

Reviews

Genetic risk factors in Alzheimer's disease

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Abstract

Following a brief introduction and discussion of the pathological features of Alzheimer's disease, the main emphasis of this review article will be the genetic factors that have been implicated in this disease. These can be divided into two main categories. First, the three genes in which mutations are known to result in early onset autosomal dominant familial Alzheimer's disease will be discussed. These are well characterised but account for only a small proportion of Alzheimer's disease cases. Late onset, sporadic Alzheimer's disease is more common and evidence suggests that there is a genetic component to this type of disease. A number of genetic risk factors have been implicated that might increase the risk of developing sporadic disease. Many of these are controversial and studies have shown conflicting results, which are discussed in this section. Finally, a brief discussion of some of the mechanisms suggested to play a role in the pathogenesis of Alzheimer's disease is included. It is hoped that this will show why particular genes have been implicated in Alzheimer's disease and how they might be able to influence the development of the disease.

(*J Clin Pathol: Mol Pathol* 1998;51:293-304)

Keywords: Alzheimer's disease; susceptibility genes; genetic risk factors

Alzheimer's disease is a progressive neurodegenerative disorder, first described by Alois Alzheimer.¹ It is the leading cause of dementia in the elderly, accounting for around 50% of all dementias. The remaining 50% are caused by ~ 70 different disorders, but comprise mainly Lewy body dementia (20-30%) and multi-infarct dementia (10-20%), with the final 10% being the atypical dementias such as Pick's disease, Huntington's disease, and Creutzfeldt-Jacob's Disease (CJD). Estimates of the prevalence of Alzheimer's disease are variable, probably because of uncertainty in diagnosis, but increase from 0.3% in the 60-69 year age group, to more than 10% in those over 80 years of age.² It is estimated that there are more than 600 000 people in the UK suffering from the disease.

Alzheimer's disease is characterised by progressive dementia beginning in middle to late life, with death occurring an average of 8-10 years after diagnosis.³ The clinical manifestations of the disease are extremely variable, with symptoms including memory loss, confusion, personality changes, impaired coordination, and speech problems.

Alzheimer's disease can be categorised according to its age of onset or mode of inheritance. The early onset disease is defined as having an age of onset before 65 years of age, and these cases are usually familial, being inherited in an autosomal dominant fashion. This type of Alzheimer's disease, known as familial Alzheimer's disease is rare, accounting for ~ 10% of all Alzheimer's disease cases. Most Alzheimer's disease cases are of the late onset type, occurring in individuals over 65 years of age, and are sporadic, with no strong family history.

Pathological features of Alzheimer's disease

Pathologically, the Alzheimer's disease brain is characterised by two types of lesion: senile or neuritic plaques and neurofibrillary tangles. It has been shown that intellectual decline correlates with the densities of neurofibrillary tangles and senile plaques in the diseased brain.⁴ It is believed that the formation of these lesions is likely to result in the destruction of cell bodies, dendrites, and synapses, thus leading to severe impairment of neurotransmission and resulting in a decline in cognitive abilities.

SENILE PLAQUES

Senile plaques consist of a spherical extracellular core of proteinaceous fibrillar deposits, or amyloid, surrounded by degenerating nerve cell processes (fig 1). They are found particularly in the hippocampus, neocortex, and amygdala, and in the walls of cerebral and meningeal blood vessels. The predominant protein in the senile plaque core is a 4 kDa peptide,⁵ known as A β , β -amyloid, A4, or β /A4. A β contains between 39 and 43 amino acids, differing at the C-terminus, with the 42 amino acid form being the most predominant form in amyloid deposits.⁶ A β is derived from a larger precursor protein known as the amyloid precursor protein (β APP) via proteolytic cleavage.^{7 8}

In addition to A β , a number of other senile plaque components have been identified. The

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Accepted for publication
1 September 1998

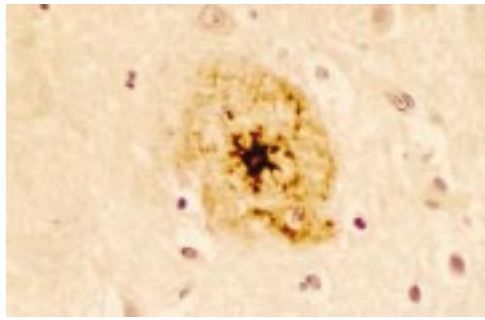


Figure 1 Typical Alzheimer's disease senile plaque immunostained for A β . The heavily stained dense core, composed of aggregated amyloid fibrils is seen clearly.

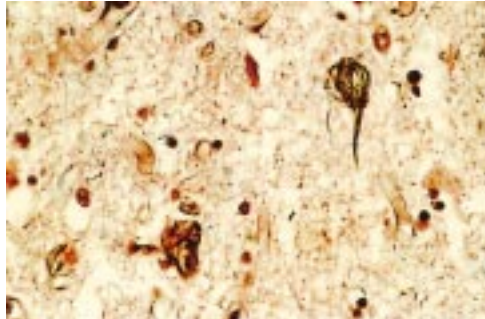


Figure 2 Alzheimer's disease brain tissue showing neurofibrillary tangles stained by Garvey's method. The darkly stained areas that are clearly visible are the hyperphosphorylated tau backbones of the neurofibrillary tangles.

serineproteinaseinhibitor (serpin) α_1 antichymotrypsin has been shown to bind with high affinity to A β and is found in the core of senile plaques.⁹⁻¹¹ Immunohistochemical studies have also detected apolipoprotein E (apoE), associated with both senile plaque amyloid and neurofibrillary tangles in the Alzheimer's disease brain.¹²

NEUROFIBRILLARY TANGLES

Neurofibrillary tangles are abnormal proteinaceous deposits, which form as intraneuronal inclusions but become externalised as their neuronal source degenerates (fig 2). They occur in cell bodies and apical dendrites of degenerating neurons surrounding the core of senile plaques. Neurofibrillary tangles are a common feature of dystrophic neurons and are not specific to Alzheimer's disease, also being characteristic of other neurodegenerative diseases, such as Parkinson's disease. Their main structural component is an abnormal fibre known as a paired helical filament. It is likely that the sole component of these paired helical filaments is a hyperphosphorylated form of the microtubule associated protein tau.¹³

Hyperphosphorylated tau in neurofibrillary tangles is also known to be ubiquitinated,¹⁴ suggesting a role for the ubiquitin dependant proteolysis pathway in Alzheimer's disease.

Genetics of Alzheimer's disease

In a number of families Alzheimer's disease is inherited as an autosomal dominant disorder, although in most cases inheritance appears to be multifactorial. Monozygotic twin studies indicate a variable concordance of between 18%¹⁵ and 41%,¹⁶ showing that Alzheimer's disease cannot be explained completely by a single autosomal dominant gene. A relative risk of 3.5 has been demonstrated for those with at least one first degree relative suffering from dementia.¹⁷

Autosomal dominant gene mutations

Three genes have been identified to date in which mutations result in early onset familial Alzheimer's disease, inherited in an autosomal dominant fashion. These are the amyloid precursor protein (APP), presenilin 1 (PS-1), and PS-2 genes (table 1).

THE APP GENE

The APP gene maps to chromosome 21q21.1,⁸ and mutations in this gene are estimated to account for up to 5% of familial Alzheimer's disease cases. A number of mutations have been identified, at codons 717,¹⁸⁻²⁰ 670/671,²¹ 692,²² and 693.²³ Mutations in the APP gene lead to early onset disease with age of onset typically between 43 and 62 years.²⁴

Mutations in the APP gene might result in altered metabolism of APP, leading to increased production of the A β protein, or an increased production of the 42 amino acid form of A β (A β ₁₋₄₂). This is the predominant form in senile plaques, and is thought to be more amyloidogenic, forming amyloid fibrils more rapidly than the shorter forms of A β .⁶

THE PS-1 GENE

A second familial Alzheimer's disease locus was identified by genetic linkage analysis at chromosome 14q24.²⁵⁻²⁷ The gene responsible was subsequently identified as S182 or PS-1, and mutations in this gene are thought to cause up to 80% of familial Alzheimer's disease cases, with onset as early as 29 years of age,²⁸ ranging to 62 years of age.²⁴ To date, more than 35 missense mutations, and one in frame deletion²⁹ have been identified in the PS-1 gene in affected families.

The fact that most PS mutations are missense ones suggests that Alzheimer's disease pathology is a result, not of the absence of the PS-1 gene product, but of its abnormal functioning as a result of the mutation. The function of the PS-1 protein is unknown, but it is known to be a transmembrane protein and, therefore, might function as a cell surface receptor, ion channel, or membrane structural protein.³⁰ The PS-1 protein is homologous to SEL-12 in *Caenorhabditis elegans*,³¹ a protein known to be involved in cell signalling during development. The PS-1 gene product is known to be essential during development because

Table 1 Genes involved in early onset, autosomal dominant familial Alzheimer's disease (FAD)

Chromosomal location	Gene	Estimated FAD cases	Age of onset (years)	No. of mutations	Possible mechanism
21q21.1	APP	Up to 5%	43-62	6	Altered APP processing Increase in A β /A β ₁₋₄₂ Increase in A β ₁₋₄₂
14q24	PS-1	Up to 80%	29-62	35+	Altered PS-1 metabolism Increase in A β ₁₋₄₂
1q31-42	PS-2	~20%	40-88	2	

APP, amyloid precursor protein; PS-1, presenilin 1.

Table 2 Susceptibility genes in which polymorphisms have been reported to influence Alzheimer's disease risk

Gene	Chromosomal location	Risk factor	Onset	Familial/sporadic	Other information
APOE	19q13.2	$\epsilon 4$ allele	LOAD and EOAD	Familial and sporadic	
APOE	19q13.2	-491 A allele	LOAD	Sporadic	APOE $\epsilon 4$ independent
APOE	19q13.2	TATA box T allele	LOAD	Sporadic	Modifies APOE $\epsilon 4$ effect
VLDL-R	9	Trinucleotide five repeat allele	LOAD	Sporadic	In APOE $\epsilon 4$ carriers only
LRP	12	Exon 3 CC genotype	LOAD	Familial and sporadic	
LRP	12	TTTC repeat polymorphism	LOAD	Sporadic	
A2M	12p	A2M-2 allele	LOAD	Familial	No interaction with APOE
PS-1	14q24	Intron 8, allele 1	LOAD	Sporadic	
BChE	3q26.1-2	K allele	LOAD (over 75 years)	Sporadic	In APOE $\epsilon 4$ carriers only
ACT	14q32.1	Signal sequence A allele	LOAD	Familial and sporadic	In association with APOE $\epsilon 4$
ACT	14q32.1	Microsatellite A10 allele	LOAD	Sporadic	In association with APOE $\epsilon 4$

EOAD, early onset Alzheimer's disease; LOAD, late onset Alzheimer's disease; ACT, α_1 antichymotrypsin; A2M, α_2 macroglobulin; APOE, apolipoprotein E; BChE, butyrylcholinesterase; LRP, low density lipoprotein receptor related protein; PS-1, presenilin 1; VLDL-R, very low density lipoprotein receptor.

PS-1 gene knockout mice exhibit skeletal deformations, impaired neurogenesis, and neuronal cell death, leading to death shortly after birth.^{32 33}

THE PS-2 GENE

Although mutations in the PS-1 gene account for most cases of familial Alzheimer's disease, there are some affected families in which both APP and PS-1 mutations have been excluded. A third locus was identified at chromosome 1q31-42,³⁴ and the mutated gene was identified subsequently as STM-2 or PS-2.³⁵ Only two mutations have been identified in the PS-2 gene to date, leading to Alzheimer's disease with an onset between 40 and 88 years of age, typically later than that seen in PS-1 linked cases.²⁴

The PS-1 and PS-2 proteins were found to be highly homologous, sharing 67% identity. The PS-2 protein is proposed to have a similar function to the PS-1 protein, although they are unable to compensate for each other. It has been demonstrated that mutations in both the PS-1 and PS-2 gene cause over production of the amyloidogenic $A\beta_{1-42}$ form in both transfected cells and transgenic mice.³⁶⁻³⁸ Presenilin mutations cause dominant gain of function, with mutant genes leading to an increase in $A\beta_{1-42}$ production, even in the presence of wild-type alleles. It has also been reported that PS-1 mutations lead to altered protein metabolism, resulting in accumulation of N-terminal and C-terminal PS-1 protein fragments in brains of transgenic mice.³⁹ However, it is not clear how this accumulation of fragments contributes to neurodegeneration.

Susceptibility genes

Only a small proportion (~10%) of Alzheimer's disease cases are caused by mutations inherited in an autosomal dominant fashion. Most cases are late onset and apparently sporadic, probably as a result of a combination of environmental and non-dominant genetic factors. A number of genetic risk factors have been implicated in Alzheimer's disease, which are not sufficient to cause the disease, but may

greatly increase the risk above that of the general population; these are susceptibility genes (table 2).

APOLIPOPROTEIN E (APOE) GENE

The gene for apolipoprotein E (apoE) is located in a cluster of apolipoprotein genes at chromosome 19q13.2. This region was first implicated in late onset Alzheimer's disease by linkage studies.⁴⁰⁻⁴² apoE is a 34 kDa glycoprotein encoded by a polymorphic gene resulting in three common alleles, named $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$. The APOE $\epsilon 4$ allele is a well established risk factor for late onset Alzheimer's disease, with a significantly higher frequency observed in Alzheimer's disease cases than in controls.⁴³⁻⁴⁵ This association has been confirmed for both familial and sporadic late onset Alzheimer's disease,^{44 45} and has also been reported in the early onset disease.^{46 47}

The APOE $\epsilon 4$ risk has been shown to be dose dependant, the risk increasing with the number of $\epsilon 4$ alleles. One study has reported an odds ratio of 2.8 for individuals with one $\epsilon 4$ allele, increasing to 8.1 for individuals homozygous for the $\epsilon 4$ allele, compared with individuals with no $\epsilon 4$ alleles.⁴³ It has also been shown that each $\epsilon 4$ allele lowers the age of onset by seven to nine years in late onset familial Alzheimer's disease.^{43 48 49}

A gender difference has been suggested for APOE associated risks^{50 51} but other studies have failed to confirm this difference.^{52 53} However, the former studies^{50 51} only considered data from families, so the proposed sex specific risk might only apply in familial Alzheimer's disease.

In contrast to the increased risk conferred by possession of the $\epsilon 4$ allele, a protective effect has been proposed for the $\epsilon 2$ allele. It has been observed in several studies that individuals possessing the $\epsilon 2$ allele have a lower incidence of Alzheimer's disease.^{53 54} However, other studies have failed to find this association,⁵⁵ and some studies have conversely suggested that the $\epsilon 2$ allele acts as a risk factor in the early onset disease.^{56 57}

Recently, two polymorphisms within the APOE gene regulatory regions have been reported to play a role in Alzheimer's disease. A G/T biallelic polymorphism in the APOE gene TATA box may modulate the influence of the APOE ϵ 4 allele on the risk of disease,⁵⁸ whereas an A/T polymorphism at position -491 was associated with Alzheimer's disease risk independently of APOE ϵ 4 status.⁵⁹ Homozygosity for -491A was found to confer an increased risk of disease, possibly by altering the level of apoE protein production. The A allele was associated with higher constitutive levels of APOE promoter activity, as a result of differences in affinity for nuclear proteins between the two alleles. However, a subsequent study has failed to reproduce the association between the -491 regulatory region polymorphism and Alzheimer's disease,⁶⁰ although results suggest that the frequency of the -491 A/T polymorphism may vary considerably between different ethnic groups.

Although the APOE ϵ 4 allele is a well documented risk factor for Alzheimer's disease, it is neither necessary nor sufficient for development of the disease. This implicates the involvement of other factors, either genetic or environmental, acting either independently or in association with the APOE ϵ 4 allele. One study has reported that the presence of herpes simplex virus type I (HSV-I) DNA in the brain, together with possession of the APOE ϵ 4 allele, is a strong risk factor for Alzheimer's disease.⁶¹ In addition, various genetic factors have been reported to modify the risk associated with APOE ϵ 4 (see below) but none, as yet, have been as convincing, or reproducible in different study groups, as the APOE effect.

VERY LOW DENSITY LIPOPROTEIN RECEPTOR (VLDL-R) GENE

Receptors for apoE containing lipoproteins such as the VLDL-R have been proposed to act as risk factors for Alzheimer's disease, by influencing the metabolism of apoE in the brain. The VLDL-R gene is located on chromosome 9,⁶² and contains a polymorphic trinucleotide tandem repeat (CGG)_n in the 5' untranslated region,⁶²⁻⁶³ with alleles ranging from four to nine repeats. It has been observed in a Japanese population that homozygosity for the five repeat allele, in association with possession of the APOE ϵ 4 allele, confers an increased risk of developing Alzheimer's disease above that associated with possession of the APOE ϵ 4 allele alone.⁶⁴ However, subsequent studies in white populations⁶⁵⁻⁶⁹ have failed to find any association, although there are marked differences in allele frequencies between the Japanese and white populations.⁷⁰ Thus, it is possible that the VLDL-R five repeat allele acts as an Alzheimer's disease risk factor in Japanese populations only, although a further study in a Japanese population has also failed to find any association.⁷¹ It seems unlikely that the VLDL-R polymorphism plays a role in the late onset disease in white populations, but further studies are required to confirm its importance in Japanese populations.

In addition to VLDL-R, other lipoprotein receptors have also been investigated as candidate risk factors for late onset Alzheimer's disease. Studies of the low density lipoprotein receptor (LDL-R) gene have shown no association with Alzheimer's disease.⁶⁸ The LDL-R related protein (LRP) gene, located on chromosome 12, has also been suggested as a candidate risk factor in the late onset disease. The LRP protein is the main apoE receptor in the brain, and is also responsible for endocytosis of secreted APP, suggesting a possible role in the pathogenesis of Alzheimer's disease. Studies involving a TTTC repeat polymorphism in the 5' end of the LRP gene have shown conflicting results.⁶⁸⁻⁷²⁻⁷⁴ Studies using an alternative marker, a silent C/T polymorphism in exon 3 of the LRP gene, suggest that the CC genotype is over represented in patients with Alzheimer's disease compared with controls, in both familial⁷⁵ and sporadic⁷⁶ late onset disease. Linkage data implicating a locus on chromosome 12 in late onset Alzheimer's disease⁷⁷ also suggest that the LRP locus might be important.

α_2 MACROGLOBULIN GENE (A2M)

α_2 Macroglobulin is a serum pan-protease inhibitor that has been implicated in Alzheimer's disease because of its presence in senile plaques⁷⁸ and its ability to bind A β ,⁷⁹⁻⁸⁰ attenuating both fibril aggregation and neurotoxicity.⁸⁰⁻⁸¹ Along with apoE and β APP,⁷⁵⁻⁸² α_2 macroglobulin is a major ligand for the LRP receptor, which has also been implicated in Alzheimer's disease.

A splice acceptor deletion in exon 18 of the α_2 macroglobulin gene, known as A2M-2,⁸³ has been reported recently to be associated with an increased risk of Alzheimer's disease.⁸⁴ Using a sibling study approach, the frequency of the A2M-2 allele was found to be higher in probands than in unaffected individuals. The odds ratio associated with possession of at least one copy of the A2M-2 allele was ~ 3.6, similar in magnitude to that associated with homozygosity for the APOE ϵ 4 allele in this study.

These findings are interesting in the light of reports implicating a locus on chromosome 12 associated with Alzheimer's disease.⁷⁷ A2M maps to chromosome 12p, within 30 cM of the implicated chromosome 12 markers.⁸⁴ Thus, further study of the A2M gene is justified, and as the odds ratio associated with the A2M-2 allele is similar in magnitude, or larger, than that associated with the APOE ϵ 4 allele, this gene could prove to be an important risk factor in Alzheimer's disease.

THE PS-1 GENE

Although the PS-1 gene is generally thought to be involved only in early onset familial Alzheimer's disease, allele sharing between affected family members with late onset disease has been observed,⁸⁵ suggesting a further role for PS-1 in late onset Alzheimer's disease. It has been reported that a biallelic polymorphism within intron 8 of the PS-1 gene acts as a risk factor for late onset disease, with homozygosity for allele 1 being associated with an approximate doubling of risk when

compared with individuals with either one or no copies of allele 1.⁸⁶ This association has been confirmed in two subsequent studies,^{87, 88} but others have failed to find an association.⁸⁹⁻⁹²

BUTYRYLCHOLINESTERASE (BChE) GENE

The BChE gene is located at chromosome 3q26.1-26.2. BChE is expressed in most human tissues but its function is unknown. BChE activity in the brain increases in an age dependant fashion after 60 years of age, and is raised in Alzheimer's disease.⁹³ BChE has been detected histochemically in both senile plaques and neurofibrillary tangles in brains affected by Alzheimer's disease.⁹⁴⁻⁹⁶

A variant of the BChE gene known as the BChE-K variant has a point mutation at nucleotide 1615 (GCA→ACA), resulting in a change of alanine 539 to threonine. This mutation reduces the catalytic activity of BChE by one third.⁹⁷ Recently, it has been reported that the BChE-K variant is more common in patients with late onset Alzheimer's disease than in controls, other dementias, and patients with early onset Alzheimer's disease.⁹⁸ The BChE-K variant was associated with a twofold increased risk of developing Alzheimer's disease, with the strongest effect observed in the over 75 age group. When APOE ε4 carrier status was also taken into account, possession of both APOE ε4 and BChE-K alleles was found to be associated with a 36 times greater risk when compared with individuals with neither allele. However, two subsequent studies have failed to confirm this finding.^{99, 100}

α₁ ANTICHYMOTRYPSIN (ACT) GENE

The presence of ACT in the cores of senile plaques, the high expression of ACT mRNA in brains affected by Alzheimer's disease,⁹⁻¹¹ and the ability of ACT to promote aggregation of Aβ fibrils,¹⁰¹ suggest an important role for ACT in the pathogenesis of Alzheimer's disease. The ACT gene maps to chromosome 14q32.1, and contains a common biallelic polymorphism in the signal sequence, resulting in either an alanine (A) or a threonine (T) at position -17 of the ACT protein. It has been reported that the A allele of this signal sequence polymorphism is associated with an increased risk of Alzheimer's disease in APOE ε4 carriers only.¹⁰²⁻¹⁰⁴ It has also been reported that the ACT/AA genotype is associated with an increased risk of Parkinson's disease,¹⁰⁵ a disease with similar pathology to Alzheimer's disease. However, many studies in Alzheimer's disease populations have failed to confirm the role of the ACT signal sequence A allele as a late onset disease risk factor,^{93, 106-113} although Talbot and colleagues¹⁰⁹ did observe that the combination of the APOE ε4 allele and the ACT/AA genotype may result in a lower age of onset.

The A10 allele of a polymorphic dinucleotide TC/TA repeat located in the 5' flanking region of the ACT gene has also been reported to act as a risk factor for late onset Alzheimer's disease, in association with APOE ε4.¹¹³ Strong linkage disequilibrium was observed between the A10 microsatellite allele and the ACT sig-

nal sequence T allele in both the control and Alzheimer's disease groups. These findings are therefore contradictory to the reports of an association between the ACT A allele and Alzheimer's disease.

MITOCHONDRIAL DNA MUTATIONS

Some evidence suggests that Alzheimer's disease can show a pattern of maternal inheritance, whereby the offspring of mothers with Alzheimer's disease have an increased risk of Alzheimer's disease compared with offspring of affected fathers.¹¹⁴ Because mitochondrial DNA is maternally inherited, this implicates the involvement of mitochondrial DNA mutations in Alzheimer's disease. However, studies of various mitochondrial DNA mutations that have been reported to influence the risk of Alzheimer's disease have yielded inconsistent results.

Mutations in the cytochrome C oxidase genes COI and COII¹¹⁵ and in the ND2 gene, encoding subunit 2 of respiratory chain complex I¹¹⁶ have been reported to cause an increased risk of Alzheimer's disease. However, several subsequent studies have failed to confirm these findings.¹¹⁷⁻¹¹⁹ A variant of a mitochondrial tRNA gene has also been reported to be associated with the late onset disease, again with conflicting results.^{117, 119-122} It is possible that any observed mitochondrial DNA alterations in patients with Alzheimer's disease occur as a result of the neurodegenerative process, rather than being heritable risk factors.

OTHER LOCI

A complete genomic screen in familial late onset Alzheimer's disease has identified four regions of interest in the genome, on chromosomes 4, 6, 12, and 20, with a region near the centromere of chromosome 12 showing the strongest linkage.⁷⁷ In this study, the effect of the chromosome 12 locus appears to be greatest in families in which the APOE ε4 allele has little influence on the risk of Alzheimer's disease. Although the LRP gene, a candidate Alzheimer's disease risk factor, is located on chromosome 12, the Pericak-Vance study⁷⁷ shows no significant association with this gene in either familial or sporadic disease groups. One study has also reported a potential locus on the X chromosome.¹²³

THE VALUE OF ASSOCIATION STUDIES

It can be seen from the previous section on risk factors in Alzheimer's disease that the results obtained from association studies have provided conflicting data in a number of instances. Of the more than 70 000 genes believed to be expressed in the brain it would be remarkably fortuitous if the ones currently known (just over 2000) included all those associated with the disease state. It is far more likely that other genetic risk factors for Alzheimer's disease remain to be identified. However, the potential success of the association study approach is illustrated by the data obtained from APOE ε4 studies, which are robust and readily reproduced by many workers in the field.

A number of other factors could be contributing to the discrepancies noted in the literature. It is essential that the study groups used are closely matched for age, sex, and ethnic background because a number of alleles have been shown to vary with each of these parameters. There is also a problem with the accuracy of the diagnosis of Alzheimer's disease; although a clinical diagnosis of dementia is relatively straightforward, attributing a diagnosis of Alzheimer's disease is more problematical. A number of other pathologies (for example, Lewy body dementia, tangle only dementia, Pick's disease) present in a similar way and cannot be excluded easily on clinical grounds. Some would argue that all patients (both study and control groups) need to have their diagnoses confirmed histopathologically before inclusion in any study. It is quite possible that the genetics of these neuropathological diseases differ and inclusion of different phenotypes within a study group will obviously lead to a potential diminution of any association. Also, to increase the power of detection of any potential associations and resolve conflicting results between studies, study group sizes need to be increased substantially. Power calculations indicate that sample numbers should be in the thousands, rather than the hundreds, to detect potentially weak associations.

Another problem that needs to be addressed is how to accommodate the strong APOE $\epsilon 4$ effect, which is apparent in all studies of late onset disease. Better mathematical models for complex multigenic disorders are required so that the APOE $\epsilon 4$ effect can be removed and the effect of other loci established. This becomes even more important when the individual effect of a particular allele is small but in combination with other risk factors may become more potent. Synergy analysis¹²⁴ attempts to address this issue and this type of approach might be useful in future association studies. Another approach would be to study only APOE $\epsilon 4$ negative patients and controls, thereby eliminating the APOE $\epsilon 4$ effect completely.

The late onset nature of Alzheimer's disease causes obvious difficulties with study design, making pedigree analysis almost impossible. Sibling studies, which are usually very informative, would be of less value in Alzheimer's disease, because knowledge of parental genotypes is desirable to draw any conclusions regarding a particular locus. Although association studies are prone to a number of very real problems, it seems that they are the best approach that can be applied to Alzheimer's disease at this time, and can yield valuable information, as demonstrated by APOE studies.

Pathogenesis of Alzheimer's disease

CHOLINERGIC HYPOTHESIS

It has been observed that there is a cholinergic deficit in Alzheimer's disease brains, with reduced concentrations of choline acetyltransferase and acetylcholinesterase.¹²⁵⁻¹²⁷ The extent of this deficiency seems to correlate with the density of lesions, with greatest reductions

in choline acetyltransferase and acetylcholinesterase activities in areas of the brain with high numbers of senile plaques and neurofibrillary tangles.¹²⁸ The cholinergic deficit also appears to correlate with the extent of intellectual decline in patients with Alzheimer's disease.

However, Alzheimer's disease cannot be regarded as primarily a disorder of the cholinergic system, because patients have been reported with the classic neuropathological features of Alzheimer's disease, but without a reduction in choline acetyltransferase activity.¹²⁹ In addition, reduction in choline acetyltransferase activity of a similar magnitude to that seen typically in Alzheimer's disease has been reported in other neurodegenerative conditions, without associated severe cognitive decline.¹³⁰ The cholinergic deficit probably only explains part of the observed cognitive decline in patients with Alzheimer's disease. Other neurotransmitter changes also occur, including reduction of noradrenaline and serotonin in the cerebral cortex.

AMYLOID CASCADE HYPOTHESIS

This hypothesis states that the deposition of A β protein is the primary event in the pathogenesis of Alzheimer's disease, and that neurofibrillary tangles, neuronal death, and dementia occur subsequently as a result of this event.¹³¹ The cascade hypothesis assumes that A β and β APP cleavage products containing A β are neurotoxic. Two successive events are required to cause Alzheimer's disease; first intact A β must be produced, and second this must facilitate or cause cell death and neurofibrillary tangle formation.

A β has been reported to have both neurotrophic^{132 133} and neurotoxic^{134 135} effects *in vitro*. The toxicity seems to depend on the fibrillar state of A β ,¹³⁶ and can be attenuated by both apoE¹³⁷ and ACT.¹³⁸ Aggregated A β may either kill neurons directly¹³⁵ or increase the vulnerability of neurons to other insults such as excitotoxicity, hypoglycaemia, and peroxidative damage.¹³⁹ It has also been suggested that A β disrupts calcium homeostasis, resulting in increased concentrations of intraneuronal calcium. It has been shown that tau phosphorylation is regulated by intracellular calcium concentrations,¹⁴⁰ therefore suggesting a mechanism by which A β causes neurofibrillary tangle formation.

The amyloid cascade hypothesis is supported by the presence of familial Alzheimer's disease mutations in β APP that apparently alter β APP processing and favour amyloid plaque formation. The fact that individuals with Down's syndrome (trisomy 21) develop Alzheimer's disease-like pathology early in life also shows that overexpression of β APP can cause Alzheimer's disease pathology, supporting a central role for A β deposition in Alzheimer's disease.

It has been suggested that the presenilin familial Alzheimer's disease mutations also cause disease by altering the processing of β APP in a similar way to mutations in the β APP gene itself. It has been demonstrated

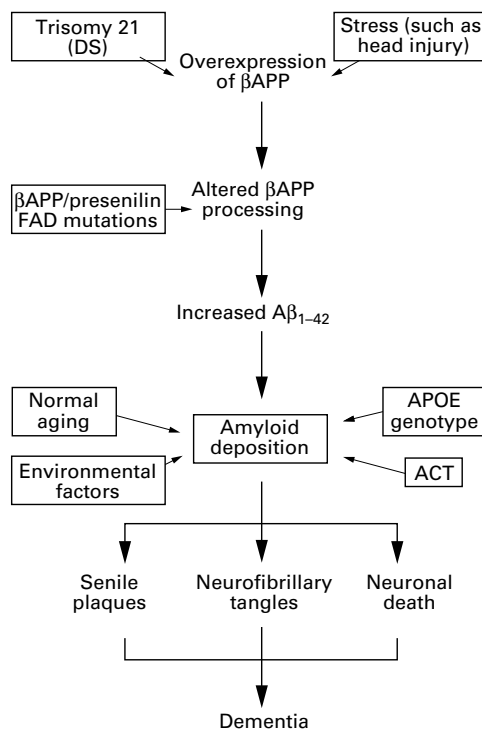


Figure 3 The amyloid cascade hypothesis: genetic and environmental factors act in combination to increase amyloid deposition, resulting in formation of senile plaques, neurofibrillary tangles and neuronal death. Ultimately, this leads to the symptoms of dementia. FAD, familial Alzheimer's disease.

that both PS-1 and PS-2 familial Alzheimer's disease mutations cause an increase in the concentration of $A\beta_{1-42}$, therefore leading to increased amyloid deposition.³⁶⁻³⁸ Other genetic and environmental factors might act on the cascade at this point by stimulating aggregation of $A\beta$ —for example, both apoE and ACT¹⁰¹ can enhance aggregation of $A\beta$ in vitro. The potential effects of both genetic and environmental factors on the amyloid cascade are depicted in fig 3.

HYPERPHOSPHORYLATION OF TAU AND NEUROFIBRILLARY TANGLE FORMATION

The main argument against the amyloid cascade hypothesis is that neurofibrillary tangle formation has been shown to precede $A\beta$ deposition,¹⁴¹ and densities of neurofibrillary tangles, rather than senile plaques, correlate closely with the extent of intellectual decline in patients with Alzheimer's disease. The presence of neurofibrillary tangles in the absence of amyloid deposits (for example in Parkinson's disease) demonstrates that amyloid is not an absolute prerequisite for neurofibrillary tangle formation.

Neurofibrillary tangles are composed mainly of a hyperphosphorylated form of the tau protein. Tau is a microtubule associated protein present in both the central and peripheral nervous systems. In the brain it is found predominantly in the axons of nerve cells, and its normal function is to promote the assembly and stability of microtubules by binding to tubulin. Native tau is a phosphoprotein, and the affinity of tau for microtubules appears to

be regulated by the level of phosphorylation.¹⁴² In brains of patients with Alzheimer's disease, tau becomes hyperphosphorylated and its ability to bind microtubules is greatly reduced,¹⁴² thus leading to destabilisation of the microtubule system. This could lead to diminished axoplasmic flow and, therefore, a reduced supply of substrates to the axons and dendrites, resulting in neuronal death.¹⁴³ Hyperphosphorylated tau, being unable to bind microtubules, is therefore available to assemble into paired helical filaments.

APOE genotype has been reported to influence tau hyperphosphorylation, with the apoE $\epsilon 3$ protein, but not $\epsilon 4$, being capable of binding to tau and preventing hyperphosphorylation.¹⁴⁴ This suggests a possible mechanism for the increased risk associated with possession of the APOE $\epsilon 4$ allele.

ACUTE PHASE RESPONSE

The presence of ACT, an acute phase protein, in amyloid plaques⁹⁻¹¹ and its raised concentrations in the cerebrospinal fluid of patients with Alzheimer's disease¹⁴⁵ suggest that the acute phase response may be important in Alzheimer's disease. Tumour necrosis factor (TNF), another acute phase protein, has also been reported to be raised in the circulation of patients with Alzheimer's disease.¹⁴⁶ Furthermore, other acute phase proteins have been detected immunohistochemically in association with senile plaques in the brains of patients with Alzheimer's disease including complement proteins,¹⁴⁷ interleukin 6 (IL-6), and α_2 macroglobulin.⁷⁹ Recent evidence suggests that α_2 macroglobulin might be genetically associated with Alzheimer's disease.⁸⁴

ACT is upregulated in response to inflammation, stimulated by factors such as IL-1, IL-6, TNF, transforming growth factor $\beta 1$ (TGF- $\beta 1$),¹⁴⁸ and oncostatin M.¹⁴⁹ IL-1 is also able to induce expression of β APP in cell culture,¹⁵⁰ and is known to be increased in the brains of patients with Alzheimer's disease.¹⁵¹

It can be hypothesised that the acute phase response is triggered in the Alzheimer's disease brain in response to cell stress (such as injury, age related neuronal damage, and viral infection) resulting in increased concentrations of β APP and ACT. This increased production of β APP might influence metabolism, causing more to be processed to produce amyloidogenic $A\beta_{1-42}$. The increased concentrations of ACT might stimulate aggregation of $A\beta$ fibrils to form amyloid plaques, and this aggregated $A\beta$ could then act as a further cause of cell stress, resulting in stimulation of the acute phase response in a positive feedback loop (fig 4).

OXIDATIVE STRESS

The identification of oxidation markers, including antioxidant enzymes¹⁵² and heat shock proteins,¹⁵³ in the neuropathological lesions of the disease suggests that oxidative stress is important in the pathogenesis of Alzheimer's disease. Evidence also suggests that the neurotoxicity of $A\beta$ might be mediated by oxygen

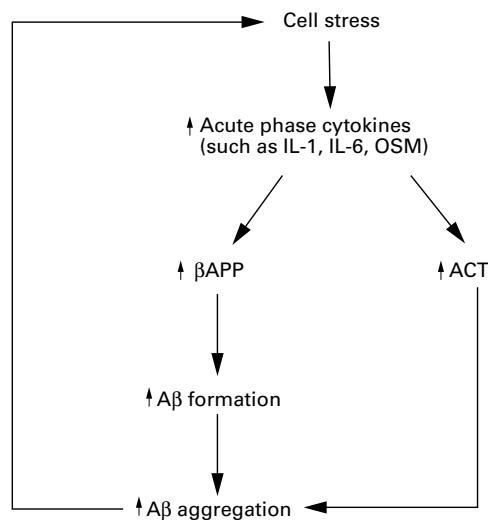


Figure 4 The possible role of the acute phase response in amyloid formation. Cell stress results in production of acute phase cytokines, which upregulate expression of β APP and ACT. This results in increased A β formation and aggregation, leading to further cell stress and activation of the acute phase response in a positive feedback loop. OSM, oncostatin M.

free radicals.¹⁵⁴ However, there is controversy over whether oxidative stress is a causative factor in Alzheimer's disease or a consequence of A β deposition. Studies in cell culture suggest that oxidative stress can shift APP processing in favour of increased A β production,¹⁵⁵ supporting the "stress first" hypothesis.¹⁵⁶ It has also been demonstrated that PS mutations are able to promote oxidative stress in neurons.^{157 158}

OXIDATIVE PHOSPHORYLATION DEFECTS

Oxidative phosphorylation (OXPHOS) generates mitochondrial ATP and is the main source of energy for a number of organs and tissues including brain, muscle, heart, kidney, and liver. The OXPHOS system is known to decline with age,¹⁵⁹⁻¹⁶¹ possibly as a result of accumulation of damage to mitochondrial DNA caused by oxygen free radicals, a byproduct of OXPHOS. Evidence suggests that OXPHOS defects may play a role in the pathogenesis of Alzheimer's disease. It has been shown that patients with Alzheimer's disease have OXPHOS defects in a number of tissues.^{162 163} Mitochondrial DNA mutations have been reported to influence the risk of Alzheimer's disease, and could lead to an increased rate of age related decline in the OXPHOS system.

APOPTOSIS

Apoptosis is a form of cell death characterised by condensation of nuclear chromatin, cytoplasmic condensation, DNA fragmentation, and maintenance of membrane integrity. Extensive loss of neurons is characteristic of certain areas of the Alzheimer's disease brain, but the mechanism of this neuronal cell death is unknown. Apoptosis has been implicated in Alzheimer's disease because of the ability of A β to induce apoptosis in cultured neurons.^{164 165} Familial Alzheimer's disease mutant APP has also been demonstrated to induce apoptosis in

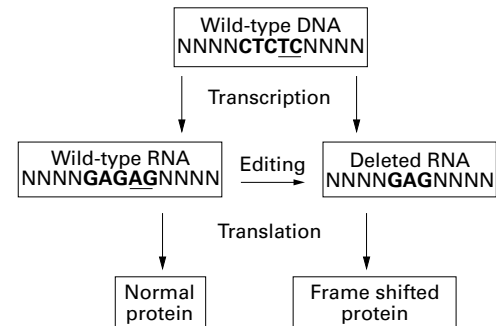


Figure 5 Mechanism by which wild-type DNA can give rise to mutant protein as a result of frameshift mutations in RNA. Such mutations have been detected in β APP and Ubi-B in Alzheimer's disease.¹⁷⁵

vitro,¹⁶⁶ and mutations in both PS-1 and PS-2 sensitise cells to pro-apoptotic stimuli.^{157 158 167} Increased concentrations of the apoptosis promoting protein Bax have been reported in response to A β in vitro,¹⁶⁸ and in Alzheimer's disease neurons in vivo.¹⁶⁹

However, the proportion of neurons in brains affected by Alzheimer's disease that actually die by apoptosis rather than necrosis is not known. DNA fragmentation has been observed in Alzheimer's disease brain cells¹⁷⁰⁻¹⁷³; however, most of these neurons lack the characteristic morphological features of apoptosis.^{171 172} The assay of apoptosis specific protein, a marker for late stage apoptosis in Alzheimer's disease brains suggests that most dystrophic neurons in affected brains exhibit necrosis rather than apoptosis.¹⁷³

FRAMESHIFT MUTATIONS

Dinucleotide deletions (Δ GA) within GAGAG motifs of the β APP and ubiquitin B (Ubi-B) genes have been suggested to play a role in both early and late onset sporadic Alzheimer's disease.¹⁷⁴ Frameshift mutations appear to be introduced during protein synthesis that are not present in genomic DNA. Aberrant forms of β APP and Ubi-B proteins were found to be more prominent in Alzheimer's disease brains than in control brains.

The mechanism by which a wild-type gene can give rise to a mutant protein is unclear. It could be via a defect during transcription, or an editing mechanism in the RNA (fig 5). Presumably, aberrant proteins arising from errors during protein synthesis would accumulate with age, possibly explaining the increased risk of Alzheimer's disease with age. However, it is possible that the mutant proteins arise as a result of damage already present in the Alzheimer's disease brain, rather than being a causative factor in the disease.

ADVANCED GLYCATION ENDPRODUCTS

Advanced glycation endproducts are a heterogeneous group of adducts arising via non-enzymatic reactions between reducing sugars and amine groups on proteins. This modification is irreversible, and advanced glycation endproducts accumulate during normal aging.^{175 176} Advanced glycation endproducts have been implicated in the pathogenesis of Alzheimer's disease; diseased brains have been

reported to contain significantly higher concentrations of advanced glycation endproducts than age matched control brains and advanced glycation endproduct modified A β has been demonstrated to accelerate amyloid aggregation in vitro.¹⁷⁷ Advanced glycation endproducts colocalise with apoE, and it has been hypothesised that advanced glycation endproduct modified proteins bind to apoE and contribute to the formation of senile plaques.¹⁷⁸ A greater binding affinity for advanced glycation endproducts has been demonstrated with apoE ϵ 4 compared with apoE ϵ 3, and this might have pathogenic consequences in vivo.¹⁷⁸

Summary

Although new discoveries are being made constantly in this field, the pathogenesis of Alzheimer's disease remains poorly understood. The question remains, what causes Alzheimer's disease and why do some individuals develop the disease while others live into old age with no signs of dementia? Molecular genetic research has shown that there is no simple answer to this question. Alzheimer's disease is a complex polygenic disorder and many of the factors involved have yet to be identified. While the disease process remains largely a mystery, development of effective treatment is obviously difficult. This difficulty is compounded by problems with accurate diagnosis, resulting in heterogeneous groups of patients who may respond differently to treatment. The discovery of further genetic factors involved in the disease could lead not only to development of a better diagnostic profile, but to an increased understanding of the disease process.

We thank Dr K Robson and Professor J Lowe (Division of Pathology, School of Clinical Laboratory Sciences, Queen's Medical Centre, Nottingham) for providing the photographs used in this article. We would also like to thank the Trent Health Research Scheme for financial support and Nottingham University for the studentship for L.T.

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Mol Path 1998 51: 293-304

doi: 10.1136/mp.51.6.293

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