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The Molecular Genetics of Alzheimer's Disease

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Abstract

The major pathological characteristic of Alzheimer's disease (AD) is the abnormal deposition of β-amyloid peptide (Aβ) in the brain. In some early onset cases, the disease develops because of mutations in the gene coding for β-amyloid precursor protein *(βAPP)*. However, the majority of AD families in the early onset subgroup are linked to a locus on chromosome 14. The genetic analysis and age of onset correlates of both the *βAPP* gene and the chromosome 14 locus are discussed. We speculate on the mechanisms by which the *βAPP* mutations cause the disease and discuss recent advances in *βAPP* processing that may be relevant to the pathogenesis of the late-onset (common) form of the disease. In addition, we review the association of the *APOE* locus with late-onset familial and nonfamilial disease. Further work is required to establish the effects of this locus on disease occurrence, age of onset, and progression. The molecular pathology of ApoE in relation to AD development and the identification of the chromosome 14 gene will greatly contribute to a general pathogenic model of AD, and will clarify the role of *βAPP* and its derivatives.

Index Entries: Alzheimer's disease; β-peptide (Aβ); chromosome 14; late-onset AD; apolipoprotein E.

Introduction

Although the clinical, neuropathological, and biochemical features of Alzheimer's disease (AD) have been exposed in intricate detail, the underlying causes have not. Given the insidious and slowly progressive nature of the disorder, most research data are gathered once the disease is advanced. Therefore, much of the information we have describes the consequences of AD rather than the initial events. The design of a rational (rather than empirical) pharmacology depends critically on the understanding of the etiology of AD and on the availability of accurate models of the disease. Several groups have gone some little way toward providing both of these with genetic studies of familial AD.

βAPP Gene Mutations

The genetic analysis of a single family (F23) led to the detection of the first clearly identified molecular cause of Alzheimer's disease: a mutation in the amyloid precursor protein gene *(βAPP)*. The background to this finding was an analysis suggesting the presence of a locus on chromosome 21 segregating with some cases of familial Alzheimer's disease (AD) *(1)*. The proposed AD locus was thought to be close to the *βAPP* gene *(2)*, a seemingly significant localization, since the *βAPP* molecule can be cleaved to produce the 39–42 residue peptide, Aβ, the cerebral deposition of which is the major pathological characteristic of Alzheimer's disease (AD). However, recombinants between the *βAPP* gene and the AD locus were reported *(3,4)*

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that excluded *βAPP* (if genetic homogeneity was assumed) as the site of the mutation causing familial AD. Genetic analysis of a large number of AD families subsequently demonstrated that the disease was most likely heterogeneous *(5)*. In particular, families with late-onset AD had not shown any linkage to chromosome 21 markers *(5,6)*, whereas only *some* early onset AD families did show linkage there. This led to the suggestion that there was non-allelic genetic heterogeneity within early onset familial AD *(3,5)*. To avoid the problems that heterogeneity posed for genetic analysis, an analysis of the cosegregation of AD and markers along the long arm of chromosome 21 was undertaken in F23 (assuming that the disease-causing gene would be the same within a single family), where NINCDS probable AD and definite AD had been confirmed *(7)*. Once linkage was established, direct sequencing revealed a point mutation in the *βAPP* gene *(8)*. This mutation causes an amino acid substitution (Val→Ile) in the βAPP^{770} molecule at residue 717, close to the carboxy terminus of the Aβ peptide. Screening other cases of familial AD revealed a second, unrelated family in which this variant occurs. Subsequent screening for the mutation in the other pedigrees has revealed a further eight families in which the mutation has occurred *(9–13)*.

Given the existence of genetic heterogeneity, one approach to detecting new disease-causing mutations has been to test for linkage to candidate regions in individual families. Using this strategy, other mutations at codon 717 have been detected *(14,15)* predicting Val→Gly and Val→Phe mutant *βAPP* isoforms. The problem of inadequate statistical power in small families has been overcome by the use of linkage-simulation studies *(16)*. More recently, a double mutation at the N terminal of Aβ was detected by linkage analysis *(17)*. Sequencing revealed a rare double mutation, and the G→T and A→C base changes predict Lys→Asn and Met→Leu at codons 670 and 671 of the βAPP^{770} transcript. These codons are positions –2 and –1 to the N terminal of the Aβ sequence.

All the mutations mentioned cosegregate with the AD phenotype, but another rare mutation *(18)* at codon 692 cosegregates with either the AD phenotype or with a cerebral angiopathy. This latter phenotype is similar to that of Hereditary Cerebral Hemorrhage with Amyloidosis-Dutch type *(19)*. In this rare condition, multiple strokes occur as a result of hemorrhage from cerebral vessels after damage resulting from amyloid deposition. The βAPP^{693} mutation, which causes this phenotype, probably reduces solubility of the Aβ metabolite. Classic AD pathology does not occur in these families.

The Significance of the *βAPP* Gene Mutations

The *βAPP* mutations demonstrate that the *βAPP* molecule can be solely responsible for the disease. The likelihood of developing the disease, given inheritance of a βAPP^{717} or $\beta APP^{670/671}$ mutation, by a person within one of these rare families is approx 10^{40}:1. Although causation can never be demonstrated by association, the possibility that something other than the *βAPP* mutations causes the disease in these families is negligible. The question of how they cause the disease is of central concern and may shed light on a general pathogenic hypothesis of AD. Their effects could be mediated through loss of function of *βAPP* or its derivatives, or via neurotoxicity of Aβ or other *βAPP* derivatives. Alternatively, deposition of Aβ could trigger other deleterious processes (an inappropriate immune response, for instance). Since deposition of Aβ is a central feature of the diagnostic neuropathology of AD, it is understandable that Aβ deposition should also be hypothetically linked to the subsequent neurodegeneration. One aspect of this hypothesis, the "amyloid cascade *(20)*," proposes that mismetabolism of *βAPP* leads to Aβ deposition. The *βAPP* gene mutants not only fired this theory, but also provided the tools to test it by contrasting their effects in relation to the normal gene. A great deal of knowledge is building on the latter subject and is briefly considered here.

βAPP Metabolism and the *βAPP* Mutations

The metabolism of *βAPP* has been extensively studied. The *βAPP* gene has at least five transcripts that arise by alternate splicing. The resulting *βAPP* isoforms are transmembrane glycoproteins, containing the 39–43 amino acid Aβ sequence, which is partly transmembranous, partly extracellular (*see* Fig. 1). *βAPP* is widely expressed in mammalian cells from an early stage. The most common transcripts in the brain are βAPP^{695} and βAPP^{751}, expressed in neurons, glia, and astrocytes. One route of metabolism of *βAPP* involves the initial secretion of the large extracellular domain (as APP_S), either after cleavage at the N-terminal end

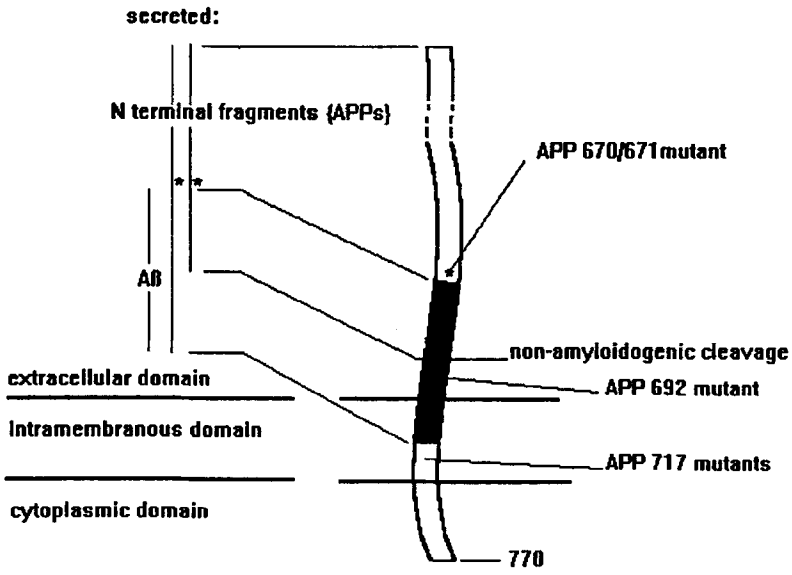

Fig. 1. The *βAPP* molecule: main secretory products and sites of the mutations causing AD (the 692 mutant causes either AD or a cerebral angiopathy).

of the Aβ sequence *(21)* or after cleavage through the Aβ sequence *(22)* (*see* Fig. 1). The latter cut precludes the formation of Aβ. Degradation of *βAPP* results in a plethora of C-terminal fragments, many of which pass through the endosomal lysosomal pathway. Fragments of *βAPP* extending from the C terminus of *βAPP* to the N terminus of the Aβ peptide (therefore potentially capable of producing the Aβ peptide) have also been localized to lysosomes *(23)*. However, Aβ has not been demonstrated in either lysosomes or other intracellular compartments to date. Since it has never been identified inside the cell, it has been postulated that Aβ is an early secretory product of *βAPP* metabolism, perhaps produced in late Golgi or early endosomes. One key aspect of *βAPP* metabolism is that Aβ is a normal cellular product, secreted in soluble form. It has been identified in the media of cells transfected with the *βAPP* gene and in the CSF of normal individuals *(24–26)*. The amount of Aβ found in cases of AD has not been reported as abnormal. The relationship between quantity of CSF-Aβ and diagnosis may not be simple, and it might be anticipated that the quantity produced would change over time with cellular degeneration and death.

Production of Aβ *per se* is then not pathogenic, but if deposition of Aβ is central to the AD process, factors that influence the solubility of Aβ may be. Much in vitro work on the solubility of synthetic Aβ has suggested that four factors influence solubility: concentration, pH, the length of the Aβ peptide, and the presence of other molecules *(27)*. Whether or not Aβ solubility is critical to the initiation of the disease process, the mechanism of cell death and degeneration is elusive. It is possible that these factors operate to different degrees in the different etiologic subgroups of AD. For instance, certain *βAPP* mutants overproduce Aβ ($\beta APP^{670/671}$), whereas others (βAPP^{717}) may produce a slightly longer variant of Aβ, although there is as yet no evidence for the latter suggestion. It is by no means clear, however, how the deposition of Aβ might initiate the disease process. In fact, there is good evidence that in many cases a moderate amount of Aβ deposition is associated with a normal mental status *(28)*. There is growing, but sometimes discordant evidence that Aβ is neurotoxic. It is unclear whether toxicity is mediated directly (via receptors or direct contact with the cell surface) as some have suggested *(29)*, or whether secondary processes mediate the damage. Less optimistically, a review of the data *(30)* notes that the results of five in vitro investigations did not consistently show toxicity. Furthermore, the in vivo toxicity studies reported in the same issue of *Neurobiology of Aging* did not faithfully reproduce neuropathology of AD. It remains probable that other unidentified factors enhance the toxicity of Aβ.

Despite the circumstantial evidence that Aβ deposition is central to the AD process, we should

consider that the function of *βAPP* and/or Aβ may be etiologically important. That *βAPP*s are a ubiquitous family of molecules, both highly conserved and expressed with a critical localization, suggests a key physiological role. A few lines of research should be noted. There is some evidence, from sequence homology studies, that *βAPP* has a G protein recognition site. In vitro studies confirm that *βAPP* does bind to one of the family of G proteins, G_o *(31)*. The significance of this finding is unclear, but it has been proposed that the binding may represent a trafficking step for either *βAPP* or G_o, or alternatively, that G_o regulates calcium homeostasis, an attractive hypothesis suggesting a mechanism of cell damage and death. Since Aβ is a normal cellular product, it may be physiologically active. The evidence that it is a neurotransmitter or binds receptors is lacking, but a recent study of artificial membranes suggests Aβ spontaneously forms channels permeable to calcium and other ions *(32)*. *βAPP* metabolism has been intimately associated with both phosphorylation status and calcium homeostasis *(33)*, and it remains possible that fluctuations in these cellular regulatory systems are mediated through, or by, *βAPP*. This idea is attractive, since these systems are natural cascades in which perturbations could account for the pathological cascade of the AD process. The relevance to the general pathogenesis of AD via any of these proposed roles of *βAPP* metabolism has yet to be determined, but useful tools for examination of mismetabolism or misfunctioning of *βAPP* are the *βAPP* mutants.

The effects on Aβ production in cells transfected with the *βAPP* mutants have been studied with the most spectacular effect to date observed in cells carrying the $\beta APP^{670/671}$ mutation. These cells produce an eightfold increase in Aβ, lending credence to the idea that excess Aβ production is at the root of the AD process in these families *(34,35)*. Alternatively, the effects of this mutation could be the result of an eightfold loss of function of the *βAPP* molecule. The effects of the βAPP^{717} mutants have still to be recorded, although it seems that overproduction of Aβ is not the mechanism *(36)*.

Chromosome 14 Gene

Chromosome 21 data excluded *βAPP* as the site of mutations in most early onset families. A number of groups began to look at linkage to other candidate loci and chromosomes in these families. Data suggestive of linkage on chromosome 14 had previously been reported *(37)*, and this chromosome was reexamined. The α_1-antichymotrypsin locus was excluded *(13)*, but linkage was demonstrated to other markers on the long arm of chromosome 14 *(13,38–40)*. The Volga-German pedigrees, included in the data set of Schellenberg and colleagues *(40)*, do not show linkage to either chromosome 14 or chromosome 21 markers, suggesting a third locus for early onset Alzheimer's disease. (This seems to be further supported by an additional set of families showing linkage to neither of these chromosomes *[41]*). For the families linked to chromosome 14, the accepted criterion (lod score >3.0) was observed for two markers, D14S43 and D14S53. In one study *(13)* no family typed for these markers gave any evidence for recombination (i.e., nonlinkage) except F126. This family was the only one in that data set showing any evidence of linkage to a chromosome 21 marker and may yet prove to have a novel mutation in *βAPP*. A multipoint analysis in that data set showed that the lod score went above 7.0 with a maximum at D14S43 ($z = 7.8$, $\theta = 0$). At present, although there are flanking markers, the multipoint map is quite flat over a relatively large genetic distance, obscuring the exact localization of the causative gene. Further recombination in other early onset families (for instance at D14S43 or D14S53) would localize the gene more precisely. Alternatively, extension of the present families may reveal recombinants as the apparent absence of recombination at D14S43 and D14S53 may partly be caused by incomplete or incompletely informative data. Whatever the strategy required to isolate the chromosome 14 AD gene, its discovery is bound to contribute significantly to our understanding of the development of AD. In particular, it will indicate whether the deposition of the amyloid peptide is the central event in all early onset familial cases of AD.

As an addendum to the subject of the chromosome 14 linkage, one important anomaly should be mentioned. The reported linkage by Hyslop et al. *(38)* shows single-point lod scores (the measure of likelihood of cosegregation of markers with disease resulting from genetic proximity rather than chance) in excess of 3.0 for four families (FAD1-4). These families were also the subject of the original linkage report to chromosome 21 *(1)*. If the original report of linkage to chromosome 21 does not reflect an underlying predisposition, then a chance linkage has occurred to the long arm of the chromosome to which true linkage was later confirmed in other

families. However, the authors suggest that this may not be a chance linkage result and invoke an epistatic model to explain the linkage to both chromosomes 21 and 14 in the same families. Taking the *βAPP* mutation families as one group, however, the combined likelihood of complete cosegregation of *βAPP* with the disease is in the order of 100 million to one in favor. Moreover, at least two large family data sets show that those families that are consistent with a linkage to chromosome 21 are not consistent with linkage to chromosome 14 and vice versa *(13,40)*. Epistasis seems an unlikely explanation, and perhaps these results reflect the difficulties of working with very large and statistically powerful families in conditions where the genetic parameters are to some extent indeterminate.

In parallel with genetic studies, the clinical features of these families have been recorded to determine clinico-genetic correlates *(11,42,43)*. In general, the *βAPP*-mutated families exhibit classic AD probably indistinguishable from chromosome 14 AD. A previous analysis of variance of age of onset in these and other families *(44)* showed that age of onset is family specific in early onset familial AD. In addition, though, a comparison of the age of onset of the *βAPP*-mutated families vs those consistent with a locus on chromosome 14 shows there to be a significant difference *(45)*. Moreover, analysis of the family not showing linkage to chromosome 14 (F126) shows it to be indistinguishable in age of onset from the *βAPP*-mutated families, but significantly different from the families consistent with a locus at chromosome 14. These data show that the *βAPP*-mutated families in this data set have a higher age of onset (generally in the 50s) than those families with mutations on chromosome 14 (generally in the 40s). This observation does not appear to be confined to mutations at the codon 717 locus. The mean age of onset of two related Swedish pedigrees with *βAPP* codon 670/671 mutations is in the same range (55 yr) *(17)*. The average age of onset for the Volga-German group and for other families showing nonlinkage to *βAPP* or chromosome 14 markers *(41)* is generally higher than for either of these groups (late 50s).

Of the total number of cases affected by AD, the early onset familial form constitutes a small proportion. Of those, approx 15% have *βAPP* mutations, the remainder having mutations in the chromosome 14 gene and perhaps other unlocalized early onset genes. Despite their rarity, these mutations are important for two reasons. First, since the clinical and neuropathological features of the disease in the mutated families do not differ from that of the common late onset form, understanding the pathogenesis of one may lead to an understanding of the other. Second, the use of these mutations in transfected and transgenic systems will provide reproducible models of the disease allowing examination of its pathogenesis.

Late-Onset Disease, Chromosome 19, and the *APOE* Gene

From the outset, it was improbable that all late-onset familial Alzheimer's disease would be the result of a single gene. A more likely model would include oligogenic (several genes) transmission, and the possibility of both genetic heterogeneity (different genes operating in different families) and etiologic heterogeneity (nongenetic factors). This model is consistent with the results of both linkage (familial) and association (unrelated individuals) studies. Taking the latter first, association of the *APOCII* gene to sporadic cases of AD was demonstrated in 1987 *(46)*. This gene is located on chromosome 19, and a series of linkage studies have consistently suggested the presence of an influential locus on that chromosome. Results from the lod-score methods of linkage analysis, which generally assume a strict Mendelian relationship between locus and phenotype, have been unconvincing. However, nonparametric methods of linkage (the affected pedigree member [APM] method) have provided evidence for a predisposing or modifying locus in the region of 19q13 *(47)*. An examination of the distribution of alleles of the *APOE* gene, which maps to this region, shows there to be a distortion of the normal frequencies in AD cases. This appears true for both familial and sporadic late-onset cases. The *APOE* gene and its products are well characterized *(48)*. Two diallelic polymorphisms at residues 112 and 158 give rise to four ApoE isoforms, three of which are common—ε2, ε3, and ε4. ε3 is the most common, and ε2 is less common and is associated with protection against atherogenesis. The ε4 allele has previously been recognized as a risk factor for hypercholesterolemia and coronary artery disease (CAD). In the first study of *APOE* and AD, an increase in the allelic frequency of ε4 in AD is accompanied by a proportionate decrease in ε2 and ε3, suggesting an effect resulting from the ε4 allele. The study examined one affected person per family

and took as controls the grandparents of large genetic reference pedigrees (CEPH). They found an allele frequency for ε4 of 16% in the controls compared to 50% in the affected probands from the families *(49)*. A review of historical controls show that there is rarely an allele frequency for ε4 in the general population over 18% *(48)*. In an examination of late-onset sporadic cases, there was a similar excess of the ε4 allele compared to age- and gender-matched controls. Since the *APOE* locus has previously been implicated in predisposition to heart disease, preliminary studies confirming the excess occurrence of the ε4 allele should carefully examine the phenotype with which it occurs. In particular, the relationship of alleles at the *APOE* locus to vessel disease, multi-infarct dementia, and AD should be carefully examined. Given the risk for vessel disease associated with the *APOE* locus, it is tempting to speculate that *APOE* is not an independent risk factor for both AD and vessel disease, but that one is causally related to the other in a subgroup of individuals. A comparison of the degree of vascular disease (determined clinically and by scan data, for instance) between those cases with the ε4 allele and those without would begin to address this issue. In addition, the effects of other loci known to be risk factors for vessel disease need to be examined.

It is clear then from the outset that since the ε4 allele occurs frequently in normals, variation at the *APOE* locus does not necessarily produce the AD phenotype. *APOE* should therefore be regarded as a genetic susceptibility locus either predisposing to AD or advancing the age of onset.

These very important findings need to be clarified and expanded to address the question of whether this locus does confer increased risk of developing AD or whether it advances age of onset in otherwise predisposed individuals. First, there is a simple need of replication both in Caucasian populations and groups with other ethnic backgrounds. One would assume that if this confers increased risk, it would do so in all populations.

Epidemiologically unbiased populations, stratified for age with matched controls, are required to determine that the distortion of allele frequencies is not artifactual. Second, if the role of the *APOE* locus is to modify age of onset, there are a number of reference families that could be used to test this hypothesis, for instance, those families that have already been shown to have the disease because of *βAPP* mutations or the chromosome 14 gene. The age of onset tends to be constant within all early onset families *(44)* (particularly the chromosome 14-linked families), but variation does occur, as does variation in rate of disease progression. An examination of these factors in relation to *APOE* status would be informative. Age of onset in late-onset families tends to have much greater within-family variation (Mullan et al., unpublished data). This variation should be similarly examined in relation to *APOE* status.

If the *APOE* locus represents an independent risk factor for AD, by what mechanism does it impinge on the disease process? One idea arises from the localization of ApoE to the plaque of AD. It has been suggested from the results of one study that the relationship of density and number of the plaque is a function of the *APOE* genotype. The individuals with an ε4ε4 genotyping tended to have the heaviest staining for amyloid in plaque at autopsy *(50)*. ApoE protein acts as a molecular chaperone, and it has been suggested that it aids the sequestration of Aβ into plaque. This idea is supported by the observation that ApoE binds Aβ to differing degrees, depending on the isoform. The ε3 isoform forms a dimer with Aβ slowly compared to the binding of the ε4 isoform, which is much more rapid *(51)*. Understanding the molecular mechanism by which ApoE either activates the disease process or enhances the progression is a major area of current investigation.

Summary

The molecular biology of AD has largely been confined to the molecular biology of *βAPP*. The neuropathology of AD and molecular genetic studies have played no small part in focusing research on this single molecule. Molecular studies of the constituents of the neurofibrillary tangle (NFT) have been examined to a much lesser extent. The study of *βAPP* and its Aβ derivative in relation to AD pathogenesis has introduced as many questions as it has answered: Is *βAPP* misfunction or mismetabolism important? If Aβ is central to the disease process, is excess production sufficient or does deposition have to occur? Is Aβ functionally active? What is the mechanism of Aβ toxicity? What is the relationship between Aβ deposition, tangle formation, and neurodegeneration? What additional factors expedite the process of NFT formation and cell death?

It may be that several of these questions will be answered empirically with transfected cell systems

and transgenic models of AD. For instance, the property of the $\beta APP^{670/671}$ mutant may be exploited to examine the relationship between excess Aβ production, deposition, and neuropathology. Once established, such models will enable examination of the interaction of other factors with the disease process, such as the effects of cerebral ischemia or trauma. Last, transgenic animals or cell culture systems may provide a test bed for the evaluation of new and existing anti-Alzheimer treatments. The revelation of the nature and function of the chromosome 14 gene will provide critical information on the putative role of *βAPP* or Aβ. It is sobering to realize that our present knowledge of the role of these moieties in AD pathogenesis is limited enough to allow diverse speculation on the function of the chromosome 14 gene. A list of candidate molecules could presently include those controlling cellular phosphorylation status or calcium homeostasis, protein trafficking, transduction or signaling, and *βAPP* metabolism! Given that Alzheimer's disease encompasses so many neurochemical processes as it progresses, it is natural that work on the etiology of the disorder may ultimately be most useful in unraveling its mysteries. In this respect, the cloning of the chromosome 14 gene and the discovery of the mechanism of susceptibility conferred by the *APOE* locus are eagerly awaited.

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