



## REVIEW ARTICLE

# A Review on Alzheimer Disease

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### Abstract

Alzheimer is one of the most common causes of dementia that influence nerve cells in various parts of the brain. Pathologically it is caused because of intracellular neurofibrillary tangles and extracellular amyloid protein and results in the deposition of plaques which obstruct the communication between the nerve cells resulting in this neurodegenerative disease. The genetic risk factor found to be associated with this disease is mutation in APP, PSEN1 and PSEN2 genes. Also, the diet and nutrition play quite an important role in the development as well as prevention of Alzheimer Disease. The biomarker used for the detection of the disease should be able to differentiate between different causes of dementia and should be able to detect it at early stage. Further the use of Induced pluripotent stem cells has proven to be an effective treatment for the cure of this disease. The objective of this review is to highlight about the pathway that lead to this disease and stem cell treatment of this disease.

### Keywords

Pluripotent stem cells, Amyloid protein, Dementia

## Introduction

Worldwide population is rapidly aging, and the cases of dementia are growing. It has been reported that 35 million people worldwide have Alzheimer Disease (AD) or other types of dementia and about 65 million people are expected to have dementia problem by 2030 [1].

Dementia is a clinical syndrome linked with progressive downturn of the intellectual function of the brain and the person affected is not able to carry out the daily activities properly [2]. The initial signs of Alzheimer's often are lapses in memory or struggling to look for the right words. Over time, symptoms like language, reasoning, decision making, visuo-spatial function, at-

tention and orientation memory loss grow and become progressively more severe.

Alzheimer's disease is one of the most common types of dementia [3]. AD is a progressive multifactorial neurodegenerative brain disorder with no known cause and several alterable and non-alterable risk factors are associated with its development. Age is the greatest non-genetic risk factor amongst all [4,5]. It causes functional as well as structural disturbance of brain's nerve cells. In early means of disease, it also causes synaptic dysfunction of nerve cells thereby affecting the communication within neural circuits which is important for memory and other cognitive functions [6].

The cause of the disease is still not clear, but researchers have found that people victimized by Alzheimer's have an unusual build-up of certain proteins in their brain. One of these proteins, called amyloid beta, clumps together to form 'plaques'. Another, called tau protein gets twisted into protein 'tangles'. Researchers are still looking whether these changes in the brain result's in the symptoms of AD. Several theory related to the development of AD have been postulated some of which we have covered in later part of our review.

Genetic aspect of Alzheimer's seems to be because of the dominant autosomal mutation in one of the presenilin genes located on chromosomes 1 and 14 or in the amyloid precursor protein (APP) gene located on chromosome 21. In addition to this, individuals with Down's syndrome (trisomy 21) have an increased risk of developing early-onset AD. Although the genetics of AD are more complex and less well understood. It is known that the epsilon four allele of the apolipoprotein

E (APOE) gene located on chromosome 19 is a risk factor for the development of sporadic AD [7].

Researches revealed that lower concentration of vitamin D (1,25dihydroxy-vitamin D3) is associated with all type of dementia and Alzheimer's [8,9]. The active form of vitamin D, 1,25dihydroxy-vitamin D3 (1,25-D3), regulates neurotrophin expression, which includes nerve growth factor, neurotrophin 3 and glial-derived neurotrophic factor and the survival, development, and function of neural cells [10,11]. Under *in vitro* conditions vitamin D increases the phagocytic clearance of amyloid plaques by stimulating macrophages [12,13].

Different studies have revealed that vitamin D could be a neuro-protector and in the case of dementia risk around 50 nmol/L is sufficient. This information would prove to be useful in improving the design and reducing the cost of randomized controlled trials investigating whether vitamin D supplements can be beneficial to delay or prevent the onset of dementia and AD in older adults [14].

## Changes in Brain

AD leads to nerve cell death and tissue loss and over-time the brain size shrinks affecting all the functions of the brain. Cell loss in cortex region of brain causes damage in thinking, planning and remembering ability of brain. Shrinkage is most severe in hippocampus area of brain important for formation of new memories. Apart from this shrinkage in brain's region the ventricles, fluid filled space in brain, grow larger. Compare to any healthy person an AD patient brain have fewer nerve cells and synapses but high built-up of tangles and plaques which might be the reason of these cell death. These plaques block cell to cell signalling and activate immune system cells that cause inflammation and devour disabled cells. Tau proteins collapse into twisted strands called tangles because of which nutrients and other essential supplies no longer move through the cells and they die.

## Causes of the Disease

Alzheimer's disease accounts for 60%-70% of cases of dementia. It is a chronic neuro-degenerative disease that usually starts slowly and gets worse over time. One theory is that plaques avert nerve cells inside the brain from communicating appropriately. Tangles may make it complicated for the cells to get the nutrients they require. It is understandable that as Alzheimer's develops certain nerve cells die rising numbers of nerve cells, also known as neurons, are lost as the disease evolves.

- 1. Age:** Age is the single most significant factor in the development of Alzheimer's disease. The likelihood of developing the condition doubles every five years after you reach 65 years of age.
- 2. Down's syndrome:** People with Down's syndrome are at a higher risk of developing Alzheimer's disease.

This is because the genetic fault that causes Down's syndrome can also cause amyloid plaques to build up in the brain over time, which can lead to Alzheimer's disease in some people.

- 3. Genetics:** The genetic heritability of Alzheimer's disease (and memory components thereof), based on reviews of twin and family studies, range from 49% to 79%. Around 0.1% of the cases are familial forms of autosomal (not sex-linked) dominant inheritance, which have an onset before age 65. This form of the disease is known as early onset familial Alzheimer's disease. Though its rare but small percentage of people develop AD before age 65 yr. Three genes which are linked with causing AD due to mutation in them are- Amyloid precursor protein (APP), Presenilin 1 (PSEN1) & Presenilin 2 (PSEN2) [15].

## Late-onset alzheimer's gene

Most commonly AD begins after age 65 yr and the gene associated with is apolipoprotein E (APOE). This APOE occur in 3 form of which APOE e4 one increases the risk of Alzheimer. Other gene associated with AD are- SORL1, CLU, PICALM, CR1 etc [15]. Most of autosomal dominant familial AD can be attributed to mutations in one of three genes: Those encoding amyloid precursor protein (APP) and presenilins 1 and 2.

## APP amyloid beta precursor protein

This gene encodes a cell surface receptor and transmembrane precursor protein which is cleaved by secretases to form a number of peptides which when secreted bind to the acetyltransferase complex APBB1/TIP60 to promote transcriptional activation and form the protein basis of the amyloid plaques found in the brains of patients with Alzheimer disease. This gene is found in chromosome 21. The constitutive upregulation of soluble  $\beta$ -amyloids which further results in the formation of amyloid plaques is associated with the pathogenesis of AD. The anti-amyloid therapy consisting of the monoclonal antibodies solanezumab, crenezumab & gantenerumab that targets soluble & insoluble  $A\beta$ -aggregates, but they were unsuccessful to improve the clinical outcomes of AD due to the limitation and adverse effects associated with them [16,17].

## APOE apolipoprotein E

The protein encoded by this gene binds to a specific liver and peripheral cell receptor and is essential for the normal catabolism of triglyceride-rich lipoprotein constituents. This gene is present in chromosome 19 along with apolipoprotein C1 and C2 genes. Mutations in this gene result in type III hyperlipoproteinemia (HLP III), in which increased plasma cholesterol and triglycerides are the consequence of impaired clearance of chylomicron and VLDL remnants.

Most mutations in the APP and presenilin genes increase the production of a small protein called  $A\beta$ 42,

which is the main component of senile plaques. Some of the mutations merely alter the ratio between A $\beta$ 42 and the other major forms particularly A $\beta$ 40 without increasing A $\beta$ 42 levels. This suggests that presenilin mutations can cause disease even if they lower the total amount of A $\beta$  produced and may point to other roles of presenilin or a role for alterations in the function of APP and/or its fragments other than A $\beta$ . There exist variants of the APP gene which are protective. Mutation in them causes production of excessive amounts of a toxic protein fragment called amyloid-beta peptide. As these fragments stick together and collect in the brain as amyloid plaques, the tau protein malfunctions. As the tau protein particles stick together and form neurofibrillary tangles, the brain cells die, and the signs and symptoms of Alzheimer's disease develop [15].

## Alzheimer's & Diabetes

We know there are certain diseases that connected to our diet like diabetes, but researchers have found a strong connection between food we consume and Alzheimer and dementia via similar pathway that causes diabetes 2, and so they even re-name Alzheimer to diabetes 3. The brain glucose metabolism level gets deteriorated in Alzheimer's disease. Out of the three types of diabetes, type 2 diabetes mellitus has been reported to increase the risk for Alzheimer's, but the reasons are still not known [18].

Previous studies have shown that the increase in glucagon-like peptide 1 (GLP-1) helps to normalize insulin signalling in type 2 diabetes. GLP-1 is also majorly involved in neuronal activities and brain functions. Specific role of GLP-1 receptors in synaptic plasticity and cognitive processes in a GLP-1 receptor knockout mouse model was tested and it was found that due to the lack of GLP-1 receptor function in the brain affects synaptic plasticity and cognitive processes and hence GLP-1 receptors play major role in brain functions [18].

In an animal study researchers induce Alzheimer like characteristics by interfering with insulin signal in brain. Insulin produced in our brain is important for proper brain signalling and its disruption can lead to dementia [19].

Diabetes is linked to AD because insulin resistance and diabetes both increased the development of plaques in brain [19]. As we over-indulge on sugar and grains, our brain becomes overwhelmed by the consistently high levels of insulin and eventually shuts down its insulin signalling. In a study it was found that consumption of high level of fructose and low omega-3 diet reduces the affinity of insulin for its receptor leading to chronic insulin resistance as evidenced by a decrease in phosphorylation of the insulin receptor and its downstream effector Akt in just 6 week in rats [20].

Maternal malnutrition and childhood malnutrition might have impact on child's mental and physi-

cal health. It can lead to development of type 2 diabetes mellitus, hypertension, insulin resistance, and cardiovascular diseases in adulthood. Protein energy malnutrition affects brain growth and function and affects CNS function too. Neuro-pharmacological studies revealed that long-lasting changes in brain neural receptor function could occur due to early episode(s) of malnutrition [21].

In a separate study it was found that the levels and activities of components of the insulin-PI3-AKT signalling pathway decrease in Alzheimer's. It was suggested that this decrease in insulin-PI3-AKT signalling might lead to the activation of glycogen synthase kinase-3 beta, the major tau kinase. This could trigger abnormal tau hyperphosphorylation that could lead to the formation of intracellular neurofibrillary tangles [22].

## Prevention

### Through diet

Nutritional support could slow the progression of dementia and probably improve the quality of life of AD patients without any effect on survival rate [23]. Foods like fish, fruits, vegetables, nuts, or even Indian spices have been verified to decrease the risk of AD up to 45%. As mention above in our review fructose should be consume less than 25 g/day. There are some researches that suggest decrease in Alzheimer symptoms with good level of magnesium in brain. Vitamin D too exerts beneficial effect on AD by its immune boosting and anti-inflammatory properties. Diet rich in vitamin B12, omega-3 too should be consumed.

Folic acid increases concentrations of  $\omega$ -3 PUFAs (polyunsaturated fatty acids) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that are known to be useful in the prevention/treatment of dementia and Alzheimer's disease. Both EPA and DHA enhance NO generation, suppress production of pro-inflammatory cytokines, and enhance brain acetylcholine levels, a neurotransmitter whose levels are decreased in Alzheimer's disease.

Beta amyloid is an abnormal protein that is also found in the plaques of arterial deposits. Beta-amyloid is a toxic invader that arises when the body is in 'emergency mode', resulting in inflammation, as the immune system becomes over-reactive. So, if inflammation is the key then by providing natural anti-inflammatory nutrients in diet like anti-oxidants and omega-3, the damage in brain might be reverse. In patients of AD antioxidants such as vitamin A, beta-carotene, and vitamins C and E, etc are found to be in low amount so by increasing their concentration back to normal might the key to cure the disease [20,24].

### Through astrocytes

In a study conducted on cultured adult and neonatal mouse, astrocytes were transplanted into the hippo-

campus of AD mice. Seven days later, these astrocytes were found mainly near A $\beta$  deposits and internalize human A $\beta$  immuno-reactive material *in vivo*. This study supports the role of astrocytes as an active A $\beta$  clearing cells in the brain, which may have important implications for future development of therapeutic strategies for AD [25].

### Through stem-cells

The neural stem cell transplantation induces a robust enhancement of BDNF-mediated hippocampal synaptic density and rescues the spatial learning and memory deficits of AD mice, without altering A $\beta$  deposits. This study suggested that modulation of neurotrophin levels could provide a viable approach in the development of stem cell-based therapies to treat AD in future [26].

Researchers transplanted the human umbilical cord blood derived mesenchymal stem cells into the hippocampus of the AD mice they found reduction in neuronal apoptosis which rescues memory deficits of host mice.

The BFCNs play essential roles in various aspects of cognitive function, such as learning, memory and attention, and the cholinergic blockade disrupts the cognitive function of normal humans. Numerous studies have shown the severe devastation of basal forebrain cholinergic innervation and resultant declined cholinergic neurotransmission in the brains of AD patients, and even in the early stage of AD patients. Also, the most severely affected areas in AD brain are within the temporal lobes, especially within the hippocampus. These studies point out that the degeneration of BFCNs essentially contribute to the cognitive deficits and pathogenesis of AD, suggesting that BFCNs might be an ideal type of donor cells to ameliorate the cognitive symptoms associated with AD [27,28].

The optimal strategy directing the differentiation of pluripotent stem cells into BFCNs *in vitro* has not been established, which is mainly due to the unclear molecular basis of the differentiation and development of BFCNs *in vivo*. A number of endogenous neurotrophic factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), basic fibroblast growth factor (bFGF) and bone morphogenetic protein 9 (BMP9), have been reported to participate and promote the survival, growth, and differentiation of cholinergic neurons, and probably BFCNs in the brain [29,30].

Both mouse and human ESC-derived BFCN progenitors have been transplanted into the NBM of transgenic AD model mice, 5XFAD and APP/PS1, and specifically differentiated into mature and functional cholinergic neurons *in vivo*. These exogenous cholinergic neurons exhibited typical basal forebrain cholinergic projection and migration patterns, and morphologically and functionally incorporate into the endogenous projection system. Importantly, AD mice with transplanted BFCN pro-

genitors exhibited improved learning and referenced memory abilities in the behaviour test, demonstrating the feasibility of using ESC-derived BFCNs for the development of stem cell therapy for AD.

The scientists from StemCells Inc. have successfully isolated a highly purified, expandable population of neural stem cells from human brain tissue by using monoclonal antibodies against the cell surface markers. Then, the human neural stem cells have been prepared under controlled conditions and cGMP standards and named HuCNS-SC cells. The rigorous preclinical studies have shown that these HuCNS-SC cells can survive long-term with no evidence of tumor formation or adverse effects, and engraft, migrate, differentiate into neurons, astrocytes and oligodendrocytes.

### Induced pluripotent stem cells

Not all stem cells are the identical. Some stem cells can build any kind of cell in the body. These are called 'pluripotent' stem cells and are found in early embryos. They are the initial point for every kind of cell in the body. These embryonic stem cells can be reserved for many years in a laboratory, because they can keep dividing, producing more stem cells which are also pluripotent. They are potentially the most helpful type of stem cell.

Current research is using a type of stem cell called induced pluripotent stem (IPS) cells to study Alzheimer's disease. These lab-grown stem cells are made by 'reprogramming' specialised cells such as skin cells. The resulting IPS cells can produce all the different types of cells in the body. This means they could act as a source of cells that are otherwise difficult to obtain, such as the neurons found in the brain [31].

Scientists have recently used IPS technology to grow neurons in the lab that show some of the key features of Alzheimer's disease. The researchers took skin cells from Alzheimer's patients and reprogrammed them to make IPS cells. They then developed a method for growing neurons from these IPS cells in a dish. The lab-grown neurons release the beta amyloid protein that forms plaques in patients' brains. This gives scientists a valuable opportunity to study neurons similar to those affected by the disease in the brain, e.g. to gain a better understanding of how and why protein plaques and tangles are formed, and to search for and test new drugs [31].

### Biomarkers

If we could diagnose Alzheimer's before symptoms started the hope is, future treatments would then target the disease in its earliest stages, before irreversible brain damage or mental decline would occurred. It is currently diagnosed only via clinical assessments and confirmed by post-mortem brain pathology. The development of validated biomarkers for Alzheimer's disease

is essential to improve diagnosis and accelerate the development of new therapies.

The majority of AD cases are sporadic (risk age > 60 years), and < 2.5% have a genetic disposition. An ideal biomarker would be one that could help in early detection and can differentiate AD from other type of dementia.

ELISA is performed for checking beta-amyloid (1-42), total tau and phosphor-tau-181 from cerebrospinal fluid is most accepted method for diagnosis.

Intra-neuronal inclusions of the microtubule-associated protein tau are significantly higher compare to healthy person with a cutoff of > 600 pg/ml [25,31]. Tau is markedly hyperphosphorylated (39 possible sites) in AD, which results in a lack of function and axonal transport dysfunction. The detection of tau phosphorylated at position 181 is significantly enhanced in AD compared to controls, with a cut-off of > 60 pg/ml [22]. Also, there is deposition of extracellular A $\beta$  plaque, which gets cleaved from amyloid precursor protein (APP) by secretases, and processing of amyloidogenic pathways produces a 42-amino-acid peptide [A $\beta$  (1-42)] that can aggregate in the brain under certain conditions. In AD patients there is comparatively reduction in the A $\beta$  with cut-off of < 500 pg/ml [25,31,32].

### Future Aspects

Diseases like Alzheimer's require an early diagnosis in order to achieve effective treatment. Since the number of Alzheimer's disease cases are growing at an alarming rate it becomes imperative to employ advanced technologies to combat this disease. In the recent years, many researchers have being conducted and still going on the biomarkers, proteomics and genomics. Despite of these researches there are still a variety of challenges that need to be overcome. Availability of technology alone cannot help fight the disease, standardization of methods and techniques are of prime importance in terms of maintaining consistency and achieve an appreciable amount of reliability.

### Conclusion

In this review we have stated some rationale and possible strategies for the treatment of AD. Various studies have shown that the causative metabolic pathways include's extracellular amyloid plaques, intracellular neurofibrillary tangles, synaptic deterioration, and neuronal death which ultimately leads to AD as a neurodegenerative disorder. About 70% of AD risk at any given age is attributable through genetics. The most common genetic risk factor for AD is the epsilon 4 allele of the gene for apolipoprotein E (ApoE). Apart from the genetic and molecular aspect vitamin D deficient diet, active form of which regulates nerve growth factor seems to be another cause of AD. Also, in AD brain glucose metabolism decrease causing diabetes 3

reasons of which are still not clear. Finally, we would like to conclude that biomarkers and stem cell therapy could be emerging techniques in early diagnosing & treatment of AD.

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