Causes of Alzheimer's disease

David G. Munoz,* Howard Feldman†

Abstract

IT IS NOW UNDERSTOOD THAT GENETIC FACTORS play a crucial role in the risk of developing Alzheimer's disease (AD). Rare mutations in at least 3 genes are responsible for early-onset familial AD. A common polymorphism in the apolipoprotein E gene is the major determinant of risk in families with late-onset AD, as well as in the general population. Advanced age, however, remains the major established risk factor for AD, although environmental variables may also have some role in disease expression. Some pathogenic factors directly associated with aging include oxidative damage and mutations in messenger RNA. Other factors unrelated to the aging process may, in the future, be amenable to therapeutic intervention by way of estrogen replacement therapy for postmenopausal women, anti-inflammatory drug therapy and reducing vascular risk factors. Older theories, such as aluminum playing a role in the pathogenesis of AD, have been mostly discarded as our understanding of pathogenic mechanisms of AD has advanced.

Review

Synthèse

From *the Departments of Pathology and Clinical Neurological Sciences, University of Western Ontario, London, Ont., and †the Division of Neurology, Department of Medicine, University of British Columbia, Vancouver, BC

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Physicians communicating the diagnosis of Alzheimer's disease (AD) to a patient's family are often confronted with the questions: What caused it? Could it have been anything I did? General practitioners and specialists caring for elderly patients are more interested in understanding the etiopathogenesis of this disorder now that there are approved treatments for some of the symptoms of AD and strategies for disease modification and there exists the potential for prevention in the future.[1–3] Several factors have contributed to the increased interest in and awareness of the burden AD places on society, expressed by both the medical community and general public[4,5] The first relates to demographics; the prevalence of AD is the highest in people 85 years of age and older (approximately 26%),[4] and this is the fastest growing segment of the population. In addition, the vague concept of senility has been replaced with specific causes and phenotypes of dementia that can now be accurately identified.

Our understanding of AD has developed in 3 stages. Although the 1907 description by Alois Alzheimer clearly identified the salient clinical and pathological features of the condition,[6] it was not until the seminal work of Blessed and colleagues[7] that the disease was recognized, not as a rare neurological disorder, but as the most common cause of dementia. The discovery of the frequent histopathological marker lesions in normal elderly individuals and the close relation between the severity of the lesions and the degree of dementia was the major advance in the second phase. The cloning of the gene mutation coding for β-amyloid precursor protein on chromosome 21 heralded the third phase.[8] The pace of discovery has rapidly accelerated, and we are now gaining a good understanding of the disease process. Rational therapies derived from this understanding are now being developed.[9]

Structural abnormalities

Although cerebral atrophy is a typical manifestation of AD, it does not distinguish normal aging from AD accurately enough to be diagnostic; this applies to neuroimaging as well as gross inspection at post mortem. However, microscopic examination reveals the critical features of the disease — a cerebral cortex peppered with neurofibrillary tangles and senile plaques. AD can therefore be said to be the dementia associated with these histopathologic abnormalities (see Figs. 1a and 1b).

Neurofibrillary tangles

Neurofibrillary tangles consist of aberrantly phosphorylated fibrillary proteins aggregated within the neuronal cytoplasm. Their presence signifies the failure of the neuron to properly maintain its cytoskeleton, which is required to support the extraordinarily complex branching shape of its numerous processes. A small number of neurofibrillary tangles are a universal consequence of aging. However, it is an increased number and the architectonic distribution of the tangles that promote the cardinal pathology and define the stages of the disease, as described by Braak and Braak.[10] The development of tangles is a major and possibly the main mechanism of neuronal death in AD.[11]

Some groups of neurons are preferentially affected by tangles in AD. For example, neurofibrillary tangles frequently occur in areas of the hippocampus that are involved in processing experiences prior to storage as permanent memories. This correlates with the clinical deficits observed in the early stages of AD in learning and in the creation of new memories, as well as with the relative preservation of established memories. The neurons at the basal forebrain that provide most of the cholinergic innervation to the cortex are also prominently affected; the resulting cholinergic neurotransmitter deficits are often treated with cholinesterase inhibitors. Donepezil, a piperdine-based acetylcholinesterase inhibitor and currently the only approved symptomatic treatment for AD in Canada, has been shown to have consistent mild-to-moderate treatment effects in clinical trials.[12,13]

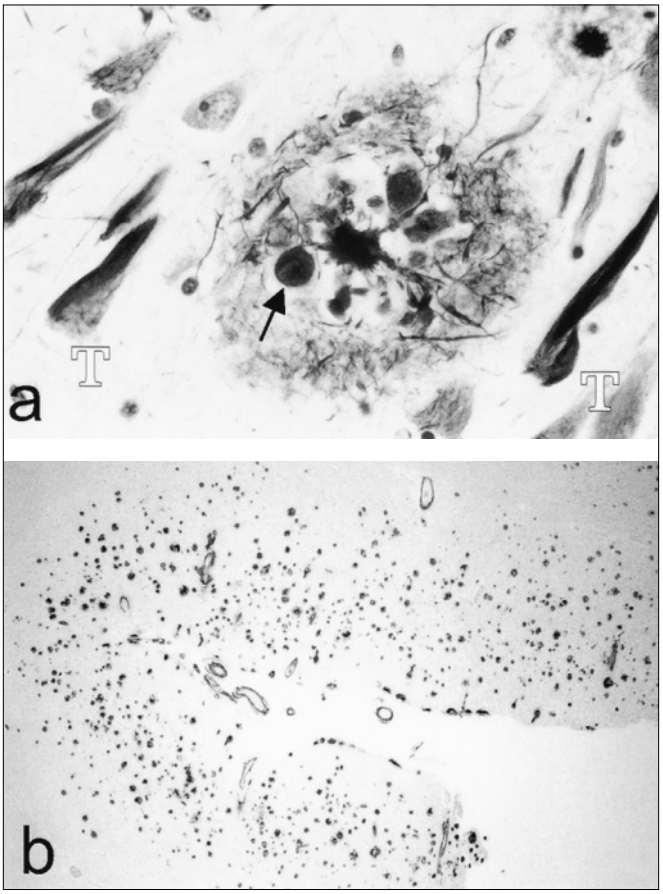

Fig. 1a: The main lesions of AD. Neurofibrillary tangles appear as flame-shaped dark structures (white T). A senile plaque, shown in centre, consists of a central core of amyloid surrounded by numerous distended neurites, one of which (arrow) clearly originates from a slender axon. (Silver stain; magnification × 400). Fig. 1b: Amyloid deposits in the form of senile plaques (appear as dots) occupy a significant fraction of the cerebal cortex, as shown here. (Stained with an antibody to β-amyloid; magnification × 20).

Senile plaques

Senile plaques are more complex; they consist of extracellular deposits of amyloid material and are associated with swollen, distorted neuronal processes called dystrophic neurites. Like amyloid elsewhere in the body, complex sugar polymer components (glycosaminoglycans) are thought to be critical in the assembly of these deposits. The specificity of cerebral amyloid is provided by its major peptide component, β-amyloid, a short 40–42 amino-acid fragment of the transmembrane protein, β-amyloid precursor protein (β-APP) (Fig. 2).

Starting in the 5th decade of life progressively greater proportions of individuals develop cortical senile plaques, until the 8th decade when approximately 75% of the population is so affected. The fact that the density of senile plaques does not increase with age[14] suggests that brains switch from plaque-free to plaque-bearing status in a short period of time; the mechanism responsible for this change is unknown. Plaques start as innocuous deposits of nonaggregated, putatively non-neurotoxic β-amyloid (diffuse plaques). However, in some individuals they undergo an orderly sequential transformation into the mature senile neuritic plaques that are associated with the development of AD.[14] It is thought that the enzyme butyrycholinesterase may play an essential role in this maturation process.[15] Although the number of senile neuritic plaques increases with age, the number remains low in most cognitively intact individuals.[16]

The regional distribution[17] and chemical composition[18] of both senile neuritic plaques and neurofibrillary tangles

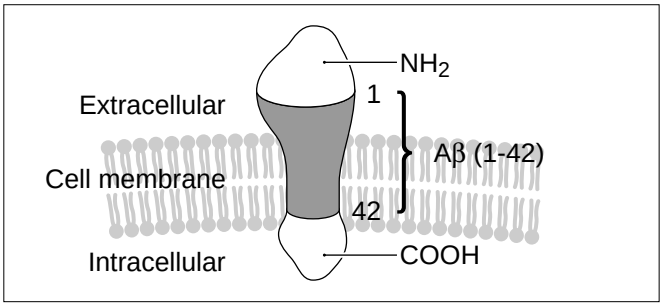

Fig. 2: Schematic showing amyloid precursor protein (APP) and the β-amyloid (Aβ) cleavage product.

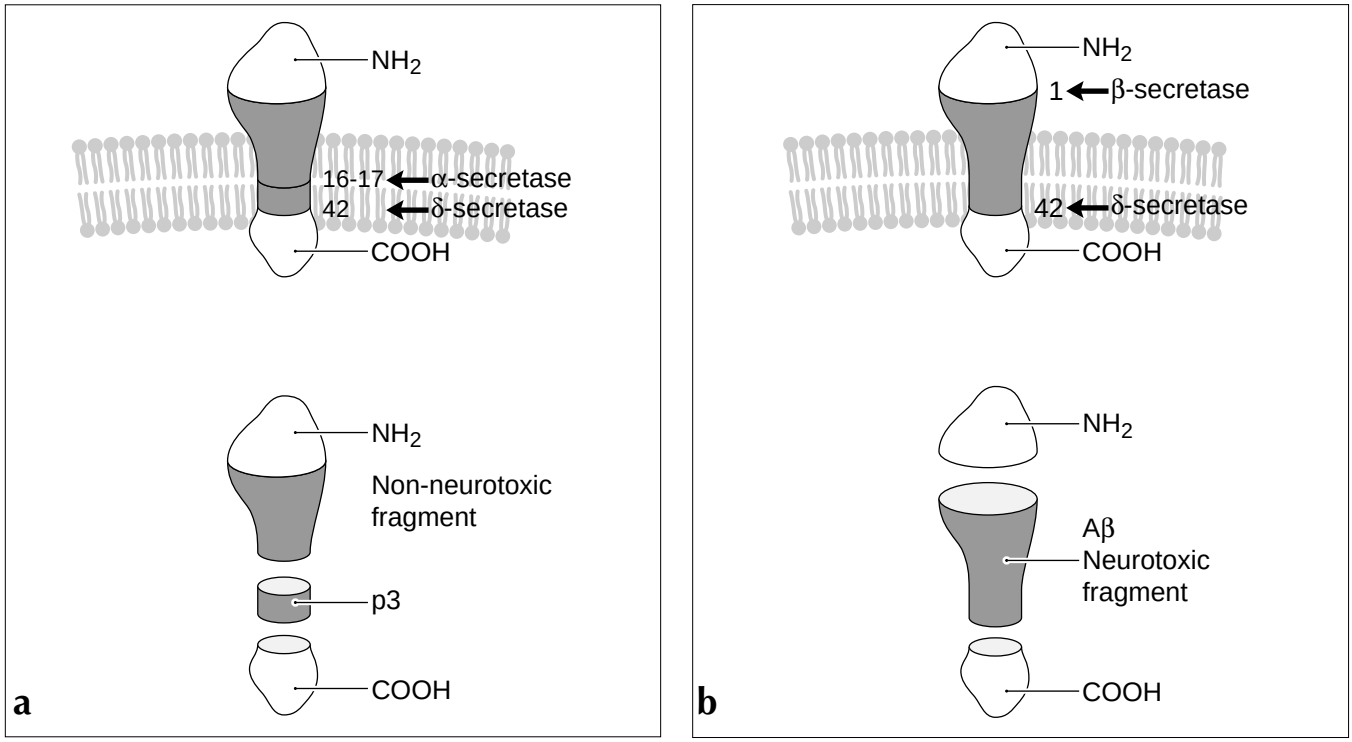

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Fig. 3a: APP cleavage pathway via α-secretase. α-Secretase cleaves between residues 16 and 17, producing soluble, non-neurotoxic segments. Fig. 3b: APP cleavage pathway via β-secretase. This pathway produces the Aβ (1–42 fragment), which is putatively neurotoxic. This cleavage of the transmembrane region has been proposed to occur inside lysosomes[25] or in the trans-Golgi network during the secretory process.[26]

are similar in those who age normally and in those who develop AD. Thus, plaques and tangles bear a relation to dementia similar to that of atherosclerosis and infarcts — as with atherosclerosis the primary lesions are common in aging, but clinical manifestations will appear after a certain density of these lesions is reached. In AD this level will vary among individuals and will depend on genetic and environmental risk factors, as well as comorbid brain pathology. For individuals with mild cognitive impairment who do not meet the formal diagnostic criteria for dementia it is uncertain if there is a relation between the density of plaques and tangles and the degree of cognitive impairment.[4,19,20]

One of the major problems is that of clinical definition — trying to determine when dementia reaches a diagnosable status and what the actual diagnostic criteria should be. A variety of clinical diagnostic criteria have been operationalized[21–23] in an attempt to implement uniform case identification. The essential elements supporting the diagnosis of AD include an acquired decline in cognitive functions, an impairment of daily living activities and a progressive course of disease. However, each set of criteria that has been developed differs in its emphasis and detail. Erkinjuntti and colleagues,[24] using data from the population-based Canadian Study of Health and Aging, reported that the prevalence of dementia in Canada in people over the age of 65 could vary between 3.1% and 29.1%, depending on the clinical diagnostic criteria used.

The role of genes

Several point mutations in the gene coding for β-APP on chromosome 21 are sufficient to cause early-onset autosomal dominant familial AD with complete penetrance; the clinical phenotype of these cases is entirely consistent with typical AD. Some mutations increase the production of β-amyloid, while others favor the formation of long (42 amino acid) forms of β-amyloid, which aggregate more readily than the short (40 amino acid) forms. In addition, mutations in 2 other genes coding for the novel proteins presenilin 1 and 2 are reported to account for the majority of early-onset, familial, dominantly inherited AD.[8] The normal function of the presenilins remains unclear, but recent findings suggest that the interaction of the presenilins and β-APP in the neuronal cell body is critical for organizing vesicular traffic.[25] When this process is interrupted the delivery of synaptic vesicles to presynaptic terminals is impaired and neurotransmitter deficit may exceed neuronal loss. In addition, excess β-APP is metabolized along a pathway leading to the production of β-amyloid, predominantly the longer (42 amino acid) form.[26,27] In this scenario plaques may represent signposts of the abnormality, rather than the relevant pathology and therapeutic target. Figures 3a and 3b illustrate the pathways involved in producing both non-neurotoxic and putatively neurotoxic fragments dur-

ing APP cleavage. The resulting toxicity of the fragments is dependent on the type of secretase involved and the location of the cleavage.

The familial forms of AD account for only 4%–8% of cases. Most individuals, with either familial or sporadic AD, become affected after the age of 65; the disease is thus considered late-onset. A common genetic polymorphism affecting the gene for apolipoprotein E (*APOE*) is firmly established as the major risk factor (other than age) for the development of AD. This lipoprotein is involved in synaptic repair, particularly in response to tissue injury. As well, it has an important role in the maintenance of neuronal structure and cholinergic function.[28,29]

The *APOE* gene exists in 3 allelic forms (alternative varieties): ε2, ε3, and ε4, with frequencies in white people of 8%, 78% and 14% respectively. The ε4 allele increases the risk of developing AD 5- to 15-fold; the reported odds ratios (OR) and 95% confidence intervals (CI) associated with the ε2/ε4 and ε4/ε4 are 2.6 (95% CI 1.6–4.0) and 14.9 (95% CI 10.8–20.6) respectively.[30] By contrast, the ε2/ε3 (OR 0.6 [95% CI 0.5–0.8]) and the rare ε2/ε2 (OR 0.6 [95% CI 0.2–2.0]) confer a modest protection. The *APOE* genetic risk factor is present in both sexes, all racial and ethnic groups studied so far, and at all ages, although the strength of the association varies depending on these factors.[28] From a different point of view, those with an ε4/ε4 genotype have a 95% chance of demonstrating AD by 80 years of age.[31]

Although determining *APOE* genotype in a blood sample is technically simple and routinely done in a number of laboratories in Canada, the consensus has been that it should not be offered as a routine presymptomatic test.[32] The rationale is that there is no preventive intervention and that some ε4 carriers will not get AD; the genetic counselling is therefore very complex. There is, however, considerable discussion as to whether the genotype may have some role in facilitating an AD diagnosis in symptomatic individuals.[33]

The marked increment in risk produced by a single amino acid difference in the *APOE*-allelic protein makes it a potentially attractive therapeutic target; more information regarding its role in AD will allow rational treatment strategies to be developed and tested. Emerging autosomal genetic risk factors currently being investigated include a susceptibility locus on chromosome 12,[34,35] polymorphisms of the very-low-density lipoprotein receptor genes, as well as an intron mutation of the presenilin gene.[36]

Two other genetic observations are of particular relevance to emerging treatment strategies. The report that the HLA-A2 allele is associated with an earlier age of onset suggests that modulation of inflammation plays a role in the development of the disease.[37] In addition, the reported mutations in the mitochondrial genome,[38,39] which can either be inherited or acquired, would contribute to the oxidative damage that many suggest plays a central role in the development of AD.

Role of environmental factors

The finding that monozygotic twins may not both develop AD[40] suggests that environmental factors also play a role in the development of AD. One hypothesis is that AD may represent a chronic active inflammatory disease. The brains of AD patients show evidence of mild active inflammation, including microglial and complement activation, and the presence of inflammatory cytokines.[41] Moreover, the recruitment and activation of microglia is associated with maturation of plaques in elderly individuals.[42] Although the inflammation is likely secondary to more fundamental injuries, it may participate in a morbid cycle of tissue damage, as it does in systemic diseases like rheumatoid arthritis. Epidemiological retrospective studies have been conducted to determine the risk of developing AD among patients receiving anti-inflammatory drugs or having conditions such as rheumatoid arthritis in which these drugs are routinely used. More than 21 independent studies, including the Canadian Study of Health and Aging,[43] have reported a decreased prevalence of AD among patients taking anti-inflammatory agents on a long-term basis, although these findings are not universal.[44] In contrast, Stewart and colleagues[45] reported that acetaminophen, a pain reliever with no anti-inflammatory activity, is not associated with AD. Perhaps the best supporting evidence to date is the findings of a study of divergent twins in which the use of anti-inflammatory drugs was the only factor identified that differed between affected and unaffected members of the twin pairs.[46] If nonsteroidal anti-inflammatory drugs (NSAIDs) have a preventive effect, their mechanism of action is unclear; elderly patients on chronic high doses of NSAIDs show a marked reduction in the density of activated microglial cells but no difference in the number and density of plaques and tangles when compared with those not taking NSAIDs.[47]

Which anti-inflammatory drugs are effective, at what doses and for what duration has not been fully determined, as most of the epidemiological data has been retrospective. Some studies have focused on NSAIDs, while others have also included steroids. There has been 1 double-blind, randomized, placebo-controlled trial of indomethacin reported to date; it tested the effect of indomethacin on cognitive and functional outcome measures in patients with AD over a 6-month period.[48] However, the trial was small and of short duration, and the study's positive results await confirmation in larger scale trials before they can be generally accepted because long-term use of NSAIDs is also associated with significant side effects in elderly people.

Several epidemiological studies have shown that women on estrogen replacement therapy may be less likely to be diagnosed with AD,[49,50] and a few small trials claim improvement in female patients with AD who are taking estrogen.[51] The widespread effects of estrogen in the brain[51] make these results plausible, although the mechanisms have not been fully elucidated. Preliminary data suggest that the

combined use of estrogen and tacrine, a cholinesterase inhibitor, may be associated with greater therapeutic efficacy.[52] The fact that estrogen replacement therapy may increase the risk of breast and endometrial cancer highlights the need for confirmatory large-scale, randomized clinical trials before a treatment approach that includes the use of estrogen among postmenopausal women is adopted.[53,54] It is of interest that some studies have found that estrogen replacement therapy is not associated with a reduced risk of developing AD.[55,56]

Clinicopathological correlation studies conducted over the last few years have shown that, although all patients whose brains showed severe AD-type lesions exhibited dementia, patients with moderate lesions may or may not

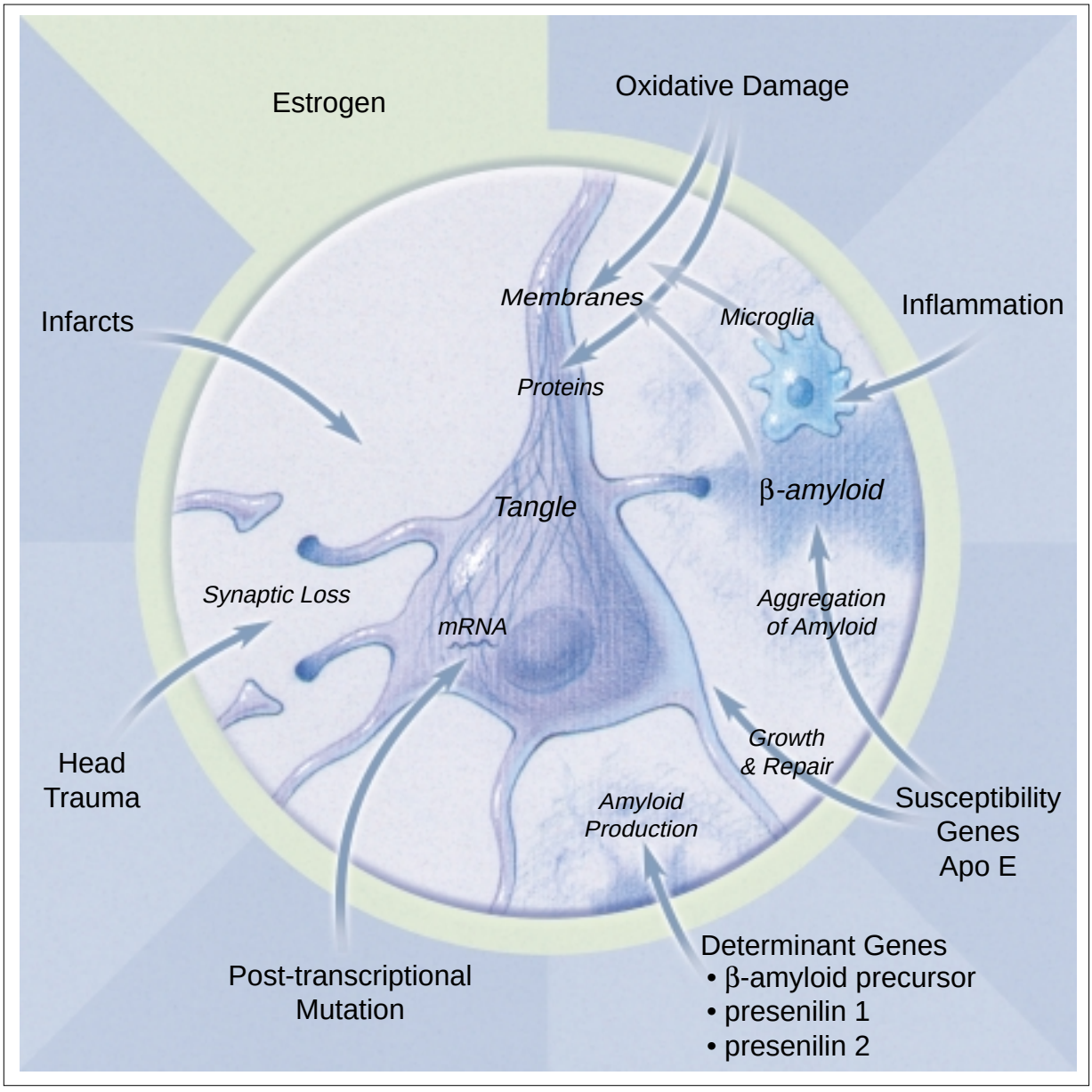

Fig. 4: Within the brain (represented by the circle) a neuron that has developed a tangle sends out 2 processes. One makes a synaptic contact, which is endangered as the tangle decreases the neuron's capacity to tend its branches. Another process is engulfed in an extracellular amyloid deposit, forming part of a senile plaque; an inflammatory cell (microglia) is in the vicinity. Factors influencing the development of AD appear outside the brain. The determinant genes influence the production of amyloid peptide, and the susceptibility gene (ApoE) is involved in the aggregation of amyloid and growth and repair functions of neurons. Age-related oxidative damage to neuronal membranes and proteins is enhanced by amyloid and inflammation; iron may participate in this process. Head trauma increases the development of plaques and tangles, whereas the mechanisms by which infarcts increase the risk for dementia in patients with AD lesions are unknown. Estrogen has multiple effects on the brain, several of which may influence the development of dementia.

have. A seminal insight was provided by results of the Nun Study conducted by Snowdon and colleagues;[57] the presence of cerebral infarcts, even if small and scarce, raised the risk of dementia by as much as 20 times for those with AD-type lesions. This may be the most common mechanism by which vascular injuries contribute to dementia, since infarcts in the absence of AD-type lesions had few cognitive effects.[57] These results have also been confirmed by others.[58] Thus, the vigorous treatment of hypertension and other vascular risk factors and the promotion of healthy diet and exercise could potentially reduce the incidence of dementia.

Mechanisms associated with aging

Aging is the major risk factor of AD in the general population. Recent research has identified 2 potential mechanisms related to aging that may contribute to the development of the disease.

One is the concept that free radicals (reactive oxygen species) produced during cellular respiration may play an important role in the process of aging and in the development of AD.[59] Ample evidence has accumulated that oxidative damage to proteins and membrane lipids and an upregulation of antioxidant enzymes is associated with AD.[60,61] The toxic effects of β-amyloid are mediated, at least in part, through the generation of free radicals by the peptide.[62,63] The recent demonstration of redox-active iron deposits associated with senile plaques and neurofibrillary tangles is relevant in this respect because iron can catalyze the formation of damaging free radicals.[64] Further, a University of British Columbia research team reported that patients with AD exhibit increased serum levels of the iron-binding protein melanotransferrin, indicating that there may be aberrant handling of iron in the brains of those with AD.[65] The measurement of melanotransferrin levels in serum may prove to be a useful diagnostic adjunct if the initial studies are replicated.

The concept that oxidative damage may be an important mechanism in aging has led to a large-scale clinical trial of vitamin E (α-tocopherol) to treat patients with moderate to severe AD.[66] Although the results demonstrated that a daily dosage of 2000 IU of vitamin E slowed the progression of the disease, there was no indication that vitamin E therapy was associated with symptomatic benefit or a reversal of disease effects. It is important to recognize that the redox balance in the brain is complex, and that additional measures will be required to provide more substantive treatment strategies aimed at the attenuation of these disease-related mechanisms.

Another possible mechanism related to aging is messenger RNA; mutations in messenger RNA have been reported in elderly humans and older rodents.[67] The deletion of 2 consecutive bases in a protein results in an altered reading frame and, thus, a protein with an amino acid sequence unrelated to that specified in the original gene. The predicted abnormal forms of 2 proteins relevant to the pathogenesis of AD, β-amyloid precursor protein and ubiquitin-B, and their corresponding altered messenger RNA have been found in the brains of patients with AD, but not in those of controls.[67]

Other relevant mechanisms of disease: interactions

Although a history of head trauma of sufficient severity to result in unconsciousness may increase the risk of developing AD,[43,68] this likely makes a minor contribution to the burden of the disease. However, individuals with a history of traumatic head injury and the *APOE*-ε4 genotype have a demonstrated increased risk for AD;[69] this highlights the crucial interactions between genetic and environmental variables that can culminate in disease expression.

A number of epidemiological studies have reported that poor education is an important risk factor for AD;[43,70,71] others have found no correlation, however.[72,73] The brain reserve hypothesis states that education, through the modification of synapses, increases the complexity of cerebral circuitry. Thus, when a biological destructive process occurs the better educated are able to call on their 'reserves' before a critical threshold of brain complexity is crossed and dementia is manifested clinically.[74] If this were true, one would expect the AD lesions in the brains of better-educated individuals to be more severe; several studies[58,75] have now shown this is not likely to be the case. Individuals with more education may have an advantage when performing the tests used for the diagnosis of dementia, however. An alternative explanation is that the greater prevalence of AD in people with less education might reflect more comorbid cerebrovascular disease and cerebral infarcts.

Patients with severe epilepsy develop senile plaques at a younger age than the norm for the general population,[42] but it is unclear if they are at greater risk for developing AD.

Mechanisms ruled out by research

Aluminum is neurotoxic. When injected into the brains of experimental animals it produces an acute encephalopathy accompanied by neuronal inclusions that resemble neurofibrillary tangles; they are now known to be of a different nature, however.[76] The acute encephalopathy seen in some dialysis patients has been traced to the aluminum in dialysis water, indicating that aluminum is also neurotoxic for humans if it reaches the brain, but neither the clinical syndrome[77] nor the pathology resemble AD.[78] Studies have found that aluminum levels are not elevated in the brains of patients with AD[79] or in association with plaques[80] or tangles;[81] aluminum levels are also not elevated in the bone,[82] blood,[83] or cerebrospinal fluid[84] of AD patients.

Thus, results of preliminary studies indicating an association between the aluminum in drinking water and AD[85] were not confirmed by more in-depth studies.[86,87] Epidemi-

ological studies investigating aluminum as an AD risk factor, including the Canadian Study of Health and Aging,[43] concluded that exposure to aluminum in tap water does not increase the risk of AD.[88,89] Furthermore, prolonged intake of antacid medications that contain aluminum in doses 1000s of times greater than drinking water is not associated with the development of AD.[43,87,88,90–94] Finally, there was no increased risk detected for workers exposed to aluminum dust and fumes.[95]

Conclusions

While we await confirmation of the potential preventive effectiveness of NSAIDs, estrogens and antioxidants the only current risk factors for dementia amenable to prevention are those associated with cerebrovascular disease. This argues for attentive treatment and prevention of hypertension, with appropriate lifestyle modifications to avoid smoking and high-fat diets.

In our role as scientists and educators we not only carry the important responsibility of advancing knowledge and the treatment of AD through a molecular-genetic understanding of the disease but we must also ensure that rapidly advancing genetic information is understood and used in an ethical and appropriate manner. In the future it is quite likely that there will be both primary and secondary prevention trials that will be guided by our knowledge of both the genetic and environmental risk factors for AD.

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Reprint requests to: *Dr. Howard Feldman, S192-2211 Wesbrook Mall, Vancouver BC V6T 2B5; fax 604 822-7703.*