Molecular Genetics of Alzheimer's Disease

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Alzheimer disease (AD) is the most common cause of dementia. In the past decade, many advances in the understanding of the etiology of AD have been reported. Familial early onset AD is a heterogeneous disorder that can be caused by mutations in at least three different genes. Current studies are focused on identifying genetic risk factors for late onset AD. In this article, the authors will review the progress in understanding the pathogenic implications of the genes mutated in familial early onset AD and the mapping studies to identify additional genes involved in late-onset AD.

Introduction

Alzheimer's disease (AD), originally described by Alois Alzheimer in 1907 [1], is the most common cause of cognitive impairment in the elderly. Clinically, the disease starts with progressive memory impairment and later spreads to other cognitive domains including language, orientation, and behavior. Given the existence of other clinically similar dementias, a definite diagnosis of AD can be made only postmortem, with the observation of numerous amyloid plaques and neurofibrillary tangles (NFTs) in the hippocampus and neocortex.

Epidemiology and Clinical Considerations on Alzheimer's Disease

Epidemiologic studies in developed countries have estimated dementia prevalence to be 1.5% at age 65 years. Prevalence doubles every 4 years until it reaches approximately 30% in 80-year-old individuals [2–4]. The incidence of AD is lower in men, and in people of African and Asian origin. In the Western world, AD is estimated to be more common than vascular dementia [5–7]. Patients with dementia have a substantially shortened life expectancy, with an average survival of 8 years from diagnosis. A longer survival time is reported for AD patients than for those with vascular dementia [8–10].

Pathophysiology

Alzheimer's disease involves progressive degeneration and neuronal death in brain regions, such as the hippocampus and basal forebrain, which are involved in learning, memory, and emotional behavior. Besides neuronal loss, the presence of neuritic plaques and NFTs are required for a definite diagnosis of the disease [11].

Neuritic plaques

Plaques are insoluble extracellular deposits composed mainly of Aβ peptides. Aβ peptides are derived from a type I transmembrane protein, B-amyloid precursor protein (APP), via proteolytic processing [12]. APP is cleaved by β-secretase or α-secretase followed by γ-secretase. Aβ peptides are generated when APP is cleaved by β-secretase and γ-secretase. This is the major metabolic pathway of APP in brain tissue, although the non-amyloidogenic α-secretase pathway is the major pathway in other tissues (Fig. 1). Aβ peptides are heterogeneous in length, but the major peptides are 40 (Aβ40) and 42 (Aβ42) amino acids in length. Studies of Down syndrome brains have demonstrated that Aβ deposition is an early and invariant step in AD neuropathology. It is thought that plaques result from elevated Aβ42 levels because soluble Aβ42 spontaneously aggregates into fibrils that are indistinguishable from those found in vivo [12]. Transgenic mice overexpressing familial AD (FAD) mutant APP exhibit extensive plaque deposition in the same brain regions that are affected in AD, and they also exhibit elevated soluble Aβ levels [13]. However, given that Aβ levels also are elevated in brain regions that do not show plaques, other factors must influence Aβ deposition [13].

Neurofibrillary tangles

The other pathologic hallmark of AD is the NFT, an intraneuronal deposit of microtubule-associated protein tau. NFTs are believed to be formed when tau becomes hyperphosphorylated, causing it to dissociate from tubulin, leading to the production of insoluble tau-aggregates called paired-helical filaments [14]. Tau hyperphosphorylation seems to be regulated by the activity of several kinases including α-kinase, glycogen synthase kinase–3, and creatine kinase–1 [15]. Characteristically, in AD, tau deposition is limited to neurons, and the main component of the pathologic tau deposits is the shortest isoform of tau, which lacks exon 10 (3R). Neuronal loss in AD seems

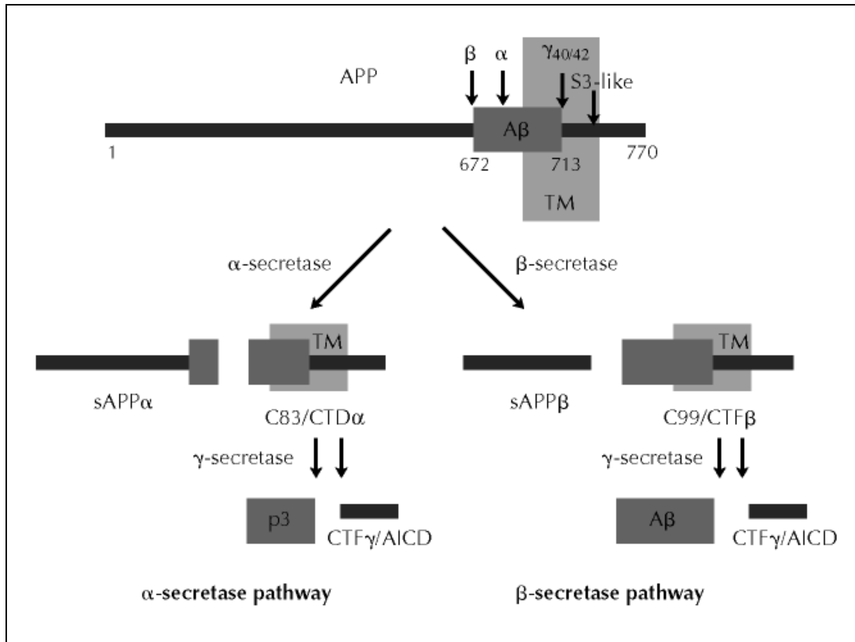

Figure 1. Amyloid precursor protein (APP) cleavage and α- and β-secretase pathways and production of Aβ-amyloid peptide. AICD—APP intracellular domain. CTF—C-terminal fragment; sAPP—soluble APP; TM—transmembrane.

to correlate with the formation of NFTs at least in some cortical and subcortical regions [16]. To date, no transgenic animal has been reported that convincingly reproduces the Aβ plaques and the NFTs found in AD.

Biologic Markers

The sensitivity of a clinical diagnosis of AD for neuropathologic AD (with or without other pathologies different from AD) can be as high as 90% in specialist dementia clinics, but may be much lower in the primary care situation [17,18]. However, at the present time, there is no single biologic marker that can be used to diagnose AD with high specificity and sensitivity. In the past decade, cerebrospinal fluid Aβ and tau levels have been proposed as tools to improve the clinical diagnosis of AD and to predict those at risk for developing dementia [19,20]. However, with the specificity of the combined tests at 80% to 90% and the sensitivity of the biomarkers for the clinical diagnosis of AD as high as 90%, there still is not enough precision to improve substantially on clinical diagnosis [20]. A recent study found a strong link between a clinical diagnosis of AD and a distinctive profile of cerebrospinal fluid Ab and tau protein [21]. Several studies have reported elevations in plasma and cerebrospinal fluid levels of Aβ40 and Aβ42 peptides in typical late-onset AD in comparison with control subjects [22,23]. The levels of brain sulfatides recently have been proposed as a promising marker for early clinical stages of AD. Han *et al.* [24] reported a significant decrease in cerebrospinal fluid brain sulfatides and in the ratio of brain sulfatides to phosphatidylinositol in subjects with mild cognitive impairment. This ratio accurately differentiated very mildly impaired subjects (clinical dementia rating 0.5) from control subjects on an individual basis [24]. Further study is needed to determine how useful this measure will be as a biomarker of disease.

Genetics of Alzheimer's Disease

Alzheimer's disease can be divided into two genetically distinct subtypes: 1) FAD, in which the disease is transmitted as an autosomal dominant trait, and 2) sporadic AD, which shows lower familial clustering. These subtypes are clinically indistinguishable, except that FAD has an earlier age of onset and sometimes shows additional neurologic features not generally present in sporadic AD [25–27]. AD also is also often divided into two groups based on age of onset—early onset (onset before 65 years of age) and late-onset (onset after 65 years of age) AD [28].

Causative Genes for Familial Alzheimer's Disease

Genetic linkage studies in large multigenerational FAD kindreds over the past two decades have established that early onset FAD is a genetically heterogeneous disorder that can be caused by mutations in the following three genes: β-amyloid protein precursor gene (APP) on chromosome 21, presenilin 1 (PS1) gene on chromosome 14, and presenilin 2 (PS2) gene on chromosome 1 [29–31].

Amyloid protein precursor gene mutations

The first mutation reported in the APP gene was observed in a very rare neurologic disorder—the Hereditary Cerebral Hemorrhage with Amyloidosis Dutch type (HCHWA-D). Cerebral hemorrhages in HCHWA are caused by β-amyloid deposition in cerebral blood vessels [32]. Linkage studies in this disease showed that the APP gene was the site of the pathogenic mutation [33] and a segregating mutation, E693Q [34], was discovered; this work showed unequivocally that mutations in APP could lead to β-amyloid deposition in brain vessels. Subsequently, mutations have been reported in APP that cause FAD [29], with

18 pathogenic mutations described so far, accounting for 10% of FAD cases (http://www.alzforum.org/res/com/mut/app/table1.asp). Typically, families segregating APP mutations have an age of onset of disease before 65 years of age. Beta-secretase cleaves between residues 671 and 672 of APP yielding the N-terminus of the Aβ peptide. Gamma-secretase cleavage of the membrane-associated C-terminal stub of APP generates the C-terminus of the Aβ peptide. Gamma-secretase cleaves APP at multiple sites within the transmembrane domain generating Aβ peptides that vary in length from 37 to 43 amino acids. The major peptide is Aβ40; however, the longer peptides are more amyloidogenic [35]. Gamma-secretase cleavage of APP also produces C-terminal fragment–γ/APP intracellular domain, a soluble C-terminal fragment that translocates to the nucleus and modifies transcription [36]. FAD mutations within the APP gene occur predominantly at the N-terminus and C-terminus of the Aβ region, suggesting that those mutations affect Aβ production. The Swedish mutation at the β-secretase cleavage site increases Aβ40 and Aβ42 levels, while the FAD mutations close to the γ-secretase site increase Aβ42 levels without increasing Aβ40 levels; some FAD mutations lead to a decrease in Aβ40 levels (*eg,* T714I) [37,38]. Recent studies using mass spectrometry show that other Aβ species alos may be increased by FAD mutations including Aβ38 and Aβ39, although these are not consistently observed with all FAD mutations.

Presenilin mutations

With the realization that the APP gene accounts for only a minority of cases of autosomal dominant AD, linkage studies in large kindreds led to the identification of a major locus on chromosome 14q [39,40]. Sherrington *et al.* [30] identified mutations in the presenilin 1 (PSEN1) gene. Database searches quickly demonstrated that a homologous gene (PSEN2) was located on chromosome 1, and mapped within a region showing linkage to AD in Volga German kindreds. Sequencing of PSEN2 in these families led to the identification of a missense mutation that segregated with disease [31]. The two presenilin genes are highly homologous at the DNA sequence, protein sequence, and gene structure levels [41]. Mutations in these two genes are thought to cause up to 80% of FAD cases. To date, there are more than 130 PS1 mutations, but less than 10 missense mutations in PS2. Most of the PS1 mutations are missense mutations predominantly located in the highly conserved transmembrane domains. Most of these mutations are characterized by an age at onset younger than 60 years of age and complete penetrance [42]. However, *PSEN2* mutations tend to be associated with a later age of onset and may exhibit incomplete penetrance and variable clinical expression, overlapping with late-onset AD [43,44].

The PS genes code for highly homologous polytopic transmembrane proteins, with a high degree of conservation across species. Presenilins are located in intracellular membranes [45] and are predicted to have eight transmembrane domains and a hydrophilic intracellular loop region. PSEN undergo a physiologic endoproteolytic cleavage to generate stable N- and C-fragments [46]. There is growing evidence that PSEN form the catalytic center of γ-secretase, a multiprotein aspartyl protease. In vivo and in vitro experiments have demonstrated that mutant PS increase Aβ42 levels [47,48]. PS mutations are gain of function with respect to Aβ generation, which is consistent with the inheritance pattern of early onset FAD cases. The reason for the disparity in the number of FAD mutations in PS1 compared with PS2 is thought to be because PS1 is the major γ-secretase, and thus mutations in PS1 have a bigger impact on Ab production. Knockout of PS2 in mice has an effect on Aβ levels and no observable phenotype, while PS1 knockout causes embryonic lethality and a decrease of Ab levels.

A splice-site mutation in intron 8 of PS1 [27], which is associated with an unusual FAD phenotype with paraparesia, produces "cotton-wool" amyloid plaques, that are morphologically different from those observed in most AD cases. These "cotton-wool" plaques do not contain amyloid fibrils in the core and are not surrounded by dystrophic neurites, suggesting that Aβ42 has a neurotoxic effect before neuritic plaques are formed [27].

Alzheimer's Disease Susceptibility Genes

Although researchers have a much greater understanding of AD pathogenesis as a result of the genetic and cell biology studies of FAD, 99% of AD cases do not carry mutations in these genes. Genetic linkage and association studies are being used to identify genetic risk factors for late-onset AD. Association studies test whether alleles of a given polymorphism are similarly distributed in AD cases compared with age- and ethnically matched control subjects. If the frequency of one of the alleles or genotypes is significantly over- or under-represented in the cases compared with the control samples, this suggests association with disease. This association may occur because the alleles of the polymorphism result in differences in risk for AD, because the marker is in disequilibrium with another variant that influences the risk of developing disease or because of type 1 error (a false-positive). Polymorphisms in more than 100 candidate genes have been assessed using association methods. However, none of these polymorphisms have shown consistent evidence of association from study to study except apolipoprotein E (*APOE*; reviewed in [49]). Possible reasons for the lack of replication in these studies are small sample size, population stratification between the case and control groups and genetic heterogeneity. Genetic linkage studies in late-onset AD families and sibling pairs have been used to identify AD susceptibility genes [50•,51•]. APOE4 was identified as a risk factor for late-onset AD through a combination of linkage and association studies. Although linkage has been reported to several other chromosomal regions, the specific genes have not yet been identified.

Apolipoprotein E polymorphisms

Apolipoprotein E exists as three isoforms in all populations, although the relative frequency of the isoforms can vary [52]. APOE3 is the major isoform in all populations. APOE2 and APOE4 vary from APOE3 at residues 112 and 158. Approximately 50% of AD cases carry one or more *APOE4* alleles [53–55]. The *APOE4* allele shows a dose-dependent increase in risk for AD; heterozygotes have a threefold increased risk for disease, and homozygotes have an eightfold risk for disease in Caucasians [53–56]. Most individuals who are homozygous for the *APOE4* allele develop AD by age 80 years of age [28]. *APOE4* alleles are associated with an earlier age of onset, with each allele causing a decrease in onset of approximately 5 years. In contrast, the *APOE2* allele decreases risk for AD and increases age of onset of disease [56]. Although the association between AD and *APOE4* has been confirmed worldwide, it had been found to be weak or nonexistent among Hispanic and African-American patients [57]. However, a recent study [58] has found that the age-related risk for cognitive decline is associated with the *APOE4* allele and the apparent protective effect of the *APOE2* allele in African-American subjects was similar to patterns observed in Caucasian subjects. APOE is a 34-kd plasma glycoprotein encoded by a gene on chromosome 19. In the central nervous system, APOE plays a role in cholesterol delivery during membrane remodeling that is associated with synaptic turnover and dendritic reorganization. The accumulation in astrocytes of high concentrations of cholesterol induces the synthesis of APOE, which binds to phospholipids and cholesterol derived from degenerating terminals to produce a high-density lipoprotein–like (HDL) complex. The APOE-HDL–like complex is secreted into the extracellular space to be recognized by APOE receptors located on specific neuronal targets. In the hippocampus granular neurons show an increased number of low-density lipoprotein (LDL) receptors in the initial phases of the re-innervation process [59]. In vitro, the APOE-LDL receptor–related protein (LRP) expressed by embryonic neurons modulates the internalization of APOE-cholesterol-LDL complexes, releasing cholesterol into the neuron [60]. In the brain, APOE is expressed in astrocytes [61]. APOE modulates the transport and distribution of cholesterol, especially under stress conditions such as neuronal growth and brain injury [62]. In vitro, APOE binds to synthetic Aβ peptide (the primary constituent of the senile plaque) with high avidity [63]. Transgenic experiments have demonstrated that APOE4 increases risk for disease by promoting Aβ deposition [62]. APOE4 most likely influences fibril formation or clearance of Aβ, thus accelerating Aβ deposition [64]. The *APOE4* allele is strongly associated with increased numbers of neuritic plaques and cerebral amyloid angiopathy in AD [65].

Candidate loci for familial Alzheimer's disease

The first genome-wide screen for late-onset AD was performed in 16 families and the chromosomal regions with a logarithm of the odds (LOD) score higher than 1 were then genotyped in 38 additional families [66,67]. In this study, the region showing strongest evidence for linkage was on chromosome 12. In a second report, the same authors extended their analysis to 466 families. This study also reported evidence for linkage on chromosome 12 in a 36-cM region [66,68]. Additional studies have also reported evidence for linkage on chromosome 12 in Caucasian and Caribbean Hispanic families, although the precise location of the linkage peak differs between studies [69–71]. In several of these studies, the strongest evidence for linkage was observed in the subset of families/sibling pairs with no *APOE4* alleles [69–71]. The differences in the exact location of the critical linkage region between studies could reflect the different analysis tools used for the statistical analysis or could represent genetic heterogeneity. It is possible that there are two susceptibility loci for AD on chromosome 12 and that the strength of the effect of each locus may differ in each study, leading to differences in the precise localization of the maximum evidence for linkage. Several candidate genes have been analyzed on chromosome 12, including alpha-2-macroglobulin and the LDL receptor–related protein 1 [72,73]. However, the results of these association studies have not been consistent.

Another chromosomal region that has been replicated is on chromosome 9 [68,74•,75•]. Several of these studies report two peaks on chromosome 9, suggesting that there may be two susceptibility genes. One of the chromosomes that had been most strongly associated with late-onset FAD is chromosome 10. There are four linkage studies reporting evidence of one or more susceptibility loci on chromosome 10 [50•,51•,68,76•]. In one of these studies, plasma levels of Aβ42 were used as a quantitative trait rather than AD diagnosis [76•]. This represents the first attempt in AD genetics to use an endophenotype to map genes that may underlie disease susceptibility. Similar approaches have been used in other complex diseases with some success. For example, electrophysiologic measurements and maximum number of drinks in a 24-hour period have been used as endophenotypes for alcoholism [77,78]. Among the linkage studies on chromosome 10, there are discrepancies again with regard to the location of linkage peaks. Several candidate genes have been examined on chromosome 10 including insulin-degrading enzyme PLAU, and αT catenin [79–82]. All three of these genes have been implicated in Aβ metabolism and therefore are strong biologic candidates. However, association studies have not provided consistent evidence in support of any of these genes.

A study using a covariate-based linkage method to reanalyze the genome scan data from affected sibships reported linkage to a region of 20p in sibling pairs that lacked *APOE4* alleles [83]. Two-point analysis provide evidence of strong epistasis between 20p and a region on chromosome 21 near the APP gene, which was limited to the oldest group of sibling pairs and to those patients lacking *APOE4* alleles [83]. Other linkage studies also have

reported evidence for linkage in *APOE4*-negative sibling pairs and in sibling pairs with the highest age of onset on chromosome 21 [74•,75•]. Many other regions have been reported in individual studies, but they do not show consistent evidence of linkage between studies. One of the difficulties in comparing results between these studies is that most of the studies use overlapping datasets, so the results are not independent. Furthermore, studies do not use APOE genotype in the same way to stratify the datasets.

Genetic modifiers of the phenotypic presentation of Alzheimer's disease

In the past decade, several groups have attempted to dissect the clinical phenotype to try to identify a more genetically homogeneous subgroup of families and increase the statistical power to detect linkage.

Age of onset

Age of onset is clearly an important covariate for the identification of genes for AD. Segregation analysis of large pedigrees with AD suggested that multiple loci associated with age at onset exist [84]. In this study, when age of onset was examined as a quantitative trait, it was estimated that up to four additional major genes as well as several minor AD genes remain to be identified [84]. Presence of *APOE4* alleles is correlated with earlier age of onset of AD [85–88]. The role of *APOE4* in modifying age of onset has been examined in AD families with known causative mutations. Early studies reported an association between the presence of the *APOE4* allele and age of onset in small kindreds carrying APP mutations, but not those carrying PS mutations [89–92].

In 1987, a Colombian family with early onset autosomal dominant AD was described [93]. Screening of the PS1 gene revealed a missense mutation at codon 280 of PS1 [89]. In subsequent years, 24 additional families with the E280A PS1 mutation have been identified [94]. This large family with similar environmental exposures and homogeneous disease etiology is a unique resource for the study of modifier genes. Kaplan-Meier Product Limit and Cox Proportional Hazard Models were used in the statistical analyses of age onset in these kindreds. *APOE4* allele carriers are more likely to develop AD at an earlier age than subjects without the *E4* allele (hazard ratio [HR]=2.07, 95% confidence interval [CI]=1.07 to 3.99, *P*=0.030). Individuals with low education were more likely to develop AD later than those with higher education (HR=0.476, 95% CI=0.26 to 0.87). Low educational level was associated with rural residence (*P*<0.001) (Fig. 2A, B, and C) [95]. This study demonstrates that the APOE isoform and environmental factors independently influence age of onset in a kindred with an FAD mutation.

Scott *et al.* [96•] recently used age of onset as a covariate in a genome-wide screen [68] and observed significant non-parametric multipoint LOD scores for several different intervals of age of onset. They observed a LOD score of 3.2 at D2S2944 on chromosome 2q34 in families with an age at onset between 50 and 60 years, and a LOD score of 4.6 at D9S741 in the chromosome 9p region previously linked to AD in families with an age at onset between 60 and 75 years. A LOD score of 2.8 was detected at D15S1507 with age at onset 79 years or older, and a peak LOD score of 3.1 was obtained at D15S153 (62 cM) in families with mean age at onset of greater than 80 years.

Another study, using a combined dataset of FAD and familial Parkinson disease families, reported evidence for a locus on chromosome 10q controlling the age of onset of both disorders [97].

Psychosis

Psychotic symptoms occur in 30% to 40% of patients with AD and are associated with more severe cognitive deficits and a more rapidly deteriorating course [98]. The presence of psychotic symptoms in AD cases confers increased risk of similar symptoms in affected siblings [99]. The presence of psychotic symptoms in AD therefore may represent a distinct subphenotype that can be used to identify more homogenous subgroups of the disease [100]. A linkage study performed in a sample of AD families with two or more members with AD plus psychotic symptoms found evidence of linkage on chromosome 2p and on chromosome 6q [101•]. Evidence of linkage to 6q has been reported in schizophrenia families and in bipolar families, suggesting that a gene in this region of chromosome 6 may influence the development of psychotic symptoms in a variety of diseases [102,103].

Conclusions

Familial-onset AD is a genetically heterogeneous disorder. Mendelian forms of the disease show genetic heterogeneity with at least three genes that act through a common biochemical pathway to cause disease. Late-onset AD is a complex trait with genetic and environmental risk factors. To date, the *APOE4* allele is the only established genetic risk factor in this group, although several chromosomal regions (chromosomes 12, 9, and 10) appear to show consistent evidence for linkage between studies. The use of endophenotypes, such as plasma Aβ, or clinical variables, such as age of onset, or special clinical presentations of the disease could be helpful tools in the identification of additional disease modifying genes.

Acknowledgments

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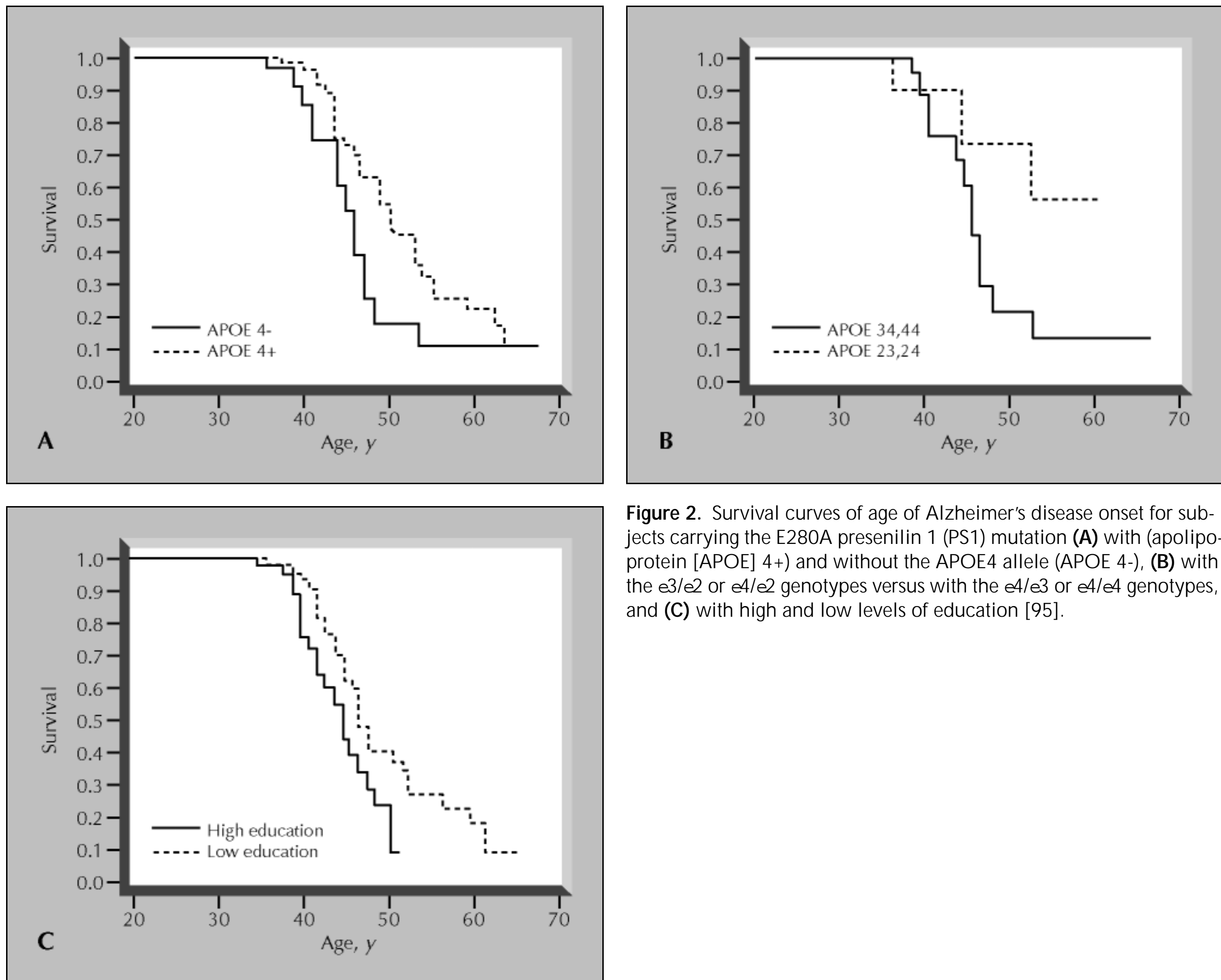

Figure 2. Survival curves of age of Alzheimer's disease onset for subjects carrying the E280A presenilin 1 (PS1) mutation **(A)** with (apolipoprotein [APOE] 4+) and without the APOE4 allele (APOE 4-), **(B)** with the ε3/ε2 or ε4/ε2 genotypes versus with the ε4/ε3 or ε4/ε4 genotypes, and **(C)** with high and low levels of education [95].

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Papers of particular interest, published recently, have been highlighted as:

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- •• Of major importance

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