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Understanding the genetic, molecular, and cellular basis of ageing as the biggest risk factor of Alzheimer's disease

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ABSTRACT: Alzheimer's disease (AD) is one of the leading causes of dementia. The disease is characterized by atrophy of brain tissue, with major physiological, molecular, and anatomical changes being observed in the hippocampus and entorhinal region of the temporal lobe. The risk of developing this disease increases with advancing age. Ageing is a chronological phenomenon wherein a considerable decline is observed in physiological functions due to the complex interplay of various exogenous and endogenous factors such as genetic construction, elevated levels of ROS, decrease in the telomerase activity, and epigenetic factors such as methylation of DNA, histone modification etc. The physiological and molecular changes in an ageing person especially in neurons overlap considerably with those observed during the progression of AD. This article highlights various factors responsible for ageing as well as AD with the latest review of literature. Understanding the factors that bring about the fated changes and how they are associated with the progression of disease can open new doors to bring about better treatment options and help cure an otherwise incurable disease.

Keywords: Alzheimer's disease; Ageing; Dementia; Neurodegenerative; Telomeres; DNA methylation.

1. INTRODUCTION

Around 50 million individuals worldwide suffer from dementia, a symptom of neurodegenerative disorders characterized by deterioration of memory, thinking, behavior, and ability to perform everyday activities, with roughly 10 million new cases diagnosed each year. Alzheimer's disease is the most frequent cause of dementia, accounting for 60-70% of all cases [1]. Neurodegenerative disorders are characterized by progressive loss of neurons in the brain, directly indicating compromise in cognitive functions. The hippocampus and entorhinal region of the temporal lobe of the brain, responsible for memory, are greatly affected. Alzheimer's disease is a major neurodegenerative disorder and one of the leading causes of dementia [2]. The most prevalent form of Alzheimer's disease is the one which begins after the age of 65, referred to as Late Onset of Alzheimer Disease (LOAD), a lesser occurring version is that of Early Onset of Alzheimer Disease (EOAD) which manifests before the age of 65 [3]. The majority of Alzheimer's disease cases are sporadic *i.e.,* they do not have a pre-genetic disposition, and the remaining ones are inherited in an autosomal dominant pattern [4]. A major risk factor for the development of AD is ageing [5, 6]. Ageing is a time-

dependent phenomenon, wherein a considerable decline is observed in physiological bodily functions. In terms of the severity of symptoms, AD progresses from mild to moderate to severe. The mild symptoms include memory loss, decreased ability to take decisions, struggling to complete normal day-to-day trivial tasks, forgetting and re-asking the same question repeatedly, and developing aggressiveness and anxiety issues. Moderate Alzheimer's is characterized by severe memory loss and extreme forgetfulness, losing the capacity to learn novel things, impairment of speech, difficulty in organizing speech, hallucinations and delusions while weight loss, seizures, extreme restlessness, difficulty in eating and swallowing, inability to walk properly, high risk of pneumonia, loss of bowel movements and increased sleeping are symptoms of severe Alzheimer's disease [7].

Alzheimer's disease, one of the leading causes of dementia, is characterized by hallmarks such as amyloid-beta plaques (formed as a result of reduced Aβ40/Aβ42 ratio), neuritic plaques, and *tau* neurofibrillary tangles [8]. The manifestations of these hallmarks have been seen to rise substantially with a progressive increase in the age of individuals. Certain inflammatory cytokines which are found in aged people are also found in people suffering from AD [8]. An ageing brain, otherwise not having AD, still shows amyloid-beta plaques and neurofibrillary tangles. Ageing can, thus, be linked to AD, and understanding the mechanisms underlying the two can provide a better insight into the disease epidemiology [4].

2. PATHOGENESIS OF ALZHEIMER'S DISEASE

AD is characterized by hallmarks such as extracellular amyloid-beta plaques containing abnormally high amounts of the protein Aβ42. Such high molecular weight monomers are harmful since they tend to increase the viscosity of the cerebrospinal fluid (CSF) along with intracellular neurofibrillary tangles [2, 9]. AD pathogenesis begins with altered cleavage of amyloid precursor protein (APP). Amyloid protein is a transmembrane protein type-1 *i.e.,* protein whose amino-terminal faces extracellularly. Under normal physiological conditions and in an undiseased state, APP undergoes non-amyloidogenic processing, by α- and γ-secretases, to produce soluble APPα (sAPPα), in a process called shedding, and a C-terminal fragment (CTF-83), which is ultimately cleaved by γ-secretase to produce extracellular fragment p3 and APP intracellular domain (AICD). sAPPα is generally associated with neuron growth, plasticity, and synaptogenesis. However, during amyloidogenic processing, APP is cleaved by β secretase into sAPPβ and CTF-99. CTF-99 is further acted upon by γ-secretase to produce AICD and amyloid-beta (Aβ) which is secreted outside the cell and eventually forms extracellular plaques, responsible for neuron loss and degeneration of brain tissue (Figure 1). The abnormally high content of Aβ42 in Aβ manifests the harmful attributes of AD [2].

3. BIOLOGY OF AGEING

An organism is said to be ageing when its chances of dying increase with its increasing age and when certain characteristic phenotypic attributes are observed as a result of declining physiological processes [10]. Telomeres are repetitive sequences present at the ends of the chromosomes which counteract the end replication problem. When an organism ages, the length of its telomeres decreases significantly, and some long-lived organisms have long telomeres, directly establishing the relationship between the two [11]. Telomeres are protected by multi-protein complexes called "shelterin" which prevent the cell from generating DNA damage responses and causing cell apoptosis (senescence). As many cell ages, it accumulates more cell divisions, which keep on shortening telomeres, ultimately leading to senescence. In cultures, mammalian cells

enter senescence after 40-60 cycles of cell division, referred to as Hay flick limit of cell. Telomere shortening is linked to increased stress levels in humans. As many cells experience senescence with age, the person's innate immunity is challenged, and age-related deterioration in overall health is observed [12].

Transcriptional activation of genes occurs via conserved TOR (Target of Rapamycin) signaling pathway. TOR pathway is typically responsible for protein synthesis via aerobic glycolysis to generate ATP and the pentose phosphate pathway to provide ribose-5-phosphates for DNA replication and thus provide energy for translation [13]. Since TOR leads to DNA replication, it indirectly leads to telomere shortening and hence, contributes a great deal to the process of ageing. Insulin activates the TOR pathway, whereas stress and tumour suppressor genes slow down the ageing process [11]. Similarly, defects in the IIS (Insulin/IGF-1) signalling pathway improve longevity and the main components of IIS signaling in mammals, include IRS, PIP3, PDK, Grb-2, Ras, Raf, MEK-1, and ERK-1 [14].

Mutant mice with defective IGF-1 have reduced insulin levels in the blood, increased survival, and increased resistance to oxidative stress [15]. Overexpression of Klotho protein (coded by *KL* gene) in mice increases longevity, and its deficiency is known to cause premature ageing in mice [16]. The results of defective IIS signaling are extremely contradictory because reduced levels of growth hormone and insulin cause growth-related defects and diabetes, respectively. However, patients suffering from Laron disease, wherein deficiency of growth hormone is observed, do not exhibit premature deaths and have increased resistance against cancer [11].

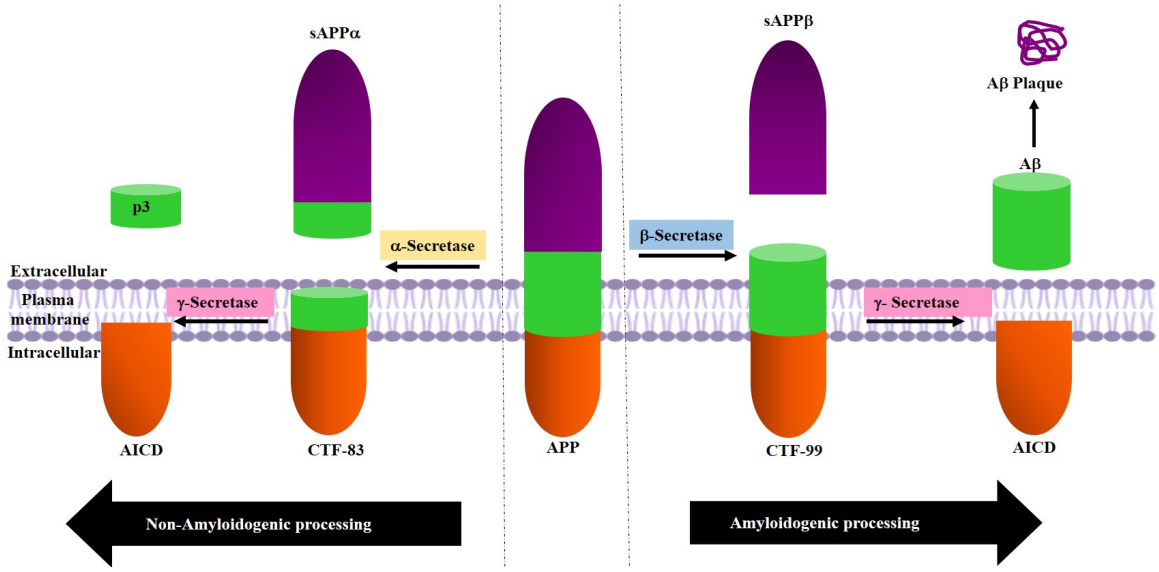

Figure 1. Amyloid precursor protein (APP) processing pathway.

Another cause of ageing is the loss of nuclear traffic as the cell ages, which occurs because nuclear pore complex (NPC) proteins are not replaced in post-mitotic cells, causing oxidative stress to accumulate [17]. As a consequence, the role of NPC deteriorates with age, resulting in the loss of compartmentalization between the nucleus and cytoplasm and a lot of cytoplasmic proteins freely enter the nucleus. This loss of compartmentalization is detrimental to neurons since, the localization of certain factors is essential for both, the generation and perception of stimulus [18]. Another critical mechanism for increasing longevity is downregulation of translation, which occurs when glucose and proteins are scarce, and is mediated by TOR and IIS signaling pathways.

Disruption of protein homeostasis, also known as proteostasis, is a significant characteristic of ageing. This pool contains protein chaperons, molecular machinery responsible for the maintenance, and the proteins that are being maintained [18]. Proteostasis ensures that protein consistency is maintained at all times by degrading misfolded and damaged proteins, ensuring proper protein folding, and allowing only healthy, correctly folded proteins to enter the cellular pool. However, oxidative stress and other endogenous and exogenous factors that appear to change the equilibrium, make it difficult to maintain this reservoir [19].

FoxOs (transcription factors; a subfamily of Fox i.e., forkhead box) are responsible for regulating responses triggered by various environmental stimuli such as insulin, growth factors, nutrients, and oxidative stress. Their function in upregulating the expression of genes involved in apoptosis, cell cycle arrest, and stress resistance is primarily responsible for their contribution to the longevity of eukaryotes. Suppression of IIS signaling is known to stimulate the FoxO pathway and thus slow down ageing [20]. The endoplasmic reticulum (ER) is another site of protein translation, however, when calcium homeostasis gets disturbed, protein glycosylation gets inhibited and there is the reduction of disulfide bonds, which results in the accumulation of unfolded protein contributing to ER stress. In normal cells, the genes encoding ER chaperons i.e., *GRP78/BiP* and *GRP94,* are upregulated, but in age-related neurodegenerative diseases, this unfolding protein response is suppressed. A mutation in presenilin-1 (PSEN1) and presenilin-2 (PSEN2) induces down-regulation of these pathways in Alzheimer's disease [1].

Autophagy helps in getting rid of cells of worn-out organelles, intracellular pathogens, and unfolded or misfolded proteins. As the clearance of degraded proteins and their recycling is essential for the development of new healthy cells, autophagy plays an important role in the longevity of cells. Downregulation of autophagy accelerates the process of ageing substantially [21]. In post-mitotic tissues such as the skeletal muscles, the mitochondrial volume density increases with age, implying that skeletal muscles produce fewer mitochondria as they age, resulting in a significant decrease in energy output. The mitochondrial volume decline affects longevity, both due to impaired ATP synthesizing machinery as well as a decreased amount of mitochondrial content, like the number of mitochondrial RNA transcripts that encode for mitochondrial proteins (such as those encoding for oxidative phosphorylation) decrease with age. The reduced amount of RNA transcripts, leads to decrease in the rate of protein synthesis [22]. Thus, the rate of protein turnover falls with progressing age and hence, a lot of oxidatively damaged proteins accumulate in the mitochondria. As people get older, their mtDNA accumulates a lot of point mutations. Both of these variables work together to generate the phenotypes associated with ageing. The unification of the cytoskeletal elements is an important prerequisite in mediating the cellular signalling as well as phagocytosis and intracellular trafficking. The disintegration of the cytoskeletal elements modifies the dynamics of the various processes leading to age-related symptoms and neurodegenerative disorders. This disruption of the structure has a profound effect on somatic as well as germ cells, further leading to various anomalies in the embryo [23, 24]. Thus, ageing is a result of the complex interplay of genes, epigenetic processes, changes in histones and RNA and other cellular changes.

4. AGEING AS A RISK FACTOR OF ALZHEIMER'S DISEASE

Ageing is known to be the greatest risk factor for Alzheimer's disease (AD). When the population reaches 65 years of age, the number of people diagnosed with Alzheimer's disease doubles every 5 years [25]. The risk of developing AD increases with ageing and there are several factors that can contribute to it.

4.1. Genetic construction

The ε4 allele of the *APO* (apolipoprotein) gene, *APOE4* is the strongest risk factor for the manifestation of late-onset AD. On the contrary, the ε2 allele (*APOE2*) provides a protective function. Both these alleles are also associated with the longevity of the organism. Several case-control studies decipher that the frequency of *APOE2* in elderly individuals and centenarians is high as compared to younger people, whereas the frequency of *APOE4* is lower in elderly individuals. Hence, a person with the *APOE2* allele develops Alzheimer's disease at a later age than someone with the *APOE4* allele. The ApoE protein isoforms are responsible for cholesterol transport in the central nervous system (CNS) and peripheral nervous system (PNS). ApoE4 protein contributes to the pathogenesis of AD by speeding up the conformational changes in amyloid-beta protein, causing it to adopt a beta-sheet conformation and thereby speeding up the process of neuritic plaque formation and aggregation. People having neuritic plaque aggregations have high amounts of Aβ40 in their CSF and on the contrary, the circulating levels of Aβ42 are very low. Middle-aged people who are cognitively functioning well, have shown this correlation, however, they had *APOE4* as their genetic composition and a lower amount of circulating Aβ42 as compared to the control subjects [4, 26]. It is confirmed that all the three isoforms of the *APOE* gene are inhibitors of Aβ aggregation, the ApoE4 protein being the least effective [4, 27]. Apart from this, there are many other genes that are associated with AD (Table 1).

4.2. Methylation of DNA

DNA methylation is an epigenetic mechanism that allows species to regulate gene expression. The eukaryotic promoter contains CpG islands and methylation of cytosine residues in these CpG islands is carried out by DNA (cytosine-5) methyl transferase enzyme (DNMT).

DNMT1, DNMT3A, and DNMT3B are eukaryotic enzymes that transfer a methyl group from S-adenosyl methionine (SAM) to the C-5 of cytosine, resulting in S-adenosyl homocysteine. S-adenosyl homocysteine is re-converted into S-adenosylmethionine through a series of reactions that form part of the one-carbon metabolism cycle [57]. Homocysteine is an intermediate in this cycle, and its elevated levels in blood plasma is linked to the pathogenesis of dementia and AD [58]. Mastroeni et al. [59] studied the effects of DNA methylation on monozygotic twins and found that the content of 5mC (5-methylcytosine) in the overall genome was decreased significantly in the regions of DNA found in the anterior temporal neocortex and the superior frontal gyrus in the twin who was diagnosed with AD as compared to the twin who was neurologically fit. The twins were chemical engineers and the twin who developed AD had been exposed extensively to pesticides. This experiment could well establish the role of epigenetic regulators in the manifestation of AD. Using immunohistochemical techniques, they investigated the differences in global DNA methylation of the temporal cortex (the relative amount of 5mC to cytosine levels in the sample) within the people suffering from AD and non-demented subjects. The results of this experiment confirmed the findings of the previous research, showing that patients with AD had lower levels of global DNA methylation in the temporal cortex. However, similar immunohistochemical studies reported contradictory results. Coppieters et al. [60] reported a substantial increase in the global DNA methylation levels in the entorhinal cortex of subjects suffering from AD while Lashley et al. [61] found no such correlation between DNA methylation and AD. Another study by Phipps et al. [62] studied global DNA methylation levels for 5mC and 5hmC (5-hydroxymethylcytosine) in the neuronal and glial cells (astrocytes) of the inferior temporal gyrus and suggested that DNA methylation is cell-specific. They concluded that patients suffering from AD had a

reduced level of global DNA methylation. However, the same results could not be derived for interneurons and glial cells.

Table 1. Genetic, cellular and molecular changes associated with ageing and AD.

Genetic/Cellular/Molecular changes	Association with	
	Ageing	**AD**
Genetics: 20-30% ageing is due to genetic factors while almost 60-80% of AD is due to the genetic factors. Genetic risk factors for AD - genes like *APOEε4* (strongest risk factor for AD), *TREM2, CD33, SHIP1, BIN1, CD2AP, CR1, ABCA7, CLU*, *EPHA1*, *PICALM*, *MS4A,* all of them have been found to have a significant contribution towards the pathogenesis of AD. Other genes involved in AD - *CASS4*, *CELF1*, *DSG2*, *HLA, DRB5, DBR1*, *FERMT2, NPP5D*, *MEF2C*, *NME8*, *SLC24H4 RIN3*, *SORL1*, *ZCWPW1* (do not contribute majorly).	[28]	[29, 30]
Genomic instability: occurs in ageing and AD due to exposure to exogenous agents like radiation and xenobiotic compounds or due to endogenous agents like DNA replication errors or ROS (Reactive oxygen species).	[29]	[31]
Mutations in genes: • *APP* gene (32 pathogenic mutations have been found out to have a significant contribution towards AD.) • Presenilin genes: *PSEN 1* (221 pathogenic mutations) and *PSEN 2* (19 pathogenic mutations) - lead to lysosomal dysfunction and increased mitochondrial autophagy. • SNPs in N-Methyl-D-Aspartate Receptor (NMDAR) genes	No direct evidence of these mutations in ageing has been formulated yet.	[29, 31-34]
• In normal ageing, Aβ accumulates intraneuronally and thus altering and declining the synaptic connections. However, excessive accumulation of Aβ leads to AD. Metal ions such as copper, iron and zinc help in Aβ aggregation in AD.	[35]	[33]
Microglial receptors active during ageing: cytokine colony-stimulating factor-1 (CSF1R); CXCR3, Astrocyte receptors active during ageing: CXCL5 Microglial senescence is associated with AD. Microglial receptors upregulated in AD: Triggering receptor expressed on myeloid cells-2 (TREM2), LRP1, Toll-like receptor 2/4 (TLR2/4), complement receptor 3 (CR3), Fc γ receptors IIb (FcγRIIb), CD33, CD36 and advanced glycation end product receptor (RAGE). Astrocytic receptors in AD: dysfunction of α 7 subtype of nAChR (α7nAChRs); upregulation of Calcium-sensing receptor (CaSR), CD36, CD47, and RAGE.	[36]	[29]
Telomere shortening/attrition: Associated with ageing and hence, with AD (telomerase deficiency).	[29]	[29]
Tau aggregation: Occurs in ageing and AD leading to memory loss.	[37]	[29]
Sirtuins: • SIRT1 is neuroprotective; Increase in *SIRT1* expression with age. *SIRT 1,2,3 & 6* are involved in AD pathogenesis.	[32]	[32, 33]
Klotho gene: • Expression decreases with age; works by activating expression of *TERF1* and *TERT* genes and Wnt signaling; *Klotho* deficiency downregulates *SIRT1* expression. Klotho gene expression decreases in AD patients; it decreases Aβ burden by promoting their clearance.	[32]	[38]
Synaptic dysfunctions: Eph are receptor tyrosine kinases, the receptor tyrosine kinases function in cell signaling. • Levels of EphB2 receptors decrease in ageing and AD Levels of EphA4 increase in AD	[39]	[29, 31]
Signaling pathways involved in ageing and AD: • Mammalian Target of Rapamycin (mTOR) signaling pathway • Nuclear Factor of Activated B-cell (NF-κB) signaling pathway • Nuclear Factor-E2-Related Factor 2 (Nrf2) signaling pathway • Wnt/β-Catenin Signaling Pathway • Adenosine Monophosphate Protein Kinase (AMPK) Signaling Pathway (these	[32, 33]	[32, 33]

Genetic/Cellular/Molecular changes	Association with	
	Ageing	**AD**
signaling pathways get compromised during AD and ageing)		
Epigenetic alterations in ageing and AD: • H4K16 acetylation and H4K20 and H3K4 trimethylation increase; H3K9 methylation and H3K27 trimethylation decrease; H4K12 histone acetylation decrease • Noncoding RNA patterns: microRNA (miRNA), long non-coding RNAs (lncRNAs) and circular RNAs (cirRNAs) expression patterns) • Increase in HDAC2 expression • DNA methylation	[29, 40, 41]	[29]
• Ageing and AD: Impairment of Protein homeostasis or proteostasis Ageing and AD: Dysfunctioning of autophagy-l lysosomal system and the ubiquitin-proteasome system (include mitophagy i.e., removal of damaged mitochondria).	[42, 43, 44, 45]	[34, 46]
Nutrient sensing systems: • Upregulation of amino-acid sensing target of rapamycin (mTOR) and low-energy state detectors (AMP kinase and the sirtuins) • Increased activity of insulin and insulin-like growth factor 1 (IGF-1) signaling (IIS) pathway, which targets the FOXO transcription factors and mTOR complexes. • AD: Malfunction in insulin signaling Disturbed energy metabolism	[28, 34, 47, 48]	[31, 34]
Mitochondrial dysfunction in ageing and AD: • Reduced respiratory chain efficiency; electron leak and reduced ATP generation. Mitochondrial proteins involved in its dysfunction: appoptosin, amyloid-binding alcohol dehydrogenase, cyclophilin D.	[49]	[29, 31]
Cellular damage and Stem cell attrition: • *INK4/ARF* locus expression of $p16^{INK4a}$ and $p19^{ARF}$ increases in ageing. Cellular pathologies observed in AD	[41]	[50]
• Low-level systemic inflammation or inflammation through factors such as interleukins, plasminogen activator inhibitor-1 (PAI-1), GFBP3 and transforming growth factor-β (TGF- β). Inflammation is a prodrome to AD	[51, 52]	[53]
Metastatic ageing: Due to ROS and miRNAs	[41]	[29, 34]
Increased oxidative stress and free radical formation (ROS)	[34, 54]	[29, 31, 34]
• Cerebrovascular changes: Cerebral amyloid angiopathy (CAA), a common cerebrovascular disorder. Glymphatic system: glial-lymphoid pathway, or glymphoid pathway is impaired in ageing and AD.	[55, 56]	[29, 31]
Sleep disorders	[29, 34]	[29, 34]

AD is also characterized by loss of synapses in the frontal cortex. Mastroeni et al. [59] study on the twins reported a significant decrease in DNA methylation in the frontal cortex. However, similar antibody-mediated studies done by Coppieters et al. and Rao et al. [60, 63] showed a considerable increase in global DNA methylation levels in the frontal cortex of subjects suffering from AD. Atrophy of the hippocampus is one of the hallmarks of AD and people with this disease experience significant memory deterioration. Since different sub-regions of the hippocampus have different patterns of methylation, the findings of global DNA methylation levels in the hippocampus are inconclusive. The levels of global DNA methylation for AD-specific genes were also investigated. Barrachina and Ferrer [64] studied methylation levels in *APP*, *PSEN1* and *MAPT* (microtubule-associated protein *tau*) in the frontal cortex of demented subjects and found no significant differences between the methylation levels of these genes within demented and non-demented subjects. Iwata et al. [65] on the other hand, using their pyrosequencing studies, drew conclusive results wherein significant differences in DNA methylation were seen in *APP, MAPT* and *GSK3B* (Glycogen synthase

kinase 3-beta) genes, however, the results were inconclusive for *PSEN1, BACE1* (beta-secretase 1) and *APOE*.

4.3. Changes in histones and RNA

Histones assist in the formation of nucleosome core and DNA packaging inside the nucleus. The packaged DNA can exist either as euchromatin or heterochromatin. For gene expression, certain histone marks (chemical modifications) are added on the N-terminal tail of histone proteins which include acetylation, methylation, ubiquitination, phosphorylation, hydroxylation, sumoylation, and proline isomerization. As the cell ages, a significant loss of heterochromatin is observed, histone tails show a lack of inhibitory marks and therefore, a lot of activating marks are observed. It is these activating marks that lead to the loss of heterochromatin. However, the effect of histone marks on the human brain is still unclear. It was also discovered that subjects with AD had reduced histone acetylation. Another study reported that the phosphorylation of H2AX protein in nucleosome of astrocytes occurs in response to dsDNA breaks, following which H2AX protein gets converted to γH2AX. This process of conversion is specifically high in the hippocampus and cerebral cortex, and these regions are severely impacted during AD. Therefore, the relationship between phosphorylation of astrocyte's H2AX protein and AD could be explored [66].

RNA is the intermediate macromolecule in the central dogma. mRNA is especially important in conveying information from the DNA to proteins, thus facilitating gene expression. MicroRNAs (miRNAs) regulate gene expression by preventing the expression of mRNA. These, miRNAs are first produced as precursor molecules, with an extended stem-loop structure, which are then cleaved by Drosha (a class 2 ribonuclease enzyme) inside the nucleus to obtain pre-miRNAs having stem-loop structure. These precursor molecules are transported from the nucleus to the cytoplasm, where Dicer cleaves them into double-stranded miRNA molecules. By base pairing with the 3'UTR of mRNA, double-stranded miRNA forms a complex with argonaute and RISC (RNA-induced silencing complex), causing gene silencing [67]. Studies on 10, 18-, 24-, and 33-month-old mice models, using microarray and global proteomic profiling established that miRNAs in ageing tissue are deregulated [5]. Furthermore, selective inhibition of the dicer protein causes neuronal tissue destruction in mouse models, which is a hallmark of AD. Long non-coding RNA (lncRNA) has also been linked to ageing. The age-associated expression patterns (age-lncRNA) are highly tissue-specific, but they are also co-expressed with a common pool of protein coding genes [68]. BACE1-AS, 51A, 17A, NDM29, BC200, and NAT-Rad18, are the six lncRNA's known to be associated with AD patients who have lower levels of RNA editing [69].

4.4. Cellular changes following ageing which contribute to AD

A person's brain volume decreases with age. According to statistics, once a person reaches the age of forty, the rate of degeneration is about 5% per decade, with the rate rapidly rising after the age of seventy. The depletion of neuronal stem cells is thought to be the cause of grey matter shrinkage [70]. It has also been proposed that an ageing brain shrinks due to a decrease in neuronal cell volume rather than its number [71]. The physical changes involving the dendrites and synapses are contrasting: on one hand, it is seen that the sprouting of synapses increases to compensate for the loss of neurons, on the other hand, loss of synaptic plasticity has also been seen. Amongst the entire brain regions, pre-frontal cortex is the most affected region in terms of its volume [70]. Some studies also suggest that it is the hippocampus that is affected the most in AD patients [72]. However, the extent of damage differs amongst individuals, it has been characterized that the hippocampus is more severely affected in men than in women [70]. It is the episodic and semantic

memory that gets affected the most in AD [70]. Episodic memory includes information on facts and semantic memory involves the memory associated with meanings. It is the disruption of episodic memory that is manifested as one of the earliest signs of AD, and it is this component that is affected the most during AD. The levels of dopamine and serotonin decrease with age, and the reduction in dopamine levels have been linked to loss of memory and motor functions. A decrease in levels of serotonin and brain-derived neurotrophic factor led to decreased neuronal plasticity and synaptogenesis [70, 73]. As people age, their nervous system loses out on a lot of weight, because the neurons undergo atrophy. This atrophy in part is attributed to the accumulation of waste products in the neurons, which stimulate their breakdown [73].

Healthy brain cells are resistant to oxidative stress, but, as the neuron ages, it displays compromised activity in terms of oxidative stress tolerance. This is due to the increased content of membrane lipids and significant damage to mitochondria due to the accumulation of ROS (Reactive Oxygen Species) leading to accumulation of damaged proteins which make neurons less effective in combating oxidative stress. Antioxidant activity is severely affected in AD, and it is also observed that amyloid-beta induces the formation of ROS, causing peroxidation of membrane lipids which disrupts synaptic potential growth [74]. Aβ proteins oxidise the LRP1 receptor, which is normally responsible for Aβ clearance, but in this oxidised state, these neurotoxic peptides accumulate. The metabolism of neurons is severely impacted as the neurons' age.

The brain only makes up 2% of the total body volume, but it consumes 20% of the body's total energy, which is generally in the form of glucose [74]. The brain utilizes glucose to generate energy for maintaining resting membrane potential, pre- and post-synaptic potentials for neurotransmitter release, and also for phospholipid remodeling and maintenance of lipid asymmetries across membranes during neuronal signaling. Therefore, as expected, the energy requirements of the brain increase when it is processing information. Even though the neurons use oxidative phosphorylation for generating the required amounts of energy, astrocytes (glial cells) use glycolysis solely for the same purpose [75]. GLUT transporters promote glucose uptake in astrocytes and neurons. GLUT-1 transporters are found in astrocytes and GLUT-3 and GLUT-4 transporters are found in neurons. Yin et al. [76] discovered that neuronal GLUTs decrease with age in aged rat brains. Therefore, with advancing age, there is reduced glucose availability (due to reduced GLUTs), reduced efficiency of mitochondria to generate energy, and increased oxidative stress leading to significant neuronal loss. As the neuron ages, glucose hypometabolism and disruption of mitochondrial integrity indicate initial signs of age-related impairment [74]. Therefore, due to less availability of glucose, the functions of the hippocampus are compromised and so is the volume of its neurons. It is also observed that as the brain ages the neuronal cells accumulate amyloid-beta protein and pro-inflammatory substances which are known to alter the signal-transduction pathways responsible for overall neuronal integrity. These attributions clearly explain the manifestation of AD as the person ages. The aggregation of lipofuscin granules, which are indigestible products of lysosomal degradation, is one of the most important indicators of neuronal ageing [74]. The CNS is composed of both neurons and glial cells, amongst the glial cells the effects of astrocytes which form the BBB (blood-brain barrier) is more pronounced. Verkerke et al. [74] analysed the astrocytes of a normal adult, an adult with the *APOE*ε4/ε4 allele, and an aged astrocyte from an elderly person in a comparative sample. In the first case, astrocytes, which make up the BBB, were found to be responsible for maintaining overall homeostasis in terms of ion concentration and neuronal transmission. In the second case, wherein the astrocytes express *APOE*ε4/ε4 allele, the astrocytes aged prematurely, the BBB was highly permeable, the cholesterol metabolism was highly disturbed, the secretion of BDNF (Brain-derived

neurotrophic factor) was significantly decreased, the homeostasis of neurotransmitters was disrupted due to accumulation of EAAT1 (Excitatory amino acid transporter 1) and GLUL (Glutamate ammonia ligase), resulting in inflammation and decreased clearance of Aβ. In the third case, astrocytes from an elderly person were taken, and it was discovered that the BBB permeability increased due to upregulation of BBB molecules (ITGB4, AQP4, GJA1), but levels of the anti-oxidant glutathione decreased which decreased resistance to ROS. These astrocytes also showed decreased levels of BDNF and neurotransmitter homeostasis (because of decreased mGluR3 expression and also showed decreased conversion of glutamate to glutamine). These also displayed an inflammatory profile. These characteristics well explain their relationship with AD [74]. The adult nervous system also has a reserve of neuronal stem cells which are known to differentiate into mature neurons and replace the lost pool of neurons. The pool of these stem cells reduce as the person ages. However, it is seen that these stem cells have a limited role in AD [5].

4.5. Inflammatory cytokines in ageing and AD

Changes in the nature and number of inflammatory molecules, leading to their dysregulation, have been associated with the process of ageing. This process is known as immunosenescence or inflamm-aging. The pro-inflammatory cytokines facilitating ageing include interleukin-6 (IL-6; also known as 'gerontologist cytokine'), tumor necrosis factor α (TNF-α), IL-1α, IL-18 etc. Other cytokines which are dysregulated in ageing and in age-related diseases include IL-2, IL-17, IL-12, IL-17R, and IL-8. The anti-inflammatory cytokines balance the actions of pro-inflammatory cytokines, thereby maintaining immune homeostasis during ageing or disease-inducing state. They include IL-10, TGF-β and IL-37 [77, 78].

The inflammation-associated molecules may act as the potential risk factors for AD. They may be used in the diagnosis and therapeutics of AD. The pro-inflammatory cytokines, which are either involved in causing AD or play a role during the progression of the disease, include interleukin-1 (IL-1), IL-6, tumor necrosis factor α (TNF-α), platelet-derived growth factor (PDGF), monocyte chemotactic protein 1 (MCP-1), IL-12, IL-7, IL-8, IL-9, IL-18, IL-1β and IL-15. However, some anti-inflammatory cytokines have also been observed in AD patients which include IL-4, IL-10, Granulocyte colony-stimulating factor (GCSF). In addition to these, the components of the complement cascade are also involved in AD pathogenesis. The single nucleotide polymorphism (SNPs) of complement genes such as clusterin (CLU) and complement receptor 1 (CR1) are major risk factors for AD. Cytokines such as IL-1β, IL-4, IL-6, IL-9, IL-17A, GCSF, Granulocyte-macrophage colony-stimulating factor (GMCSF), basic fibroblast growth factor (bFGF), interferon-gamma (IFN-γ), and macrophage inflammatory protein 1β (MIP-1β) are negatively associated with the AD progression [77]. Since the dysregulation of inflammatory molecules is one of the risk factors for AD, the knowledge of the mechanism of their action and their threshold levels in a healthy body may be exploited to control and slow down the progression of AD [79].

5. CORRELATION BETWEEN FACTORS OF AGEING AND AD

Many of the factors that play important roles in ageing, are also associated with AD. Some common factors include APOE genes, DNA methylation, RNAi etc. (Figure 2). However, AD is also characterized by specific genetic, molecular, and cellular changes Table 1.

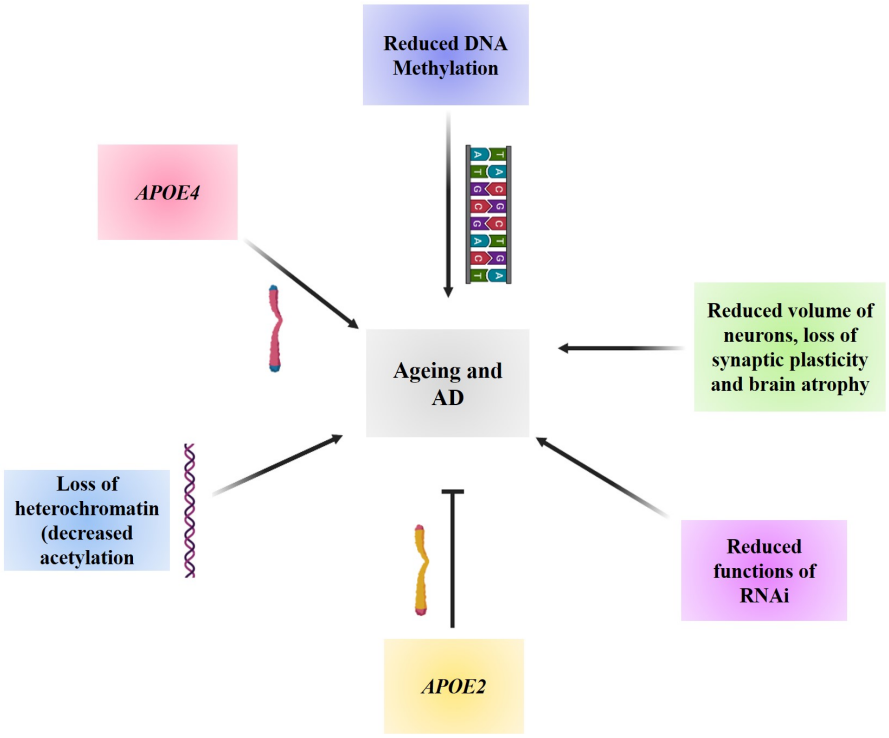

Figure 2. Epigenetic, genetic and cellular changes responsible for ageing and AD.

6. CONCLUSION

Alzheimer's is a progressive neurodegenerative disorder. This disease is characterized by atrophy of brain tissue and hence a compromised memory and cognition. It is seen to be more prevalent among aged individuals therefore; it becomes necessary to establish a relationship between factors leading to ageing and the manifestation of the symptoms of AD. From this review, we conclude that there are strong associations between cellular, molecular and genetic changes that occur in a neuron as it reaches senescence and the progression of AD. Changes in amyloid-beta clearance, reduced mitochondrial metabolism, increase in the lipid profile of the plasma membrane, altered transcription, and translation rates, changes in DNA methylation profiles, and the genetic makeup of the cell are all major contributors to neuronal senescence. This review focuses on changes, occurring at the cellular and molecular; however, there is a lot more scope to find the changes at the organ and organ system levels. Exogenous factors that contribute to ageing and how they can accelerate the development of Alzheimer's disease can also help neurologists gain a greater understanding of the disease's epidemiology and, as a result, develop better treatment options.

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