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Chapter 2

Pathophysiology of Alzheimer's disease

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2.1 Alzheimer's neuropathology

For a definitive diagnosis of AD, post-mortem microscopic histopathological examination of the brain must reveal the deposition of two types of protein aggregates: parenchymal deposits of amyloid ($A\beta$) extracellularly as 'plaques', and intraneuronal deposits of tau protein fragments as neurofibrillary 'tangles' (NFT), over and above that which occurs with normal ageing. Tau deposition may also occur as straight or paired helical filaments (PHF) surrounding plaques, and argyrophilic neuropil threads, which are predominantly neuronal dendrites containing tau deposits. Additionally, at least 80 per cent of cases have congophilic angiopathy, with cerebrovascular amyloid deposited in small blood vessel walls of the leptomeninges and cerebral and cerebellar cortex.

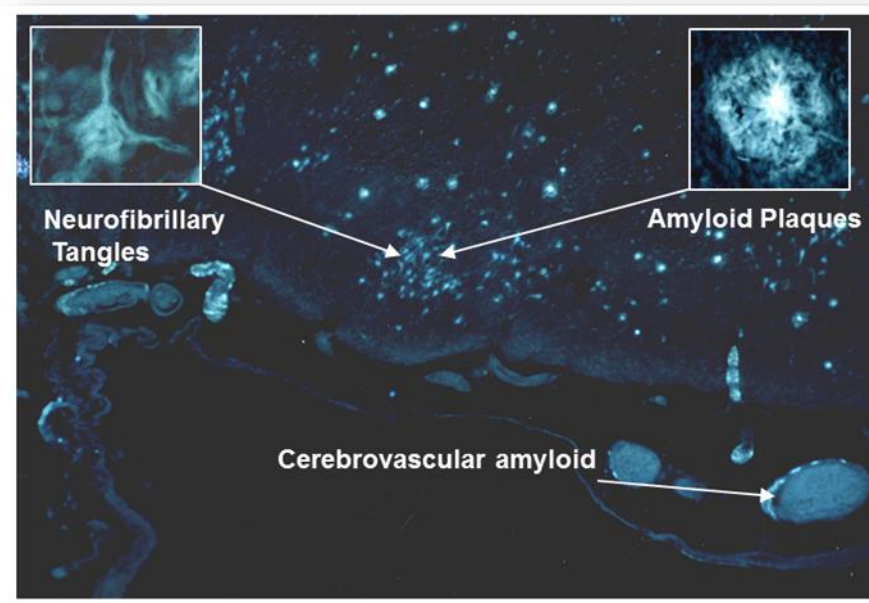


Figure 2.1 A section of temporal cortex stained with Thioflavin S, a fluorescent stain, showing clusters of amyloid plaques and neurofibrillary tangles and cerebrovascular amyloid at low magnification. Insert (top left) shows higher magnification of an amyloid plaque, (top right) shows high magnification of neurofibrillary tangles.

All three protein aggregates can be visualized using a fluorescent dye, such as Thioflavin S (Figure 2.1), which binds to proteins which form beta-pleated sheets. With disease progression there is an overall reduction in brain size, especially in the hippocampus and temporal lobe, where cortical gyri are thinner and sulci wider. Certain neurotransmitter-specific pathways are particularly vulnerable including the cortical glutamatergic system and also projections from subcortical nuclei such as the serotonergic dorsal raphe or noradrenergic locus coeruleus. Also, the cholinergic basal nucleus, and neurotransmitter deficits may cause symptoms such as depression, aggression, and memory dysfunction. These provide the rationale for the symptomatic drugs currently administered. There may be extensive gliosis, including hypertrophic astrocytes which have increased expression of glial fibrillary acidic protein (GFAP). Neuritic plaques frequently contain GFAP-positive astrocytic fibres. Early in the pathology microglial cells are activated and increase in the grey matter near neuritic plaques and NFT. These are enlarged and are activated; with increased expression of MHC Class II antigens and complement receptors, they may also express the receptor for advanced glycation endproducts (RAGE) which readily binds A β and mediates its effects. In later stages there is likely to be extensive cell loss with subsequent enlargement of the lateral and third cerebral ventricles. However, motor, sensory, and primary visual areas are generally spared until the end stages of the disease.

Dysfunctional axonal transport due to NFT formation will affect passage of a number of proteins including growth factors. For instance, nerve growth factor (NGF), which is synthesized in the cortex and hippocampus and retrogradely taken to the cholinergic basal nuclei, is known to have impaired transportation.

The importance of NFT as a correlate of dementia severity was reported in 1991 by Braak and Braak who described a well-defined route of deposition of increasing density with progressive stages of dementia. Stages 1 and 2 of NFT deposition are largely subclinical and this degree of NFT deposition is fairly common in the normal elderly. NFT are largely restricted to the transentorhinal, entorhinal, and CA1 regions of the hippocampus. At stages 3 and 4, NFT accumulates in the hippocampus and limbic system, and at the final stages, 5 and 6, this spreads to the neocortex. NFT deposition is statistically more closely linked with stages of dementia than with amyloid accumulation; one reason suggested for this may be because of the extracellular location of amyloid plaques and the ready availability of clearance enzymes including insulin-degrading enzyme (IDE) and neutral endopeptidase (neprilysin) to remove the plaques. Removal of intracellular NFT may be thought of as more difficult and may result in the extended presence of a trail of neuronal 'tombstones'. Amyloid plaques are frequently observed at the terminals of neurones which have intracellular NFT which suggests that tangles may form due to retrograde effects of A β actions at the synapse.

We now understand that the disease process is initiated at least 15–20 years before the first symptoms of cognitive impairment. With the growing availability of early diagnosis using imaging techniques and new biomarkers, there is hope that by increasing our understanding of the basic mechanisms which underlie the pathology we may actually be able to reverse what has hitherto thought to be an irrevocable process. With this comes a focus on the earliest changes likely to trigger the pathology, such as synaptic withdrawal which marks the

loss of communication within neuronal pathways. A number of studies both in human brain and animal transgenic AD models show a significant loss of synapses; in mice this is evident very early in the pathology. The focus therefore has moved away from the importance of the amyloid plaques and neurofibrillary tangles in the disease process to the soluble A β oligomers and phosphorylated tau peptides.

2.2 Genetics of familial Alzheimer's disease

The amyloid A β peptide present in parenchymal plaques or cerebrovascular deposits is a 4 kDa cleavage product of the amyloid precursor protein (APP), coded for by the APP gene. It has three main splice variants, APP770, 751, and 695, of which APP695 is the major neuronal form. APP is a multi-functional protein, important in development and synaptic plasticity. Although A β production occurs in normal neurones as well as those from AD, there are much higher brain levels of A β in AD, probably due to increased production or reduced clearance.

In autosomal dominant familial forms of AD (FAD), symptoms usually present earlier in life (i.e. before 60 years) and are due to mutations in one of three genes: APP, PSEN1, or PSEN2 on chromosome 21, 14, or 1 respectively. According to the Alzforum database <<http://www.alzforum.org/mutations>>, although many mutations are non-pathogenic, there are at least 25 APP pathogenic mutations, over 200 PSEN1, and at least 16 PSEN2 clearly pathogenic mutations which lead to autosomal dominant forms of AD. Familial early-onset AD accounts for about 1% of cases of AD; however, the underlying mechanisms provide an indication as to how the majority of sporadic (isolated or non-clustering) AD cases may occur. Those living with Down's syndrome (trisomy 21) usually develop AD pathology by their 40s, and this is thought to be due to the third copy of the APP gene provided by the extra chromosome 21. Thus gene duplication or the presence of mutations, which facilitate an increase in A β , result in AD pathology and consequent symptoms of cognitive dysfunction. Many of the FAD mutations present in the three aforementioned genes have been shown to result in an increase in total A β production or an increase in the A β 1-42:A β 1-40 ratio; that is, the two common forms of A β which comprise 42 and 40 amino acids respectively. This lends credence to the 'amyloid hypothesis' which suggests that all AD pathology and symptoms are derived from the toxic effects of A β , essentially by its overproduction or lack of clearance. Of the two forms, A β 1-42 has been shown to be more neurotoxic; it is usually found within parenchymal plaques as it has a propensity to aggregate more rapidly. A β 1-40 is found predominantly in the vasculature as it is sufficiently soluble to be cleared to the blood vessels before being deposited. There is a lot of evidence now to suggest that cerebrovascular amyloid deposition (cva) in small blood vessels may provide an important contribution to the AD pathological process.

2.3 Processing of amyloid precursor protein

The 'amyloidogenic' route, by which A β is produced during the processing of APP, is described in Figure 2.2. In the normal brain this constitutes only a small part of the processing of APP

protein, the rest is processed by the 'non-amyloidogenic' pathway where A β is not produced. The enzymes responsible for cleavage of APP are α -, β -, and γ -secretase. α -secretase (comprising three enzymes, known as ADAM 9, 10, and 17) are members of the ADAMs (A disintegrin and metalloprotease) family; ADAM 17 is also known as tumour necrosis factor- α (TNF α) converting enzyme or TACE. β -secretase cleavage is due to the activity of two aspartyl proteases, β -site APP cleaving enzyme (BACE) 1 and 2, the former of these is most important in the brain.

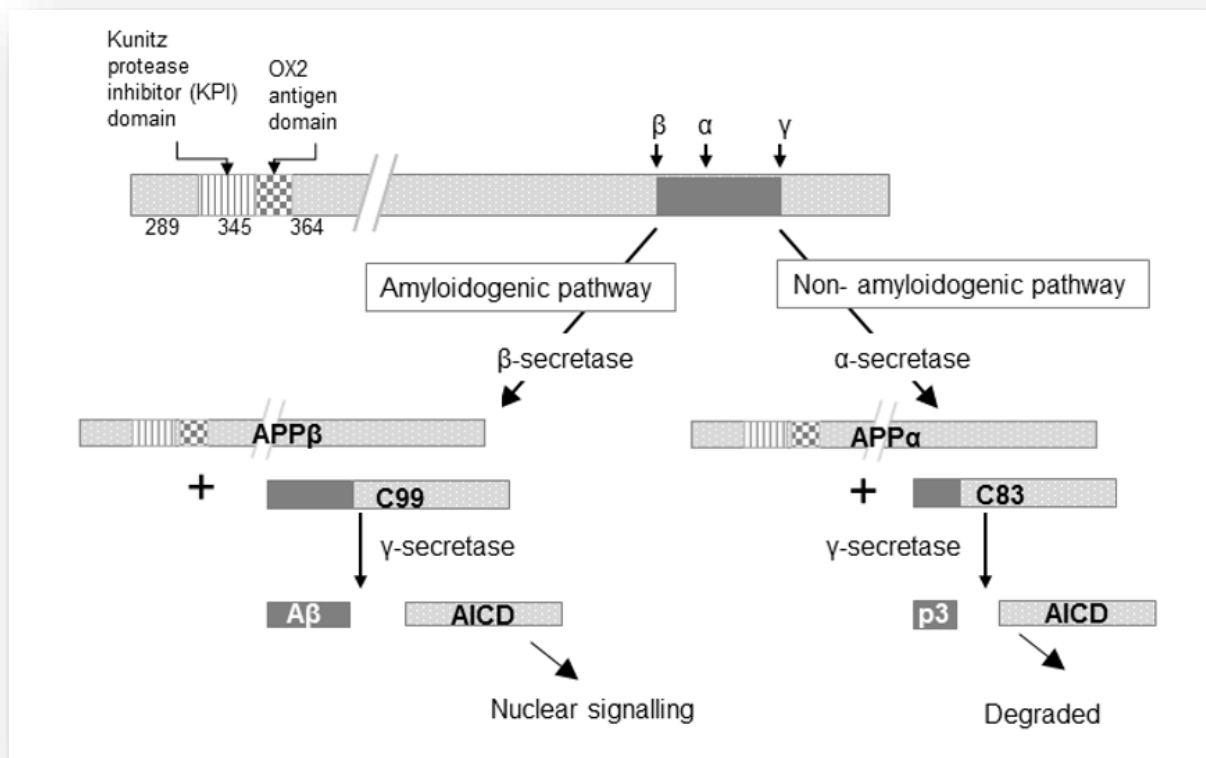


Figure 2.2 The processing of amyloid precursor protein (APP) to form A β .

This schematic shows the APP770 splice variant, and amino acid residue numbers are according to this; in the APP695 variant, most commonly found in neurones, the two N-terminal exon insertions are excluded. Cleavage may occur by α -secretase in the plasma membrane or by β -secretase during recycling through the endosomal pathway. In normal neurones the enzyme α -secretase cleaves APP about 90-95% of the time, to form the C-terminal peptide C83 (83 amino acids long) and the N-terminus called APP α (or soluble sAPP α). α -secretase comprises ADAM 9, 10 or 17. Normally, 5-10% of the time the enzyme β -secretase cleaves APP to form C99 and sAPP β . Subsequently γ -secretase cleaves within the hydrophobic membrane to form the peptide p3 (non-amyloidogenic pathway) or A β (amyloidogenic pathway) respectively. The APP Intracellular Domain (AICD) produced in the non-amyloidogenic pathway is degraded, whereas the identical fragment produced in the amyloidogenic pathway is transferred to the nucleus where it acts as a transcription factor and is stabilized by adaptor proteins such as Fe65. One of the target genes for upregulation includes that for neprilysin.

γ -secretase is a complex of four proteins, presenilin 1 or 2, nicastrin, PEN2 (presenilin enhancer 2), and APh1a or APh1b (anterior pharynx-defective phenotype 1). Presenilin is the catalytic component of γ -secretase, responsible for cleaving the APP C-terminal peptide (C99 or C83) to form either A β or a non-toxic peptide, p3. This γ -secretase complex has a large number of substrates other than APP, such as Notch1, low-density lipoprotein receptor-related protein 1 (LRP1), cadherins, ErbB4, and the cell-surface glycoprotein CD44. Mutations near the C-terminal region of A β (such as the 'London' mutation V717I) or mutations in PSEN1 or 2 lead to an increase in the A β 1-42:A β 1-40 ratio as this is the site of cleavage after either 40 or 42 residues. Conversely, the 'Swedish' double mutation at the N-terminus of the A β peptide results in an increase of both A β 1-40 and A β 1-42. This is because this mutant APP has an approximate hundredfold higher affinity for BACE1 than the normal APP protein.

2.4 The toxicity of A β

2.4.1 A β : mitochondrial damage and calcium

A β , particularly the A β 1-42 form, has adverse effects on neurones and the cellular environment of the brain, and it is suggested that an accumulation of these effects over a long period of time eventually causes enough neuronal damage to generate symptoms consistent with AD. The brain has a high rate of oxygen consumption yet low levels of protective antioxidant enzymes and therefore is vulnerable to damage from oxidation and the reactive oxygen species (ROS) produced. A β accumulates intraneuronally in endosomes and lysosomes and disrupts protein degradation. Mitochondrial dysfunction also occurs early in the disease process and is related to the presence of A β . Damage includes decreased mitochondrial membrane potential, loss of respiratory enzyme activity, production of ROS, and calcium dysregulation. An important aspect of toxicity in the AD degenerative process is the control of calcium homeostasis and there are a number of processes which may facilitate an undesirable rise in intraneuronal Ca²⁺. Extracellular A β oligomers, in particular A β 1-42, have been shown to bind to normal cellular prion protein at the plasma membrane to increase entry of Ca²⁺ into the neurone. The APP intracellular domain (AICD) peptide may also be involved by affecting the sensitivity of the channels (InsP3 and ryanodine receptors (RYRs)) that cause Ca²⁺ levels to be released from internal stores. High levels of Ca²⁺ may also cause the mitochondria to release cytochrome C, with subsequent initiation of caspase cleavage and controlled cell death (apoptosis) and/or synapse reduction via long-term depression (LTD). At the mitochondrial membrane A β may also interact with cyclophilin D to form a mitochondrial permeability transition pore (mPTP) which contributes to leakage of mitochondrial constituents such as cytochrome C.

2.4.2 A β : binding partners and synaptic dysfunction

A β is able to bind many proteins and thus interfere with their expression and function. Amyloid β -peptide binding protein alcohol dehydrogenase (ABAD) is a binding partner of A β . This enzyme is important in glucose-deficient environments and is able to increase the brain's ability to use ketones, where it can be protective, as seen after a stroke. However, in the presence of A β , this normally protective enzyme is able to facilitate apoptosis. When ABAD is overexpressed in transgenic AD mouse models, the presence of A β results in spatial and temporal memory deficits. A β also binds the transcription factor cAMP-response element binding protein (CREB), which is important in formation of memory. CREB controls expression of a number of important proteins including brain-derived neurotrophic factor (BDNF), known to facilitate long-term potentiation (LTP), a correlate of memory formation. Notably, BDNF levels are reduced in AD brain and this fact alone probably contributes significantly to synaptic loss and memory dysfunction. Still under examination are the roles of A β oligomers and hyperphosphorylated tau in the profound synaptic dysfunction seen early in AD. The presence of A β is known to be associated with a decrease in the phosphorylation of glutamate N-methyl-D-aspartate (NMDA) receptor, which is required for LTP and synaptic strengthening. This results in an increase in receptor endocytosis and reduced LTP. Calcineurin (Ca²⁺-dependent protein phosphatase) is necessary for A β -induced spine loss and endocytosis of glutamate receptors. This suppression of LTP by A β can be prevented by inhibition of caspase-3. Tau appears to be required for some of the A β induced synaptic defects as its removal ameliorates some of the synaptic and behavioural deficits seen in animal models of AD.

2.4.3 A β : inflammation

The deposition of A β into parenchymal plaques acts as a catch-all for other molecules and eventually an inflammatory response may be invoked. As we age, the balance of immune capability shifts from the humoral cell-mediated immune response and antibody production to rely further on the innate response involving proinflammatory cytokine production. Therefore with continued A β production, activated microglia produce proinflammatory mediators, such as the cytokines interleukins and tumour necrosis factor- α (TNF α), upregulate the complement system and produce ROS and excessive amounts of nitric oxide (NO) by inducible nitric oxide synthase (iNOS) which leads to neuronal cell death.

2.5 Tau

Importantly, the production of A β is linked with the deposition of tau, thought by many to be the more important of the two peptides in terms of neuronal degeneration and its associated symptoms.

Mutations in the tau gene MAPT have not been shown to be a primary cause of AD. Tau mutations are usually seen in frontotemporal dementias (FTDPs) such as frontotemporal dementia with parkinsonism associated with chromosome 17 (FTDP-17); chromosome 17

has the MAPT gene within it. Those living with FTDP-17 have tau deposits but rarely amyloid plaques. It seems that A β is 'upstream' of NFT formation and that tau mutations circumvent this step and do not invoke excessive A β production. The protein tau is a microtubule-binding protein, keeping microtubules in an assembled state by stabilizing α - and β -tubulin strands and enabling axonal transport. Tau exists in six isoforms, each with three or four microtubule-binding domains in the middle region of the protein. Microtubules facilitate passage of cargo containing nutrients, neurotransmitters, etc., from the cell body to the axon by kinesin protein complexes and towards the cell body by dynein protein complexes. Tau prevents cargo movement by obstructing its path, whereas phosphorylation of tau causes it to detach from the microtubules allowing regulated cargo movement. Tau may then become de-phosphorylated and return to its position on the microtubule. However, if the tau protein becomes hyperphosphorylated or abnormally phosphorylated, as it does in AD, this results in deposition of tau and production of NFTs. This may occur if kinases are overactivated, and kinases, particularly glycogen synthase kinase 3 β (GSK3 β) and cyclin-dependent kinase 5 (Cdk5), have been implicated in tau hyperphosphorylation. It is important to have a balance by which phosphorylation is kept optimal. This may occur by dephosphorylation of the tau by phosphatases allowing its reinstatement on the microtubule. Notably however, there is reported to be reduced phosphatase activity in AD brain; in particular, PP-2A (protein phosphatase 2) is reduced by up to 30 per cent.

2.6 Risk factors for sporadic AD

2.6.1 Age

Age is the greatest risk factor for AD. It is rare to develop dementia before the age of 65 years, yet its prevalence in the UK is given as approximately 7 per cent of people aged 65 years or above. Since AD accounts for an estimated 60–70 per cent of dementias this equates to nearly 5 per cent or 1–20 people in this age range. The prevalence of dementia then almost doubles each subsequent five years (Dementia UK, 2014).

2.6.2 Apolipoprotein E

The second most robust risk factor for AD is the presence of an E4 allele of apolipoprotein E (APOE4), located on chromosome 19. This was shown to be associated with a family with late-onset AD and later, more generally, as a strong risk factor for sporadic AD. There are three common isoforms of the protein APOE: E2, E4, and E3 which is the most frequent in the population. The frequency of the E4 allele in sporadic AD is approximately 40 per cent compared with 16 percent in age-matched normal subjects. It is estimated that one E4 allele hastens the theoretical onset of AD by 5 years, and two E4 alleles does so by 10 years. Conversely, the E2 allele is protective. The reason for this striking statistic almost certainly lies in the conformational difference between the E3 and E4 proteins which is entirely due to one amino acid difference; whereas APOE3 has the amino acid cysteine at position 112, E4 has an

arginine residue. APOE is a glycoprotein which enables transport of phospholipids and cholesterol within high-density lipoprotein (HDL) particles to neuronal sites requiring repair and remodelling of synapses. This is especially important after injury. APOE2 and E3 bind to small phospholipids rich in HDL; however, due to its conformation, E4 binds preferentially to larger triglyceride-rich very low-density lipoproteins (VLDL). A β is formed from within the APP transmembrane region and therefore originally forms an α -helix; all polymorphic forms of APOE catalyse its conversion into a β -strand formation, thus promoting aggregation. APOE4 however is less lipidated than E3 and E2 and therefore tends to promote fibrillization more readily than the other isoforms. Therefore more plaques are likely to be apparent in the brain of an E4 carrier. Furthermore, since E4 is less able to bind HDL it is less able to promote neurite outgrowth. There is a reduced capacity for synaptic remodelling which may result in a reduced synapse formation and lower 'neuronal reserve'. This reserve keeps us above the threshold of cognitive impairment. Thus, in some ways the presence of E4 has been likened to a 'knockout' of APOE and is associated with lower synaptic density and a significantly reduced repair response after head injury, resulting in a higher mortality rate. Another aspect of APOE is in its facilitation of the removal of A β from the brain. When fully lipidated, APOE binds A β and can maintain its solubility and promote its clearance from the brain. APOE is mainly produced by glial cells and is lipidated to form lipoprotein particles by means of the lipid transporters ABCA1 (ATP-binding cassette sub-family A member 1) and ABCG1 (sub-family G). APOE4 is less lipidated and is much less efficient in clearing A β from the brain. The expression of APOE and the lipid transporters are partly regulated by the retinoid X receptor (RXR), the liver X receptor (LXR), and peroxisome proliferator-activated receptor (PPAR). Further to this, an RXR agonist bexarotene has been shown to upregulate APOE and lipid transporters and to increase phagocytosis by macrophages and microglia, resulting in increased clearance of A β .

2.6.3 Genetic risk factors

Other risk factors have become known due to the Genome Wide Association Studies (GWAS) in which large numbers of individuals are examined for common genetic variants to highlight any associations with the disease. Approximately 30 polymorphisms have been identified as significantly lowering or increasing the age of onset of sporadic AD. This includes proteins involved in immune function including CR1 (complement component (3b/4b) receptor 1) and TREM2 (triggering receptor expressed on myeloid cells 2), and cholesterol metabolism and transport such as APOE, clusterin (apolipoprotein J), and ABCA7. In the light of the link between APOE and other ATP-binding cassette members it is of interest to find that carriers with inactive forms of ABCA7 have twice the likelihood of developing AD. Additionally, sortilin-related receptor 1 (SORL1) is a sorting receptor which binds APP and APOE. Without SORL1 present to guide APP, it is directed towards β -secretase. Under these circumstances there would be an increase in A β produced. Notably, in blood samples from AD patients, a reduction of approximately 50 per cent in the level of SORL1 protein was measured in AD compared with normal. Similarly, certain variants of the endothelial cell protein PICALM (phosphatidylinositol-binding clathrin assembly protein) are a significant risk factor for AD

and it is suggested that these variants may reduce A β clearance. Further studies are underway to provide mechanisms by which these diverse proteins may influence the course of the disease.

2.7 Conclusion

The consensus is that the initial trigger for commencement of the disease process involves the production of A β oligomers which, over time due to unmodulated cellular responses, result in a variety of cellular stresses. Part of this process involves the activation of specific kinases and abnormal phosphorylation of tau. One of the outcomes is defective neuronal transport but also loss of synaptic and neuronal cell communication. The pathological processes which occur during the onset and progression of AD are now known to occur perhaps 15–20 years before any symptoms appear. This knowledge changes the understanding of the disease: if we can develop sufficiently sensitive biomarkers of the disease process, with improved imaging we will have more time to identify and slow down the disease and perhaps prevent its appearance completely. This long-term approach also shifts focus somewhat towards diet, exercise, and other environmental factors.

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