Introduction

Alzheimer’s disease (AD) is one of the most serious health problems in the industrialized world. It is a progressive neurodegenerative disorder that accounts for the vast majority of age-related dementia.

According to Diagnostic and Statistical Manual of Mental Disorders (DSM IV), dementia is characterized by multiple cognitive defects that include impairment in memory without impairment in consciousness. The cognitive functions that can be affected in dementia include general intelligence, learning and memory, language, problem solving, orientation, perception, attention and concentration, judgment and social abilities. A diagnosis of dementia requires that the symptoms result in significant impairment in social and occupational functioning and that they represent a significant decline from a previous level of functioning (American Psychiatric Association 2000).

Dementia in Alzheimer patients is commonly diagnosed in the clinical setting after other causes of dementia have been excluded from diagnostic consideration. Mental state examination and collection of information from the patient’s family, friends and employers are part of the clinical examination. Memory impairment is typically an early and prominent feature of the illness.¹

Definite diagnosis of Alzheimer’s disease can be performed by only pathologically examining the brain tissue. Whereas shrinkage of neuronal volume without loss of neurons is a feature of normal aging, loss of neurons is typical of Alzheimer disease. The two characteristic neuropathological features of Alzheimer’s disease are senile plaques and neurofibrillary tangles. A

References

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recent clinicopathological study found that elderly nuns with senile plaques and neurofibrillary tangles don’t always have dementia but the risk is greatly increased (from 57 to 93 percent) if they also have suffered from strokes.1

For people over 65, the prevalence rate of all dementias is 1.5 percent in America. Alzheimer’s disease as the most common type of dementia constitutes 50-60% of all dementia cases and the prevalence increases with the increase of age.2 The onset of illness usually starts in the 60’s but in rare instances the disorder appears in people in their 40’s and 50’s. This form is known as “Early onset Alzheimer’s disease”. Four genetic loci have been associated with Alzheimer’s disease risk. 10% of Alzheimer’s disease cases are hereditary and remaining 90% are sporadic. Of the hereditary cases 70 to 80 percent are attributable to mutations in the Presenilin 1 gene, located on chromosome 14 which causes onset of symptoms at age 40 to 50 years. Another 20 to 30 percent are attributable to mutations in a related gene, Presenilin 2, located on chromosome 1, which causes onset of symptoms at age 50 years. A final 2 to 3 percent of familial cases are attributable to mutations in the β-amyloid precursor protein gene (APP), located on chromosome 21, which causes onset of symptoms at age 50.1

Presence of the ε3/ε4 or the ε4/ε4 alleles has been claimed to account for 10 to 50 percent of the risk of sporadic Alzheimer disease with onset of symptoms about age 60 years.3

Other risk factors for developing Alzheimer’s disease are being female, having a first degree relative with the disorder and having a history of head injury. Down syndrome is also characteristically associated with the development of Alzheimer dementia 4. Risk factors contributing to the development of Alzheimer’s disease are summarized in figure 1.

**Neuropathological hallmarks of Alzheimer’s disease**

Brains from AD patients are characterized by cortical atrophy in the form of gyriar shrinkage, widening of the sulci and enlargement of the ventricles. The first regions to be affected are the hippocampus and entorhinal cortex. Later in the disease, a pronounced neurodegeneration occurs in the temporal and parietal lobes. The frontal and occipital lobes may also be affected in some patients. Hallmarks of Alzheimer’s disease on a microscopic level are: Extracellular senile plaques, intracellular neurofibrillary tangles, neuronal cell loss and synaptic degeneration.5 Although abundant levels of these brain lesions are required to con-
firm the diagnosis of Alzheimer’s disease, the issue of whether they are neurotoxic, protective or simple incidental markers of the disease has remained controversial.

**Neurofibrillary tangles**

Neurofibrillary tangles are bundles of abnormal ~20-nm cytoplasmic fibers (paired helical filaments) in neuronal cell bodies, axons and dendrites. The density of neurofibrillary tangles correlates well with the loss of cells and synapses and with the progress of Alzheimer’s disease.

Intensive studies by numerous laboratories have shown that tau protein, which normally enhances the polymerization of tubulin into microtubules and stabilizes these organelles in neurons, is excessively phosphorylated in Alzheimer patients. The modification of this normally soluble protein into an insoluble filamentous polymer seems to involve a deregulation of cytoplasmic phosphorylation / dephosphorylation cascades, but the factors that trigger this imbalance are poorly understood. The gene on human chromosome 17 that encodes the tau protein is not known to be the site of disease-causing mutations in familial forms of AD.

A recent report by Santacruz and his colleagues suggests that tau protein and not the neurofibrillary tangles are the neurotoxic entities. The authors have engineered mice to express a mutant form of the tau gene, known to cause frontotemporal dementia. Expression of the mutant tau gene resulted in age-related loss of neurons, atrophy of the forebrain and tau-positive-lesions along with a dramatic age-dependent decline in spatial memory. They then deactivated the mutant tau gene to assess the potential for the recovery of brain function. They found out that the number of neurofibrillary tangles continued to increase in the absence of mutant tau protein. Surprisingly, although the levels of neurofibrillary tangles increased, there was a plateau in the rate of neuronal loss and brain atrophy.

Neurofibrillary tangles composed of paired helical filaments containing hyperphosphorylated tau molecules are found in a variety of neurological diseases besides AD.

**Amyloid plaques**

AD is characterized by abundant extracellular masses of ~8-nm filaments composed of Aβ.

Other molecules present in amyloid plaques are: proteoglycans, inflammatory molecules and apolipoprotein E.

**Amyloid β precursor protein (APP) and Aβ peptide (Aβ)**

Aβ peptide (Aβ) is the main component of amyloid plaques which are hallmarks of Alzheimer’s disease. Aβ is produced by the proteolysis of amyloid β precursor protein.

The gene coding for β APP is located on the 21. chromosome and encodes for a 110- 130 kDa membrane glycoprotein (Figure 2). β-APP reaches the membrane after being synthesized at the ribosome and is proteolytically cleaved by α-, β- and γ-secretase enzymes. Proteolysis produces Aβ peptides of different length. The main ones are Aβ peptides consisting of 40 and 42 amino acids (Aβ 40 and Aβ 42) (Figure 3).

Candidates for α-secretase activity are three members of the ADAM (a disintegrin and metalloprotease) family: ADAM-9, ADAM-10 and TACE (tumor necrosis
factor- α converting enzyme). The protein responsible for β-secretase activity was identified as aspartyl protease and was named BACE (β-site APP cleaving enzyme). A large complex of different proteins, including neprilysin, insulin-degrading enzyme and presenilins is suggested to be responsible for the γ-secretase activity.

α-secretase cuts β APP between 687 lysine and 688 leucine residues which correspond to the 16 and 17. residues of the A β peptide. Because this cut is within the A β peptide sequence, A β peptides which are hallmarks of Alzheimer do not arise from this cut (Figure 2). The two pieces that arise are: the soluble N-terminal fragment of APP (α-APPs) and the C-terminal 10 kDa fragment C83 anchored in the membrane. Further γ-secretase cleavage of the C-terminal fragment releases a 3 kDa peptide p39 (Figure 3).

β-secretase cuts APP at the N-terminus of A β right after 671. residue. The peptides that arise from this cut are: the soluble N-terminal part of APP (α APPs) and the C-terminal fragment C99. Subsequent cleavage of the C99 protein intermediate at the C-terminal side of the A β peptide by γ-secretase generates the amyloidogenic form of the protein. γ-secretase processing is a heterogeneous event forming A β peptides with different termini. The enzyme usually cuts after Valine at position 40 or after Alanine at position 42. A β 40 and A β 42 are the most common forms of A β peptides. (Figure 4).

A β 42 has a greater tendency to form fibrillary tangles characteristic for Alzheimer’s disease, A β 42 induces lipid peroxidation and protein oxidation in vitro and in vivo possibly by generating radicals. The neurotoxicity of A β 42 is related to the generation of H2O2 but the chemistry involved in generating the oxidation products is unclear.

Transgenic mice that overexpressed the mutant form of APP from a vector whose expression can be regulated via deoxycycline was generated. Deoxycycline administration was shown to inhibit transgenic APP expression by greater than %95 and reduced A β production to levels found in non transgenic mice. Suppression of A β production was found to abruptly halt the progression of amyloid pathology.

Alzheimer disease and genes

There are two types of Alzheimer’s disease: "Early Onset Alzheimer’s disease (EOAD)" and "Late Onset Alzheimer’s disease (LOAD)" (Table 1). Rare and highly penetrate "Early Onset Alzheimer’s disease (EOAD)" mutations are transmitted in an autosomal dominant fashion, while "Late Onset Alzheimer’s disease (LOAD)" shows no obvious familial segregation pattern.

EOAD represents only a small fraction of all AD cases (≤5%) and shows an autosomal dominant transmission within affected families. EOAD is seen between ages 30 and 65. To date, more than 160 mutations in 3 genes have been reported to cause EOFAD. Genes that have been found to be associated with EOAD are A β precursor protein (A β APP) on chromosome 21, presenilin 1 (PSEN1) on chromosome, and presenilin 2 (PSEN2) on chromosome 1 (Table 2). The most fre-
The table presents the age of onset and genes associated with Early Onset Alzheimer’s Disease (EOAD) and Late Onset Alzheimer’s Disease (LOAD).

### Table 1
**Early Onset Alzheimer’s Disease (EOAD)** | **Late Onset Alzheimer’s Disease (LOAD)**
---|---
Age | Onset between 30 and 60 | Onset after 60
Genes | Amyloid β precursor protein (APP), Presenilin 1 (PSEN1), Presenilin 2 (PSEN2) | Apolipoprotein E4

LOAD is classically defined as AD with onset at age 65 years or older and represents the majority of all AD cases. The ε 4 allele of the apolipoprotein E gene on chromosome 19q13 (APOE) has been found to be a risk factor for the onset of LOAD (Table 2). Apolipoprotein E has 4 isoforms: ε 1, ε 2, ε 3 and ε 4. Carriers of APOE ε 4 have been found to have increased risk of developing AD depending on the number of alleles they carry.3,16

In contrast to all other association-based findings in AD, the risk effect of APOE ε 4 has been consistently replicated in a large number of studies across many ethnic groups. In addition to the increased risk exerted by the ε 4-allele, a weak, albeit significant, protective effect for the minor allele, ε 2, has also been reported in several studies. Unlike mutations in the known EOFAD genes, APOE ε 4 is neither necessary nor sufficient to cause AD but instead operates as a genetic risk modifier by decreasing the age of onset in a dose-dependent manner.3,9

Despite its long-known and well-established genetic association, the biochemical consequences of APOE ε 4 in AD pathogenesis are not yet fully understood. Apolipoprotein E has been found in senile plaques and fibrillary tangles which are characteristic for Alzheimer’s disease.17 ApoE is responsible for the efflux of cholesterol from neurons 18 and was found to form stable complexes with Aβ both in vitro and in vivo.19-21

A growing body of evidence suggests that high cholesterol levels might be linked to Alzheimer’s disease. ApoE 4 allele has been linked to high cholesterol levels22 and hypercholesterolemia is considered a risk factor associated with AD.23 Epidemiological studies indicate that the prevalence of AD is reduced among people treated with inhibitors of cholesterol biosynthesis statins24,25 and these results are supported by animal studies.26,27

High cholesterol levels were found to increase APP and apo E expression in human NT2 neuron progeni-

### Table 2
Represents genes associated with “Early onset Alzheimer’s disease”, their chromosomal locations and the Aβ phenotype resulting from defects in these genes.

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Gene defect</th>
<th>Age of onset</th>
<th>Aβ phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Mutations in β APP</td>
<td>50’s</td>
<td>Production of full length Aβ peptides or Aβ 42 peptides</td>
</tr>
<tr>
<td>14</td>
<td>Mutations in Presenilin 1</td>
<td>40’s and 50’s</td>
<td>Production of Aβ 42 peptides</td>
</tr>
<tr>
<td>1</td>
<td>Mutations in Presenilin 2</td>
<td>50’s</td>
<td>Production of Aβ 42 peptides</td>
</tr>
</tbody>
</table>

### Table 3
Represents ApoE4 polymorphism which is associated with “Late onset Alzheimer’s disease”, its chromosomal location and the Aβ phenotype resulting from ApoE4 mutations.

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Gene defect</th>
<th>Age of onset</th>
<th>Aβ phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>ApoE4 polymorphism</td>
<td>60’s and above</td>
<td>Dense Aβ plaques and vascular deposits</td>
</tr>
</tbody>
</table>
tor cells. A cholesterol rich environment also induces processing of APP, leading to the formation of Aβ and Aβ peptide fragments. The binding of cholesterol to ApoE is abolished completely in the presence of Aβ 1-42.28

Another proof for the association of disturbances in cholesterol metabolism and Alzheimer’s disease came from a study where Alzheimer patients were treated with lipid lowering agents (LLAs). Patients who took LLAs were found to have slower cognitive decline than patients who did not receive LLAs.29

Amyloid β precursor protein (APP) and presenilin mutations and their effects on the APP Metabolism

Up to date, 20 mutations have been found in the APP gene, 144 in the Presenilin 1 gene and 20 in the Presenilin 2 gene.30

The finding that the APP β gene is on the 21. chromosome explains why β-amyloid plaques and Alzheimer symptoms are seen in patients with Down syndrome in their 40’s (Down patients have trisomy 21).51,52

All APP mutations are located close to and change the α-, β-, γ-secretase cleavage sites. β APP mutations were shown to increase the production of Aβ peptides, especially of Aβ 42 (Figure 4).13,33-37

PSEN mutations found in Alzheimer patients also increase levels of the Aβ 42 peptide 9.

Presenilins are transmembrane proteins located in the membranes of the endoplasmatic reticulum and the golgi vesicles and play a role in the proteolytic cleavage of APP but their exact role is not yet clear.

Future aspects of Alzheimer’s disease treatment

If AD is a result of a chronic imbalance between Aβ production and Aβ clearance, which can be due to numerous distinct initiating factors, how should we treat and prevent the disorder?

Figure 4

Proteolytic cleavage by β-secretase and γ-secretase enzymes

β-secretase cleaves after residue 671. This cut results in the secretion of the soluble β-APPs peptide and retention of the C-terminal domain in the membrane (~12 kDa). The C-terminal 12 kDa domain is further cleaved by γ-secretase. γ-secretase cleaves at either valine 711 or alanine 713 which leads to the release of the 40 residue Aβ 40 or 42 residue Aβ peptide (Aβ 40 or Aβ 42). APP mutations are close to cleavage sites of α-, β-, and γ-secretases. These mutations change cleavage of the amyloid β precursor protein and increase production of the 42 residue Aβ peptide (Aβ 42). Figure shows locations of frequently seen mutations.

Six broad strategies have been put forward for the treatment of Alzheimer’s disease.

First, one could attempt to partially inhibit either of the two proteases, β- and γ-secretase, that generate Aβ from APP (Figure 4). In the case of the former enzyme, compound screening and medicinal chemistry are being vigorously pursued to identify potent small-molecule inhibitors that can fit the large active site of this aspartyl protease and still penetrate the blood-brain barrier. In the case of the latter, potent membrane-permeable inhibitors are already in hand. However, their testing in humans has barely been attempted because of the theoretical concern that most such compounds might interfere importantly with signaling by Notch proteins and other cell-surface receptors, which would probably lead to serious disorders.

Secondly, one could attempt to prevent the oligomerization of Aβ or enhance its clearance from the cortex. This approach is exemplified by the use of active or passive Aβ immunization, in which antibodies to Aβ decrease cerebral levels of the peptide by promoting microglial clearance and/or by redistributing the peptide from the brain to the systemic circulation. Active immunization with synthetic Aβ 1-42 peptide produces robust benefits in APP transgenic mice without detectable toxicity. However, when this approach was extended to AD patients, a small but unacceptable fraction of the study subjects developed a usually transient nervous-system inflammatory reaction that was fatal in a few cases, precluding further testing with this preparation. Those who were enrolled in the clinical trial surely had a significant plaque and tangle burden; it is possible that if this treatment were possible very early, even pre-symptomatically, this unacceptable adverse effect could be avoidable. An interesting example of worsening when toxic load is leached from the nervous system can be seen in the treatment of Wilson’s disease, in which copper accumulates in the nervous system as well as in the liver and other organs due to abnormalities of copper transport. When first treated, those with nervous-system involvement often worsen before they improve; the copper that was inactive in the nervous system is freed by the treatment and transiently increases before the new baseline of low copper is manifest as a result of chronic treatment. Analysis of a subset of Aβ-immunized patients suggested that the treatment may have slowed disease progression, although definitive conclusions on this await the report of the analysis of the whole trial. Several alternative preparations intended to provide Aβ antibodies by either active or passive routes have been formulated and one or more of these is likely to reach clinical testing before long.

The third broad approach to the treatment of AD is anti-inflammatory. This strategy is based on evidence that a cellular inflammatory response in the cortex is elicited by the progressive accumulation of Aβ. Additionally, it was recently shown that some anti-inflammatory drugs may have direct effects on the cleavage of APP by γ-secretase, independent of their inhibition of cyclo-oxygenase 2 and other inflammatory mediators. Some such drugs have been shown to reduce cytopathology in APP transgenic mice. Clinical trials of compounds based on these findings are currently under way (http://www.clinicaltrials.gov/ct/gui/show/NCT00463587?order = 20). It is worth noting that the recently reported trial of naproxen that failed to show benefit in AD is not directly interpretable because although naproxen is a potent cyclo-oxygenase inhibitor, it does not affect APP processing. Thus, this trial will have to be repeated with a different compound.

The fourth approach is based on modulating cholesterol homeostasis. Chronic use of cholesterol-lowering drugs has recently been associated with a lower incidence of AD. Concurrently, high-cholesterol diets have been shown to increase Aβ pathology in animals and cholestrol-lowering drugs have been shown to reduce pathology in APP transgenic mice. These effects seem to be caused by a direct (though poorly understood) effect of cholesterol on APP processing. A particular advantage of this approach is that statin drugs are generally well-tolerated and have already been widely prescribed. Again, clinical trials are under way.

The fifth approach is based on the surprising observation that Aβ aggregation partially dependent on Cu+2 and Zn+2 ions. This has led to the suggestion that chelation of these ions may have therapeutic potential. Clinical trials of such a chelating agent, clioquinol, which has been used successfully in the transgenic model, are currently under way.

Another approach apart from interfering with Aβ production and Aβ clearance is the use of stem cells for the treatment of neurodegenerative diseases. Brazelton et al. have shown that genetically marked adult mouse bone marrow cells develop into cells that express neuronal proteins after intravascular delivery.
into lethally irradiated normal adult mouse hosts. Confocal microscopy images of individual cells have shown that hundreds of marrow-derived cells in brain sections expressed gene products typical for neurons. Marked stem cells were shown to express cells of the cerebellum, hippocampus and cortical regions of the brain.65

Another study by Mezey et al. shows that transplanted bone marrow cells migrate into the brain of mice and differentiate into cells that express neuron-specific antigens capable of developing cells of the myeloid and lymphoid lineages.66

Discussion

Recently, more attention has been paid to the importance of people's lifestyle's role in the development of dementia and Alzheimer's disease (AD). Because dementia is among the most salient health concerns of older adults, knowing whether behavioral factors may modify risk is of great practical interest.

Genetic screening might be of particular importance for individuals with Alzheimer's disease risk such as people with a first degree relative with the disorder and for people with head injury. Carriers of alleles associated with Alzheimer's disease could make certain lifestyle changes to prevent the development of dementia.

Mental exercise provided by frequent engagement in intellectually demanding activity at work may facilitate the maintenance of inherent cognitive reserve, leading to more sophisticated cerebral networks in old age and allowing aging individuals to tolerate dementia neuropathology longer into the progression of the disease.67

In addition there has been an increasing ability to accurately identify those at a very early stage of disease, "mild cognitive impairment".68 This progress is a necessary corollary to therapeutic strategies aimed at slowing disease pathogenesis, since obviously the intention is to maintain people at high levels of function rather than maintaining them in an impaired state.

Conclusions

Clinical AD research has reached a pivotal point. There is a general, though not universal, belief that we now have a skeletal understanding of the disease pathogenesis. As a field, we have greatly improved our diagnosis of the disease and have learned how to assess its severity and rate of progress. Thus, we appear to have all the technologies and tools necessary to test whether a particular treatment is showing efficacy. As outlined above, we have at least six different approaches that target different aspects of this crucial central pathogenic pathway. Clinical trials are by nature slow and complex and side effects may be difficult to predict. However, it is hoped that one or more of these six approaches, either alone or in combination, will yield clinical benefit.

Such an outcome would validate the enormous amount of basic work done on the molecular genetics and molecular biology of the disease. This in turn would further focus drug-discovery efforts so that further rapid clinical advances would be expected. Such translational research is necessarily iterative, as improvements in diagnosis and understanding of pathogenesis help to refine each other. In a general sense, successful treatments for AD would also validate the more general concept of pathogenic-knowledge-based therapeutics. The current "third golden age" would have clinical relevance analogous to that conferred on basic discoveries of neurotransmitters by the success of L-dopa therapy.69

References


