pathology is to cease it from getting accumulated in the brain. The outcome that occurs on removing these pathological lesions in AD patients should be intensely focussed in the research studies.

5. Conflict of interest:

There is no conflict of interests among the authors

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Table :

Table 1: Gives a brief idea about the sources and functions of the targets discussed in this article.

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