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Review

Advances and Challenges in the Prevention and Treatment of Alzheimer's Disease

Roberta Diaz Brinton[1,2] and Rose S. Yamazaki[1]

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Alzheimer's disease (AD) is the most common cause of dementia and accounts for one-half to three-fourths of all cases of dementia. In the United States, AD is the leading cause of a loss of independent living and subsequent institutionalization. Approximately 4 million Americans are currently diagnosed with Alzheimer's disease—which results in greater than $100 billion dollars in health care costs. This review provides a description of the cognitive and neuropathological features of AD and the challenge that aging populations around the globe pose to health care systems and to societies. A review of new and promising therapeutic strategies for the prevention of AD is discussed which includes estrogen replacement therapy and anti-inflammatory therapeutics. Pharmaceutical approaches that delay the progression of the disease, such as antioxidants, are discussed as well as therapeutic strategies for improvement of cognitive function in AD patients, including the new generation of compounds aimed at enhancing cholinergic function. This section is followed by a review of the current status on nerve growth factor trials. The final section addresses the issue of the genetic linkages of AD, the impact of transgenic and gene knockout mouse models of AD on research in the field and the potential use of gene therapy to treat AD.

KEY WORDS: Alzheimer's disease; estrogen replacement therapy; anti-inflammatories; anti-oxidants; cholinergic therapies; nerve growth factor.

INTRODUCTION

It is the best of times and worst of times in the field of Alzheimer's disease (AD) research and clinical care. It is the best of times because several therapeutic strategies have recently emerged that hold great promise for significantly reducing the incidence of AD in our time. It is the worst of times because we still do not yet have a cure for AD and within little more than two decades an avalanche of AD cases could be upon us. But there is the promise of light at the end of this dark degenerative tunnel. Strategies that are now just emerging as effective in reducing the risk of AD coupled with increasing knowledge regarding the genetic basis of AD promise to hold insights into the prevention of and potential cures for AD. In the review that follows, a brief description of the cognitive and neuropathological features of AD is covered followed by a review of the challenge that aging populations around the globe pose to health care systems and to societies that will care for persons afflicted with AD. A review of new and promising therapeutic strategies for the prevention of AD is discussed which include estrogen replacement therapy and anti-inflammatory agents. Following this section, a discussion of pharmaceutical approaches that delay the progression of the disease, such as antioxidants, as well as therapeutic strategies for improvement of cognitive function are presented, including the new generation of compounds aimed at enhancing cholinergic function and the current status on nerve growth factor trials. The final section addresses the issue of the genetic linkages of AD, the impact of animal models of AD and the potential use of gene therapy to treat AD.

Alzheimer's Disease

The most common cause of dementia is Alzheimer's disease (AD) accounting for one-half to three-fourths of all cases of dementia. AD is the leading cause of loss of independent living and subsequent institutionalization (1,4). Approximately 4 million Americans are currently diagnosed with Alzheimer's disease—which results in greater than $100 billion dollars in health care cost (6).

AD is characterized by a progressive loss of cognitive function over a period of 5 to 15 years before death ultimately occurs (1). The first symptoms of AD express as deficits in memory function. These deficits are usually severe enough to interfere with normal daily living and exceed the annoyance that many of us experience with momentary lapses in memory. At first AD victims are unable to recall new information following a delay of several minutes or longer. Social skills are often maintained and often these individuals in the early stage of the disease appear normal to the casual observer. Later, most victims of AD will exhibit deficits in higher cognitive functions such

[1] Department of Molecular Pharmacology and Toxicology, University of Southern California, Pharmaceutical Sciences Center, 1985 Zonal Avenue, Los Angeles, California 90033.

[2] To whom correspondence should be addressed. (e-mail: rbrinton @hsc.usc.edu)

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as abstract reasoning, judgement and language as well as deficits in visuoperceptual abilities and skilled motor movement. The memory deficits increase in severity with increasing duration of the disease. As an example, a person with AD may not remember an event 1–2 minutes after that event has occured. At worst, victims of AD can lose the ability to recognize their spouse of many years and even their own children. Affective disturbances are also apparent and can include apathy, depression, agitation, anxiety, progressive paranoia, or delusions (1).

Two types of lesions, the neurofibrillary tangle and the beta amyloid plaque characterize AD pathology (2, see Figure 1). Neurofibrillary tangles are intracellular fibrils formed mainly by paired helical filaments linked together by hyperphosphorylated tau protein (2). These fibrils can be found in axons and dendrites and are also commonly found in the extracellular space in the form of neuropil threads and can be associated with the plaques containing β-amyloid deposits. The second lesion is the neuritic plaque, which are accumulations of β-amyloid protein (Aβ) in the extracellular space containing dystrophic neurites with abnormally phosphorylated tau (see Figure 1). It is the combination of these plaques and tangles which lead to the postmortem definitive diagnosis of AD and can, in sufficient abundance, lead to dementia (3).

The Challenge of Alzheimer's Disease in the Coming Decades

The life span of humans is increasing (5,7) and with this expanded life span comes the risk of developing AD since age continues to be the greatest risk factor for developing Alzheimer's. The prevalence of AD doubles about every four and half years following the seventh decade of life (1,4 see Figure 2). Persons in the eighth decade of life have a 50% risk of developing AD. The number of Americans at risk for developing Alzheimer's disease is expected to continue to increase dramatically as members of the post WWII baby boom turn 65 during the years of 2010 to 2025. In the US alone, 12–15 million Americans will have Alzheimer's disease (note that this is not risk but projected incidence) by the middle of the next century unless a cure or prevention is discovered (6).

The challenge of an aging population is not unique to the United States. The developed as well as developing world face similar challenges of increasingly aged populations. In Europe, the challenge of an aging population is particularly evident in Scandinavia and the European Union. In the European Union, 18% of the population was over the age of 60 in 1990. This percentage of aged in the population is projected to rise to 30% in the year 2030 (6).

Alzheimer's disease is a cross cultural disease, affecting those of European, African and Asian racial heritage. In Japan, the life span exceeds that of Americans and is the most long-lived population in the world (6). Moreover, the birth rate in Japan is below replacement levels, so that the ratio of active workers to retirees in the future will cause significant social, economic and political strain. In the developing world, countries such as China, India and other countries with enormous populations are aging at a rate similar to that of the developed nations (6). Each month approximately 800,000 people reach the age of 65 in the developing countries of the world. In 15 years there will be 400 million individuals over 60 in economically poor countries, double the number in economically advantaged countries. Thus, the challenge of Alzheimer's disease is a national and global issue of monumental economic and social proportions. With this challenge comes a tremendous opportunity, to sustain life—life that is consistent with the features of evolved human existence.

PHARMACEUTICAL STRATEGIES TO PREVENT AND TREAT ALZHEIMER'S DISEASE

Hormone Replacement Approaches

The most encouraging and exciting news regarding the prevention of AD has come from retrospective observational and epidemiological studies of women who received estrogen replacement therapy. Remarkably, the data indicate that estrogen replacement therapy in postmenopausal women resulted in a 40–60% reduction in the risk of developing AD (reviewed in 4, see Figure 3). These clincal observational data have been recently reviewed by Birge (4) and the reader is referred to that reference for an in-depth analysis.

Three areas of interrelated research are at the forefront of developing hormone replacement therapies for the prevention and possibly the treatment of AD. These areas include development of new or discovery of existing estrogenic steroids that lack effects in reproductive organs, discovery of novel nuclear estrogen receptors, and development of selective estrogen receptor modulators or SERMs.

The efforts to develop new or discover existing estrogenic steroids that lack effects in reproductive organs, started with investigations of the impact of the endogenous active estrogen, 17 β-estradiol, on cognitive function of women with AD and on the neurotrophic and neuroprotective effects of 17 β-estradiol. Nearly a decade ago Fillet and colleagues were the first to show that estrogen replacement therapy could significantly improve cognitive function in women diagnosed with Alzheimer's Disease (rev in 10). Exploring the mechanisms by which estrogens could improve cognitive function, Brinton and her colleagues found that 17 β-estradiol induced cellular and morphological features of memory formation and promoted survival of neurons involved in memory function and affected in AD (10). A number of investigators have found that 17 β-estradiol protects neurons against oxidative damage induced by beta amyloid as well as other oxidants such as hydrogen peroxide and glutamate excess (11,12). Recent data from the Simpkins laboratory indicate that the neuroprotective effect of select estrogenic steroids is also independent of a nuclear estrogen receptor. Simpkins and colleagues have found that 17 β-estradiol can potentiate the actions of glutathione to protect neurons against beta amyloid induced toxicity (24). Interestingly, the steroid specificity of the neuroprotective action is dependent upon the presence of a hydroxyl group in the C3 position on the A ring of the steroid molecule and is independent of the activation of nuclear estrogen receptors (11,25). This chemical structure profile includes the non-estrogenic stereoisomer, 17 α-estradiol, which does not induce neuronal process outgrowth (26) but does exert neuroprotective effects (see Table 1). Finally, 17 β-estradiol has been found to regulate growth factor systems both with respect to nerve growth factor receptors as well as the mRNA for nerve growth factor (NFG) and brain derived growth factor (BDNF) (15,16,17,18,19). In animal and *in vitro* studies, 17 β-estradiol has been found to induce and increase activity of choline acetyl-

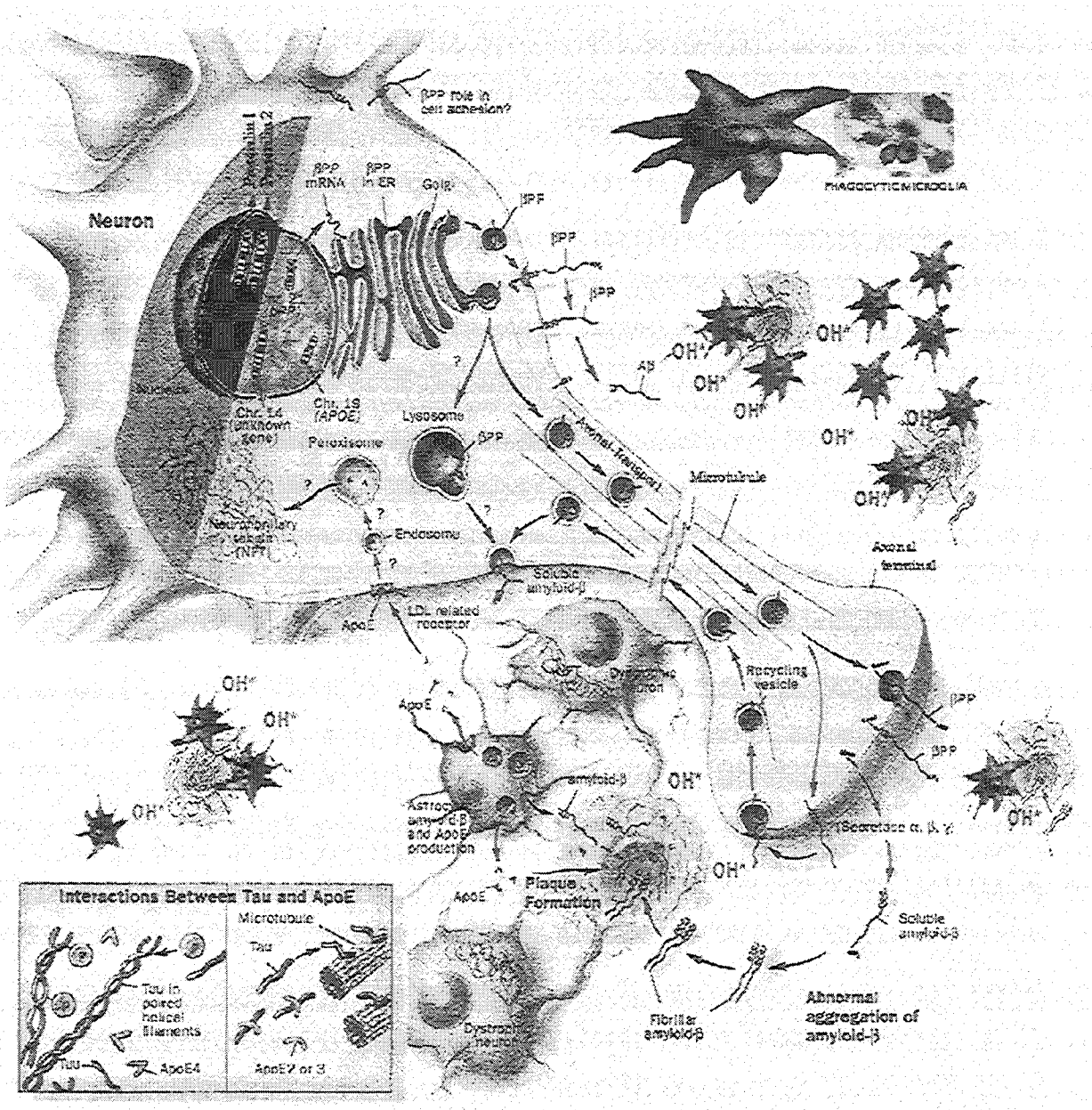

Fig. 1. Cellular, biochemical and genetic mechanisms of Alzheimer's disease. Generation of amyloid precursor protein (APP), following maturation in the endoplasmic reticulum and Golgi, is metabolized by one of at least three pathways. In the "α-secretase" pathway, APP is cleaved within the Aβ domain preventing formation of β-amyloid and leading to the secretion of the ectodomain of APP. The remaining C-terminal fragment is internalized and degraded in the endosomal-lyso-somal pathway. The "β-secretase" pathway leads to a proteolytic clip at the N- terminus of the Aβ domain yielding a C-terminal APP fragment containing intact Aβ. Additional cleavage by γ-secretase at the C-terminus of the Aβ is then necessary for the secretion of Aβ. Monomeric Aβ is converted into a β-pleated-sheet form, then aggregates into fibrils where upon it is toxic to neurons. The ApoE 4 allele and the protease inhibitor α1-antichymotrypsin (ACT) promote Aβ filament formation. In addition, ApoE2 and 3 prevent phosphorylated tau from forming paired helical filaments whereas the ApoE4 allele does not. Formation of the aggregated beta amyloid in plaques generates free radicals that can induce oxidative damage in neighboring neurons. In addition, invasion of the amyloid plaque by microglia induces an inflammatory response and generation of high levels of oxygen free radicals. These free radicals damage surrounding neurons, increasing the degenerative process. See text for further details on each of the mechanisms. (Figure modified from 38.)

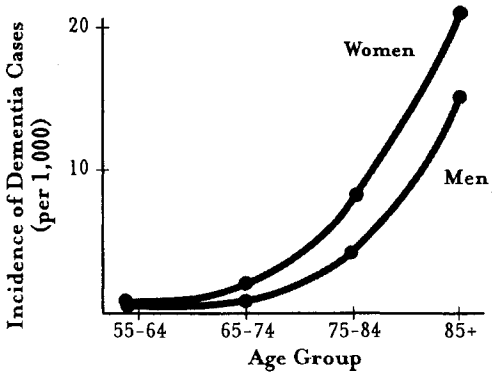

Fig. 2. Incidence of Alzheimer's Disease in Men and Women. The number of individuals with AD approximately doubles every 5 years after the age of 70. In addition, the exponential increase in AD occurs earlier in women than in men. While it is well established that women live longer than men, the increase in the incidence of AD in women is not soley due to their increased numbers in the aged population. Compared to their age matched male counterparts, the incidence of AD is still 2-3 times greater in women than in men. (Figure adapted from 92.)

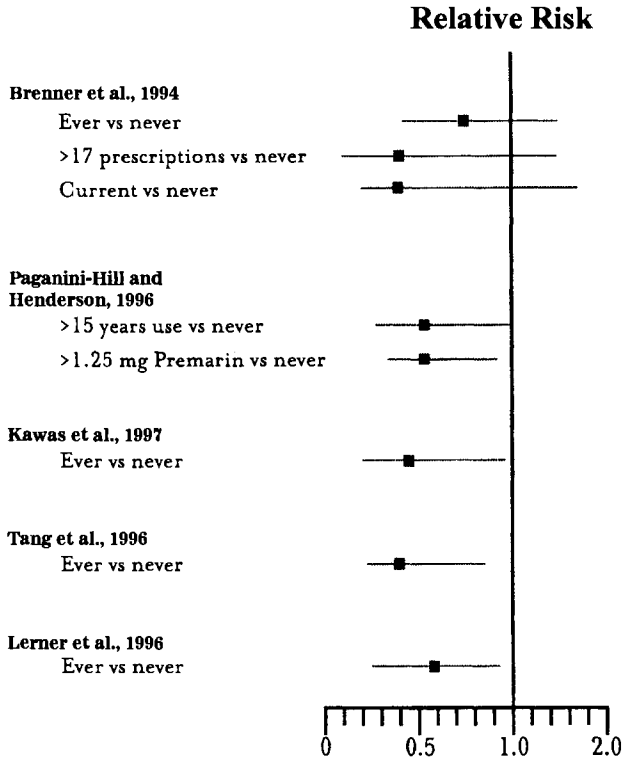

Fig. 3. Effect of Estrogen Replacement Therapy/Hormone Replacement Therapy on Risk of Alzheimer's Disease. Five independent epidemiological studies consistently demonstrated a 40-60% reduction in the risk of AD for women who have received estrogen replacement therapy. The majority of hormone users in these studies were receiving unopposed estrogens (reviewed in 4). The squares represent the point risk estimates while the lines through the squares indicate the confidence intervals. The intervals at the bottom indicate the odds ratio. An odds ratio of 1 represents the risk of developing AD in the general population. An odds ratio greater than 1 indicates an increased risk while an odds ratio lower than one indicates a reduced risk. Note that all of the retrospective observational studies conducted thus far show an odds ratio less than 1 for those women who received estrogen replacement therapy indicating a reduced risk of developing AD.

transferase, the rate limiting enzyme for acetylcholine synthesis, in both the basal forebrain and target areas of cholinergic neurons (13). Moreover, Simpkins and colleagues have found that 17 β-estradiol protects cholinergic neurons from degeneration following lesion of cholinergic fibers (14). Interestingly, a retrospective analysis of the tacrine data that were the basis of FDA approval of tacrine for the treatment of AD (20,91), revealed that only those women who had been receiving unopposed estrogen replacement during the tacrine (a cholinesterase inhibitor) trial had any improvement in cognitive function (see Figure 4). Collectively, these data provide the foundation for understanding potential mechanisms that mediate the protective action of estrogen replacement therapy (see Table 1) and the structure activity relationships required for induction of these protective mechanisms.

Data indicating a reduced risk of developing AD in women taking estrogen replacement therapy have been based on existing hormone replacement therapies. The most frequently prescribed estrogen replacement therapy is in the form of conjugated equine estrogens, marketed as Premarin. Premarin is a formulation of naturally conjugated equine estrogens containing at least 20 different estrogens (8). Three multicenter prospective, randomized, double-blind, placebo-controlled trials investigating the effect of the estrogen replacement therapy Premarin are currently in progress. The largest of these is the Women's Health Initiative study which will investigate the impact of hormone replacement therapy on cardiovascular disease, cancer, osteoporosis, as well as determine the impact of estrogen replacement therapy on prevention of dementias including AD (9). This same formulation of equine estrogens was used in the PEPI (Postmenopausal Estrogen/Progestin Intervention) Trial which investigated the impact of hormone replacement therapy on cardiovascular disease. Results of the PEPI trial showed that conjugated equine estrogens significantly reduced a woman's risk for developing cardiovascular disease. Cardiovascular disease can significantly increase the severity of cognitive impairment in women with AD (3). Moreover, estrogen replacement has been shown to increase cerebral blood flow which is important in maintaining oxygen and glucose to the brain (4). As a follow-up to the PEPI trial, the HERS (Heart Estrogen Progestin Replacement) Study will investigate the impact of the hormone replacement therapies, Premarin and PremPro (a combination of conjugated equine estrogens and medroxyprogesterone) on secondary events in patients with cardiovascular disease.

Since the majority of women in the US receive conjugated equine estrogens as estrogen replacement, we investigated the impact of Premarin on cellular and morphological features of memory and neuroprotection. Results of those investigations indicate that the complex formulation of estrogens, Premarin, promotes the outgrowth of neurons affected in AD and protects these same neurons from oxidative damage (21,22). In an effort to determine which estrogenic steroid components of this complex formulation are active, we have begun a systematic analysis of the individual components of Premarin. Results of these studies have shown that estrogen steroid components of Premarin are as or more efficacious and in some instances more potent than 17 β-estradiol in promoting neuronal outgrowth (see

Table 1. Effect of Estrogenic Steroids on Incidence of Alzheimer's Disease and Cellular Mechanisms Associated with Memory Function

Effect	Estrogenic Steroid or Estrogen Replacement Therapy
Reverses memory deficits in postmenopausal women	Conjugated Equine Estrogens (Premarin) 17 β-Estradiol
Reduces risk of Alzheimer's disease	Conjugated Equine Estrogens[a]
Reduces cognitive deficit in women with AD and slows progression of disease	17 β-Estradiol, Conjugated Equine Estrogens (Premarin)
Promotes neuronal outgrowth	17 β-Estradiol[b] Equilin[b] Δ-8,9 Dehydroestrone[b] Conjugated Equine Estrogens (Premarin)
Promotes neuronal survival	17 β-Estradiol, 17 α-Estradiol, Premarin
Protects against oxidative damage	17 β-Estradiol, Conjugated Equine Estrogens (Premarin), Equilin, Δ-8,9 Dehydroestrone
Promotes glucose uptake in brain	17 β-Estradiol[c]
Promotes cholinergic neuron survival	17 β-Estradiol[c]
Promotes acetylcholine synthesis	17 β-Estradiol[c]
Increases NGF and BDNF mRNA expression	17 β-Estradiol[c]
Regulates metabolism of Alzheimer amyloid beta precursor protein to soluble APP indicative of non-amyloidogenic processing	17 β-Estradiol[c]
Protects against β-amyloid toxicity[c]	17 β-Estradiol[c]
Reduces risk of cardiovascular disease	Conjugated equine estrogens (Premarin), 17 β-Estradiol

[a] Other types of estrogen replacement therapies were grouped with Premarin in the analyses.
[b] Estrogenic components of Premarin formulation.
[c] Other estrogenic steroids not tested.

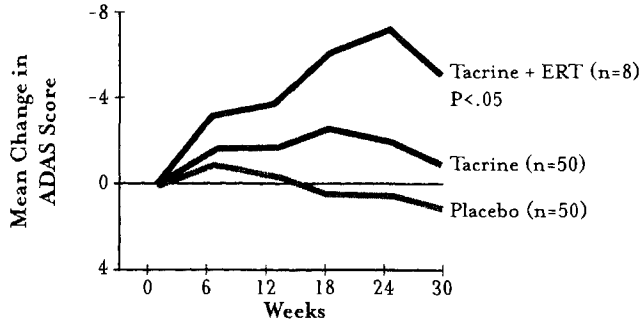

Fig. 4. Effect of Estrogen Replacement Therapy on Therapeutic Efficacy of Tacrine in Women with Alzheimer's Disease. Data depicted presents a post hoc analysis of the pivotal tacrine trial that lead to the approval of the anticholinesterase drug for AD. Of the 108 women who completed the trial, 8 were ERT users who continued ERT concurrently with tacrine. Only 2 women on placebo were ERT users and were therefore not included. Cognitive function was assessed by the ADAS-cog test. A negative or decreased score represents a lack of cognitive decline or improvement in cognitive function. Women receiving ERT concurrently with tacrine had the clearest beneficial response. ADAS = Alzheimer's disease assessment score (figure adapted from 91).

Table 1). Equilin, a major estrogenic component of Premarin significantly increased the outgrowth of neurons affected in AD (8). Surprisingly, a relatively minor but unique component in the formulation of Premarin, Δ-8,9 dehydroestrone, was the most potent neurotrophic estrogen. Remarkably, the neurotrophic effect of these estrogenic steroids does not require activation of a nuclear estrogen receptor but instead acts at a plasma membrane site. Neurotrophic estrogenic steroids (8–10) induce neuronal outgrowth by interacting with a type of glutamate receptor, the NMDA receptor, that conducts calcium ions, a major signaling pathway for regulating neuronal process growth (8,21–23). Collectively, these data provide the foundation for understanding potential mechanisms that mediate the protective action of estrogen replacement therapy (see Table 1), and the structure activity relationships required for induction of these protective mechanisms.

Data from clinical and basic science studies provide encouraging evidence indicating a protective effect of estrogen replacement therapy against developing AD and activation of cellular and biochemical mechanisms that both promote cellular mechanisms of memory formation and neuronal survival. Current efforts are focused on development or discovery of neurotrophic and neuroprotective molecules that will exert the same neurotrophic and protective effects as current estrogen replacement therapies but which will ultimately prove to be tissue selective activators of the beneficial effects of estrogens. The discovery of a second type of estrogen nuclear receptor was an important contribution to that goal.

Table 2. Effect of Anti-Inflammatory Agents on Prevention and Treatment of Alzheimer's Disease

Anti-inflammatory Agent	Effect on Prevention of AD	Effect on Cognitive Function with Persons Diagnosed with AD
Prednisone	+	+
Aspirin	+	+
Ibuprofen	+	
NSAIDS[a] Including but not limited to (Phenylbutazone, Indomethacin, Sulindac, Diclofenac, Piroxicam, Naproxen, Ketoprofen, Pirprofen, Flurbiprofen, Tiaprofenic acid, Tolfenamic acid)	+	+

[a] Select studies listed only the class NSAIDS and not the specific NSAIDs used by study participants.

The discovery of a novel nuclear estrogen receptor termed ERβ has turned a once quiet well-established dogma, i.e. the dogma of one nuclear estrogen receptor, into an exciting vista of new possibilities. The emergence of a new estrogen nuclear receptor (29) is particularly exciting for neuroscientists since the new estrogen nuclear receptor ERβ is readily apparent in brain (31,32). The classical estrogen nuclear receptor, Erα and ERβ both bind the endogenous estrogen, 17 β-estradiol with near equal affinity. The role of ERβ in brain and its functional significance for AD remains to be determined since both the neurotrophic and survival promoting effects of estrogen steroids have been found to be independent of nuclear estrogen receptors (8,24,26). This question notwithstanding, the finding of novel nuclear estrogen receptors has already solved one mystery, described below, and has the potential of making important contributions to the search for the ultimate estrogen replacement therapy in brain and other estrogen responsive organs.

The third area of advancement is the development of a class of molecules termed selective estrogen response modulators or SERMs. This area arose out of basic science and clinical observations indicating that molecules, such as tamoxifen and raloxifene, which were developed as nuclear estrogen receptor antagonists had partial or full agonist properties in select tissues (33,34,35). The reason for this pharmacological duality was impressively documented in a recent paper by Paech et. al (27). These investigators showed first that ERα and ERβ differentially affected transcription through an AP1 regulatory site. When complexed with 17 β-estradiol ERα activated transcription through the AP1 site whereas ERβ inhibited transcription. Surprisingly however, the antiestrogens, tamoxifen, raloxifene and ICI 164,384 were potent transcriptional activators operating through ERβ at an AP1 site. These data indicate that tissue selective expression of ERα and ERβ nuclear receptors can lead to very different responses and that different molecules that can bind these nuclear estrogen receptors can result in complex cellular responses depending on the receptor activator. Moreover, these data provide a mechanism whereby compounds that are antagonists at one estrogen nuclear receptor can exert agonist effects through activation of the other nuclear estrogen nuclear receptor. While the research into the development of tissue selective SERMs is in its infancy, the implications are already clearly apparent. A goal of this area of very intense academic and pharmaceutical industry research is to develop molecules that act as estrogen agonists in bone, vasculature and brain and as antagonists in breast and uterus by selectively binding to one or the other estrogen nuclear receptors. Such a molecule could be the ultimate estrogen replacement therapy for postmenopausal women. Moreover, if such a SERM lacked endocrine action in the hypothalamus and in reproductive tissues, it could have real implications for use in men to potentially prevent cardiovascular disease and AD.

Anti-inflammatory Approaches

For the past decade, there has been a slow but steady flow of intriguing clinical and epidemiological data indicating a reduced risk of developing AD with the use of steroidal and nonsteroidal anti-inflammatory drugs (NSAIDS) (40). Basic science efforts have been aimed at extending these clinical observations to the cellular level to achieve an understanding of the mechanisms by which NSAIDS can protect the nervous system from the ravages of AD pathology. Together these analyses have revealed important insights that have significant therapeutic relevancy.

The McGeers and their colleagues led the way when they postulated a role for the immune system in the pathology of neurodegenerative diseases. These investigators pursued this postulate by conducting a retrospective observational analysis of 7490 hospital discharges of elderly patients and examining the records for concomitant diagnoses of rheumatoid arthritis and AD (41,42). In those individuals with a diagnosis of rheumatoid arthritis, the rate of AD occurrence was 0.39%, or six to twelve times lower than would have been predicted (assuming independence of the two diagnoses) by the product of the rates of the individual diseases. From these findings, McGeer proposed that sustained use of NSAIDS (a mainstay of treatment of rheumatoid arthritis), corticosteroids, methotrexate or other anti-inflammatory agents could protect against the neurodegenerative process of AD. Supporting this postulate are data from other observation analyses of individuals suffering from other diseases whose treatment requires NSAIDS. Leprosy patients in Japan who were on continuous dapsone or its derivative (both of which have anti-inflammatory activity) showed a lower than expected incidence of AD (reviewed in 43). Results of a case-control study by Graves et al., (reviewed in 43) investigating the relationship between prior use of glucocorticoids and

AD revealed an odds ratio of 0.73 suggesting that AD risk may be reduced (an odds ratio of less than 1 indicates an inverse association between two variables). The Canadian Study of Health and Aging reported an odds ratio of 0.55 for AD and concurrent or prior use of NSAIDS. The Rotterdam population study of AD found an odds ratio of 0.38 associated with concurrent use of NSAIDS. In a study of 50 twin pairs who were discordant for onset of AD, Breitner and colleagues found an odds ratio of 0.25 for AD and prior treatment with ACTH or corticosteroids (44). The effect was stronger in monozygotic twins, showing an odds ratio 0.09, than in dizygotic twins and in twin pairs over 70 (odds ratio of 0.13). In a study of sibling pairs from families with a very high risk for AD, the data indicated a delayed onset of AD in those individuals with a sustained prior exposure to NSAIDS. When subjects were categorized by their genotype of APOE, the polymorphic locus for apolipoprotein E (the major known genetic risk factor for AD) there was a trend suggesting that NSAIDS were principally effective in those individuals who lacked the pathogenic ε4 allele. However, even for those individuals with a high risk for developing AD, onset of the disease was later in those individuals who had a history of anti-inflammatory therapy. For those individuals with AD, NSAIDS have been found to be associated with later onset, reduced severity and slower progression of symptoms (43,45,46). Epidemiological analyses have supported the observational studies. Collectively, these analyses, with one notable exception (reviewed in 43), indicate an inverse relationship between prior use of NSAIDS or other anti-inflammatory drugs and development of AD (43).

These clinical observations have stimulated basic science researchers to pursue the mechanism of action for NSAIDS-induced reduction in the risk for AD. Numerous studies now provide a substantial body of evidence indicating the involvement of immune and chronic inflammatory mechanisms in AD (see Table 2) (see reviews 40,41,43,47–48). Immune type cells, microglia, the nervous system's equivalent to the macrophage, and microglia released immune signals are found within beta amyloid plaques, one of the pathological lesions of AD (see Figure 1). Activated microglia are found within or near all AD lesions. These phagocytic cells produce complement proteins and IL-1β, IL-6 and tumor necrosis factor. Activated microglia attach to their targets using surface receptors for immunoglobulins and complement components. Following phagocytosis of the target, microglia can produce class II major histocompatibility antigens enabling the activation of other immune responses. There is evidence of increased activity of cytokines such as interleukin-1β (IL-1β), IL-6 and tumor necrosis factor. These cytokines are mediators of the so called acute-phase reactants to cellular injury such as fibrinogen, C-reactive protein, and α1-antichymotrypsin. The last two of these acute-phase reactants have been found to be elevated in the cerebrospinal fluid of AD patients. Interestingly, α1-antichymotrypsin is a component of the amyloid plaques.

As part of their role in protecting the nervous system from hazardous invaders and removing cells that are in the process of dying, microglia, as their macrophage counter parts do in the periphery, generate oxygen-free radicals, nitric oxide and other potential toxins. Three recent findings indicate that the main component of neuritic plaques, the amyloid peptide beta (Aβ) can induce inflammatory reactions in microglial cells by activating two different receptors in microglia, the receptor for advanced glycation end products (RAGE) and the class A scavenger receptor (SR). The first study by Yan and colleagues (49), found that binding of the soluble beta amyloid peptide to RAGE induced microglial migration along a concentration gradient, possibly leading to a deposit of insoluble amyloid (49). The gradient of soluble Aβ either leads to or induces a deposit of Aβ. Microglia are then literally stuck to a plaque of insoluble Aβ in the plaque, arresting cell migration and inducing a sustained activation of microglia which may underlie a chronic inflammatory response in AD. Independently, El Khoury et al. (50), found Aβ bound to the SR microglial receptor. The high density of Aβ peptide, the SR ligand, present in the Aβ plaques causes immobilization of microglia that come into contact with them and induces these microglia to secrete cytokines, reactive oxygen species and nitrogen species that injure neighboring neurons (see the section on antioxidants for description of degenerative cascade of events induced by oxidative stress). The most recent study by Barger and Harmon (51) also shows that secreted β-amyloid precursor protein can induce inflammatory reactions in microglial cells of the brain. Remarkably, this effect can be blocked by apolipoprotein E3 but not by apolipoprotein E4, a variant associated with AD. In addition, secreted β-amyloid precursor protein exerts a neuroprotective effect against microglial induced inflammatory response whereas the amyloidogenic beta form does not. This latter finding is extremely important as microglia are armed with yet another weapon in their biological arsenal, protease inhibitors. Microglia secreted α1-antichymotrypsin and α_2-macroglobulin are potent protease inhibitors. This mechanism too ends up being a double edge sword. The extent to which amyloid β protein rather than other nonamyloidogenic fragments is released by amyloid precursor protein may depend on the balance of proteases (47). Thus, inhibition of proteases by α1-antichymotrypsin and α_2-macroglobulin may disrupt the balance of protease reactions that regulate the soluble and insoluble forms of peptides derived from the β amyloid precursor protein (this cascade is described in more detail below).

Schmidt and her colleagues, who also reported Aβ binding and activation of RAGE in microglia (49), went on to show that, surprisingly, Aβ binds as well to a neuronally expressed RAGE. Aβ binding of RAGE induces neurons to synthesize and release macrophage-colony stimulating factor by an oxidant sensitive, nuclear factor kB-dependent pathway (52). These investigators also found that AD brain shows increased neuronal expression of macrophage- colony stimulating factor in neurons proximal to Aβ deposits and in the cerebrospinal fluid of AD patients. The neuronal release of macrophage-colony stimulating factor by Aβ-stimulated neurons interacts with its cognate receptor on microglia thereby triggering chemotaxis, cell proliferation, increased expression of macrophage scavenger receptor and apolipoprotein E, and enhanced survival of microglia (52). Together these data document that Aβ activation of RAGE in both microglia and neurons and activation of SR in microglia can lead to a self perpetuating vicious cycle of inflammatory reactions that induce oxidative damage to neurons via microglia induced inflammatory mechanisms.

While the debate of whether the inflammatory condition seen in AD is the cause of AD or is a secondary event that potentiates the degenerative process remains unsettled, the evidence for an inflammatory reaction in AD is clear. Moreover, the data indicate that anti-inflammatory therapy lowers the risk

for developing AD in persons without known genetic risk for AD and delays the onset of the disease in those individuals with a high risk for developing AD. The current anti-inflammatory agents have obvious benefits in reducing the risk or delaying the onset of AD and are generally well tolerated (see Table 2). However, they also have a number of drawbacks and potentially serious side effects (56).

Glucocorticoids are efficacious in suppression of inflammatory and autoimmune mechanisms. Prednisone, a synthetic glucocorticoid, is the most effective treatment for most inflammatory rheumatic diseases, including systemic lupus erythematosus, a disease in which brain inflammation and complement activation cause major manifestations. Prednisone suppresses the acute phase response of rheumatoid arthritis and suppresses complement activation in AD (53). Currently, prednisone is the only anti-inflammatory agent being studied in a multicenter placebo-controlled therapeutic trial for the treatment of AD which began enrollment in 1995. Pilot studies have demonstrated that low to moderate doses of prednisone are well tolerated by AD patients. Given the potential negative side effects of long-term exposure to glucocorticoids, it is questionable whether long-term exposure to prednisone will benefit or exacerbate the cognitive decline in AD patients. Elevated glucocorticoids have been associated with neuronal injury in both Cushing's disease and in animal studies and have been shown to precipitate adverse behavioral changes in a subset of AD patients (54–55).

Nonsteroidal anti-inflammatory drugs are the first-line drugs for the treatment of inflammatory diseases such as rheumatoid arthritis and gout (43,47). Their efficacy has been attributed to inhibition of neutrophil function and prostaglandin synthesis. Moreover, salicylate, the prototypic NSAID serves as a trap for hydroxyl radicals (45). The most serious side effect of NSAIDS is gastrointestinal bleeding which can develop following long-term exposure (56). Unlike the glucocorticoids, however, NSAIDS do not suppress the acute phase response that accompanies rheumatoid arthritis. Despite this difference, most of the clinical data from retrospective analyses indicating a reduced risk of developing AD with prior exposure to anti-inflammatory agents are based on NSAIDS (54).

The antimalarial drugs, hydroxychloroquine and chloroquine are slow acting remission-inducing drugs that are useful in the treatment of rheumatoid arthritis (47). Antimalarials are lysosomotropic—that is they accumulate in lysosomes, neutralizing the acidity and thereby inhibiting acid protease activity. This action may be beneficial in AD in decreasing the conversion of beta amyloid precursor protein into the fibril generating Aβ peptide.

Potent anti-inflammatory/immuno-suppressive agents such as methotrexate, azathioprine and cyclophosphamide have proven effective in the treatment of lupus, rheumatoid arthritis and polymyositis (47). However, these drugs have the potential to cause life-threatening toxicity.

In addition to developing anti-inflammatory agents that specifically target microglia, other development areas include targeting both the RAGE and SR receptors as sites for therapeutic intervention to prevent initiation of the chronic inflammatory reaction associated with AD (49,50,52,57). Discovery of the best anti-inflammatory agent to prevent AD should be a high priority.

Anti-oxidants

As the preceding section on inflammatory reactions and the mechanisms that mediate inflammation indicate, there is substantial evidence indicating that oxidative damage very likely occurs in the AD brain (58). When the homeostatic mechanisms that inactivate reactive oxygen species (ROS) are deficient, as can occur in aging, cell damage ensues which can be manifested in nucleic acid breakage, increase in intracellular calcium, protein damage and lipid peroxidation. In support of the postulate of increased oxidative mechanisms in AD, Subbarao and colleagues found that samples derived from AD cortex show increased peroxidation in vitro (59). Given that age is the greatest risk factor for AD and oxidative damage increases with age, increasing attention is focused on strategies that combat oxidative damage (60).

Potential therapeutic antioxidants include vitamin E, a lipid-soluble vitamin that interacts with cell membranes and traps free radicals, thereby protecting lipid membranes from oxidative damage, and vitamin C which acts as a free radical scavenger. Other viable candidate antioxidant therapies include co-enzyme Q-10, idebonene, the selective monoamine oxidase (MAO-B) inhibitor selegiline which can inhibit oxidative deamination and lazaroids.

The Alzheimer's Disease Cooperative Study consortium recently completed a multicenter trial of two antioxidant compounds, vitamin E and selegiline, as treatment for AD. Results of this study showed that treatment with either one or both of these antioxidants slowed the progression of the disease (62). Unfortunately, no improvement in cognitive function was found. Thus, antioxidant therapy appears to be able to slow the decline of degeneration in individuals with AD but in and of itself does not restore lost function. To definitely determine whether antioxidants can reduce the risk of AD both epidemiological analyses and prospective trials are needed in addition to long-term clinical studies investigating the ability of antioxidants to reduce the risk of developing AD.

Cholinergic Pharmaceutics for the Treatment of Alzheimer's Disease

The loss of cholinergic innervation of the hippocampus and cerebral cortex coupled with the loss of cholinergic neurons in the basal forebrain is a well described finding in the postmortem analysis of Alzheimer disease brains (63). This finding more than two decades ago has been the impetus to develop and implement strategies that would result in replacement of the lost neurotransmitter, acetylcholine. While this approach made sense based on the early neuropathological findings, it has, in the main, led to modest and short-lived effects. Unfortunately, replacement therapy does not address the root cause of the disease and from the outset is not sufficient to counteract the ravages of the disease process. Moreover, many other neurotransmitter and receptor systems are affected in AD. Thus replacement of just one of the degenerating systems does not compensate for the magnitude of the loss. Despite the modest improvement in cognitive function induced by this class of compounds, efforts to develop cholinergic modulators continue. The classes of cholinergic agents include muscarinic and nicotinic agonists, cholinesterase inhibitors (ChEIs) and indirect modifiers of acetylcholine release. Cholinergic agonists include xanomeline, milameline, SB202026, AF102B and ABT418

(64). All of these agonists are in the early stage of development. The cholinesterase inhibitors include drugs that range from the first generation cholinesterase inhibitors such as tacrine, physostigmine and sustained release physostigmine. These first generation compounds, such as tacrine, trade name of Cognex, nonspecifically inhibited acetylcholinesterase, butyrylcholinesterase and other peripheral cholinesterases. The nonspecific inhibition of other cholinesterases led to serious side effects which often made use of tacrine problematic or untenable. The second generation cholinesterase inhibitor derivatives include donepezil, ENA 713, metrifonate, galanthamine and eptastigmine. Advantages of the second generation cholinesterase inhibitors include greater predictability in their pharmacokinetics and induction of higher concentrations in the central nervous system. The greater selectivity of the second generation cholinesterase inhibitors is expected to produce fewer side effects. When ChEIs are used alone and then withdrawn, the patient looses any cognitive benefit of the therapy within a very short span of time and is subsequently indistinguishable from placebo control subjects (65). Moreover, a retrospective analysis of the tacrine data that was the basis of FDA approval of tacrine for the treatment of AD (20), revealed that only those women who had been receiving unopposed estrogen during the tacrine trial had any improvement in cognitive function (91; see Figure 4). In the main these drugs are used in combination with other therapies that are directed towards counteracting the disease process while attempting to increase levels of the neurotransmitter acetylcholine to improve cognitive performance (64).

Neurotrophic Factors for the Treatment of Alzheimer's Disease

Several neurotrophic factors have held the promise of therapeutic efficacy in the treatment of neurodegenerative disease. Of those, nerve growth factor (NGF) has the greatest potential for use in AD. Nerve growth factor has been found to be essential for basal forebrain cholinergic neuron survival (66,67). Given this vital relationship between NGF and cholinergic neurons, a great deal of effort has been put forth to create a basic science foundation for the use of NGF to protect cholinergic neurons from death and to restore cholinergic innervation to the hippocampus where acetylcholine regulates memory function (67). Animal studies using this paradigm have been very successful (68). A major obstacle in humans is drug delivery. NGF because of its large molecular weight does not cross the blood-brain barrier and is easily metabolized by peptidases when administered peripherally. While intraventricular administration in animals is easily achieved and regulated, the same is not true for humans. Thus far, human trials in Sweden with intrathecally administered NGF have not been successful and have been marked by an increased incidence of pain. Gage, Borklund and their colleagues have attempted to circumvent the delivery problem by genetically engineering fibroblasts to synthesize NGF with the long-term goal of transplanting host derived fibroblasts, that have been genetically engineered to synthesize NGF for transplant into the host to act as biopumps in the brain of the AD patient (67–68). A different strategy of addressing the drug delivery problem, is to administer drugs that either enhance the action of NGF and or increase the expression of NGF in the appropriate cell population (reviewed in 61). The drug AIT-082, which can cross the blood brain barrier, has been found to enhance NGF effects in vitro. In addition, the drugs propentofylline and idebenone, which also cross the blood-brain barrier, enhanced NGF synthesis and improved memory deficits in rats following a lesion of the basal forebrain cholinergic pathway (69). One important caveat to development of genetically engineered NGF transplants or NGF enhancers, is the inverted U-shaped dose response profile for growth factors. High concentrations of growth factors can be neurotoxic.

Genetics of Alzheimer's Disease, Genetic Testing and the Potential of Gene Therapy

Early-onset familial AD, a rare form of AD, characterized by onset before age 60 years and an autosomal dominant mode of inheritance, is linked to mutations in three genes: amyloid precursor protein (APP), and the presenilins one and two (PS_1 and PS_2) (reviewed in 70, see Figure 1). Mutations in the APP gene account for 5–20% of cases of early-onset familial AD while mutations in the PS1 gene accounts for approximately 50% of the familial AD cases whereas mutations in the PS2 gene are a rare cause of AD (reviewed in 70). The gene coding for APP, which is encoded on chromosome 21, gives rise to the Aβ peptide by proteolytic cleavage (for review see 71). Rare mutations in the APP gene cause some forms of familial early-onset AD by altering APP processing to increase either total production of Aβ or the relative amount of its most amyloidogenic form, $A\beta_{1\text{-}42}$, which is the major constituent of amyloid plaques. Consequently, all individuals who carry three copies of chromosome 21 (and thus the APP gene) due to meiotic nondisjunction (Down syndrome) also produce more $A\beta_{1\text{-}42}$ (72) and develop Alzheimer pathology at an early age.

The majority of early onset familial AD is caused by mutations in either of two related genes, termed the presenilins, PS1 located on chromosome 14 and PS2 located on chromosome 1 (70,73). Thirty point mutations that cause familial AD have been identified in the genes coding for PS1 and PS2 (reviewed in 70). The function of these proteins remained a mystery until very recently when work by Potter and colleagues (73) showed that PS1 and PS2 proteins are localized to nuclear membrane, associated with kinetochores and the centrosomes, both subcellular structures involved in cell cycle regulation and mitosis. Together mutations in the APP, PS1 and PS2 genes account for approximately 70 to 80% of early onset familial AD which suggests that other gene mutations leading to AD could be as yet undetected.

Variation in three other genes, apolipoprotein E (76; apoE4), antichymotrysin (ACT-A) and HLA-A2 (77) gene which is part of the major histocompatibility complex in humans, are associated with increased risk of or earlier onset of AD. Perhaps the most important breakthrough in this area has been the observation of an increased frequency of the ε4 allele of the apolipoprotein gene in late-onset AD cases (76). Apolipoprotein E is a major serum lipoprotein involved in cholesterol metabolism and while apoE4 does not cross the blood brain barrier it is synthesized in the brain by astrocytes (reviewed in 76). ApoE protein interacts with both beta-amyloid and tau in an isoform specific manner (see Figure 1). ApoE3 interacts with both tau and the protein MAP2c at the microtubule binding repeat domain under conditions in which ApoE4 is less tightly bound. ApoE3 and 2 are thought to serve a protective role by interfering with tau binding to itself to form paired

helical filaments and neurofibrillary tangles, while protecting the site for microtubule stabilizing interactions with beta-tubulin (76; see Figure 1). A similar mechanism occurs in the interaction between ApoE and Aβ wherein the products for the ApoE4 and ACT-A genes increase the risk of AD by promoting Aβ polymerization into neurotoxic amyloid filaments.

Curiously, the association between expression of the ApoE4 allele and increased risk of AD is not consistent across ethnic groups. The increased risk has been observed in many studies of populations of European heritage and Japanese origin whereas increased risk with ApoE4 allele is not seen in Nigerians (reviewed in 70,76). The data on African Americans is not yet conclusive (reviewed in 70,76). Estimates indicate that as much as 50% of the risk for AD may be accounted for by APOE genotype (74). This estimate may be spuriously high given the recently published data of Mayeux and colleagues who found in a random sample of over individuals age 65 and older that lack of an ApoE4 allele would only reduce the incidence of AD by 13.7% (75). Moreover, not everyone possessing the high-susceptibility ϵ4 allele will develop AD and many who lack the ϵ4 allele will develop the disease. The consensus of clinical and basic science researchers in this area is that ApoE4 is not a sufficiently reliable indicator for development of AD and therefore should not be used for genetic testing (84).

Two other genes have been found to be associated with an increased risk for AD. Expression of the HLA-A2 allele of the major histocompatibility complex gene, encoded on chromosome 6, was associated with an earlier onset of AD (77). Recently, another gene located on chromosome 12 has been associated with increased risk of AD. Current efforts are directed towards searching for a segment of chromosome 12 that is about 12–20 centimorgans in length, a span of DNA large enough to contain 20–50 genes, for the gene or genes that increase the susceptibility to AD (78).

A new area of intense interest is the search for mutations in mitochondrial genes that would account for the preponderance of cases of AD which are late onset and are not linked to mutations in the beta amyloid or presenilin genes (83). Mitochondrial gene mutations, while providing a genetic basis for AD, would not follow the Mendelian gene inheritance pattern since mitochondrial genes are maternally derived. A decrease

Table 3. Currently Developed and Characterized Transgenic and Knock-Out Mouse Models Important to Pharmaceutical Strategies for the Prevention and Treatment of Alzheimer's Disease

Mouse Model	Genetic Alteration	Notable Features
K670M:N671L-Hsiao mouse model	APP overexpression	Amyloid deposits in hippocampus and cortex positive for Aβ1-40 and Aβ1-42 with evidence of β sheet conformation appearng at 12 months of age. Evidence for inflammatory response. Brain region selective deposits of amyloid resembling distribution seen in AD. No evidence for neurofibrillary tangles. Memory deficit in older transgenic animals (reviewed in 86).
PDAPP - Athena mouse model	APP overexpression	Same as Hsiao model with onset of abnormality occuring at 6-9 month. Some complement proteins associated with a subset of plaques. Some dystrophic neurons immunoreactive for phosphorylated tau (reviewed in 86).
APP751 Sandoz mouse	APP over expression	Same as Hsiao model and shows immunoreactivity to phosphoryltated tau protein in dystrophic neurons and cell bodies (reviewed in 86).
Human PS-1 cDNA mutant mouse	Aβ 1-42(43) over expression	Elevation of Aβ 1-42(43) but no Aβ 1-40. Amyloid deposits have not been observed at 8 months of age. Mice appear healthy at one year of age (reviewed in 86).
APP-C100 transgenic mouse	APP-C100 over expression	Over expression of the 100 carboxy-terminal amino acids of the amyloid precursor protein which contains the neurotoxic Aβ1-42 peptide (87).
C104 transgenic mouse	Express carboxy-terminal 104 aminoacids of amyloid precurson protein	Extracellular β-amyloid immunoreactivity, cell loss in the CA1 region of the hippocampus, increased gliosis and microglial reactivity, spatial learning deficits and deficits in maintenance of long-term potentiation, an electrophysiological correlate of memory (88).
ERKO - estrogen receptor α knockout mouse	Knock-out of the estrogen receptor a gene	Loss of reproductive capability in homozygotes (30).
IL-6 transgenic mouse	Chronic expression of interleukin 6 (IL6)	Astrocytic over expression of IL6. Extensive synaptic damage, loss of calbindin immunoreactive neurons, increase in mRNA for Mac-1 microglial marker, acute phase response gene, and inflammatory adhesion molecule. Dose of IL6 and age related deficits in learning (89).

in mitochondrial cytochrome oxidase I and III expression has been found in the postmortem analysis of AD brains (79). This reduction in cytochrome oxidase mRNA was paralleled with reduced enzyme activity in platelets derived from persons with AD (81). More recently, Parker and colleagues found that mutations in mitochondrial cytochrome c oxidase genes were found to segregate with late-onset AD (82). These investigators found that specific missense mutations in the mitochondrial cytochrome c oxidase genes 1 and 2 but not 3 were found to segregate at a higher frequency with AD compared with other neurodegenerative or metabolic diseases. These mutations appeared together in the same mitochondrial DNA molecule and define a unique mutant mitochondrial genome. Asymptomatic offspring of AD mothers had higher levels of these mutations than offspring of AD fathers, suggesting a pattern of maternal inheritance. Mitochondrial gene mutations could make an important contribution to understanding late onset AD.

The determination of genetic mutations and genes that are associated with increased risk of AD lead inevitably to the question of genetic testing. A consensus statement addressing this issue was recently published in the Journal of the American Association (84). The consensus of the group was that "except for autosomal dominant early-onset families, genetic testing in asymptomatic individuals is unwarranted." Moreover, this group concluded that it is premature to introduce genetic testing and the adverse consequences of such testing should be avoided.

One extremely important use of the genetic data is the development of transgenic mouse models of AD. The genetic linkages of AD have greatly increased our understanding of the causes and risk factors associated with AD and in addition have provided targets for developing transgenic mouse models for the study of AD (reviewed in 86). Transgenic mouse models of AD will provide crucial *in vivo* screening systems for therapies that could lead to successful approaches for the prevention and treatment of AD. None of the current mouse models, however, completely recapitulates the histopathological, biochemical and cognitive impairments induced by AD. The most glaring omission in these models is the neurofibrillary tangle within degenerating neurons (see Figure 1). Nevertheless, these animal models of AD, while still not fully expressing all the characteristics of the disease, provide the first real opportunity to directly test pharmaceutical therapies directed against both the mutated genes and their dysfunctional products (see Table 3 for list of transgenic knockout mice relevant to the study of AD).

In the near future, gene therapy approaches will be more likely directed towards genetically engineering cells either *in vivo* or *ex vivo,* for later implantation, to produce factors that promote the survival of degenerating neurons (see section on neurotrophic factors) or that decrease the processing of APP into the neurotoxic $A\beta_{1-42}$ peptide.

Near Term Pharmaceutical Therapy for the Prevention and Treatement of AD

It is clear that AD is a multifactorial degenerative process (85). Based on this understanding, it is reasonable to assume that in the majority of cases the treatment strategy will have to take this into consideration. Together, data from epidemiological, basic science and clinical trials indicate that estrogen replacement therapy in postmenopausal women, and anti-inflammatory agents in both men and women could provide substantial reduction in risk for developing AD. In those individuals diagnosed with the disease, these same therapeutics along with antioxidants have been found to provide some improvement in cognitive function and in some instances a slower rate of neurodegenerative decline. Addition of the new generation cholinesterase inhibitors such as donepezil (Aricept) to increase acetylcholine levels could prove to have some short-term benefit in persons diagnosed with AD.

The challenge still lies in fully understanding the etiology of Alzheimer's disease. Armed with this knowledge, successful therapeutic strategies can be developed that could prevent the degenerative process from ever taking hold. If we are able to meet this challenge, and for the first time in decades rapid progress towards that goal is occurring, it will mean millions of people world wide can become elders, confident that they will age successfully with the opportunity to contribute their life's wisdom to their families and communities.

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