Cell, Vol. 120, 545–555, February 25, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.cell.2005.02.008

Twenty Years of the Alzheimer's Disease Amyloid Hypothesis: A Genetic Perspective

Review

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From Alois Alzheimer's description of Auguste D.'s brain in 1907 to George Glenner's biochemical dissection of β-amyloid in 1984, the "amyloid hypothesis" of Alzheimer's disease has continued to gain support over the past two decades, particularly from genetic studies. Here we assess the amyloid hypothesis based on both known and putative Alzheimer's disease genes.

Introduction

Perhaps the single greatest obstruction to maintaining a healthy brain with advancing age is the insidious accumulation of the pathological lesions that define Alzheimer's disease (AD), the most common form of dementia in the elderly. With the increasing longevity of our population, AD is already approaching epidemic proportions with no cure or preventative therapy yet available. AD is a progressive neurodegenerative disorder characterized by global cognitive decline involving memory, orientation, judgment, and reasoning. The disease is named after Alois Alzheimer, a Bavarian psychiatrist with expertise in neuropathology, whose 1906 meeting presentation of his patient, Auguste D., fueled a major paradigm shift in how we think about mental disorders (Alzheimer, 1907). Auguste D. was a 51-year-old woman admitted to an asylum for "delerium and frenzied jealousy of her husband." Given her relatively young age of 51, she was diagnosed with what we would now refer to as "presenile dementia." In his presentation of this patient, Alzheimer made the then bold assertion that her dementia was intimately related to gross neuropathological lesions that he observed in her autopsied brain: "miliary bodies" and nerve cells whose interiors were choked by "dense bundles of fibrils." This postulate was put forward in the early days of what could be considered the "clinicopathological era" of neurological and psychiatric disease, when scientists were attempting to correlate clinical symptoms with pathological features. While the unfamiliar notion that a "mental" disorder like presenile dementia could be due to "physical" aberrations was not readily accepted at the time, the disorder would, nonetheless, be named in 1910 after Alois Alzheimer by his mentor, Emil Kraepelin.

By the end of the 1960's, autopsy of brains taken from elderly individuals who suffered from dementia would reveal that "senility" was not simply a function of advanced age but, in most cases, was consistent with the same disease presented by Alzheimer in 1906. Clearly visible upon autopsy examination of most cases of senility at the light microscopic level were extracellular deposits of β-amyloid (Alzheimer's "miliary foci") and intracellular deposits of neurofibrillary tangles (Alzheimer's "dense bundles of fibrils"). Abundant amounts of these lesions in the brain are necessary for a confirmed diagnosis of AD. Studies of the etiology of AD were not particularly fruitful over most of the 20th century, and the majority of AD cases display no discernible mode of inheritance. However, in 1981, Heston et al. first reported that relatives of 125 subjects who had autopsy-confirmed AD exhibited a significant excess of dementing illness consistent with genetic transmission (Heston et al., 1981). Interestingly, in that same seminal study, it was observed that when compared to controls, the relatives of affected individuals derived from families with a significantly greater incidence of Down's syndrome (DS, or trisomy 21). While this connection is still not fully understood, it was particularly interesting given the high incidence of Alzheimer-type neuropathology that is inevitably observed in the brains of middle-aged patients with DS. Taken together, these observations first suggested a possible genetic link between AD and an abnormal gene or structural defect on chromosome 21. The relationship between chromosome 21 and AD pathology would become clearer a decade later, but not without the advent in the mid-1980s of critical biochemical data emanating from the analysis of AD-related β-amyloid deposits.

From AD Pathology to Genetics

The prediction of an AD gene on chromosome 21 was put forward in 1984 when Glenner and Wong reported the hard-sought amino acid sequence of the main component of β-amyloid—a 4 kiloDalton peptide that they termed "amyloid β protein" (Aβ)—based on their analysis of cerebrovascular amyloid derived from patients with DS (Glenner and Wong, 1984). This study can be considered to have initiated the "amyloid hypothesis" of AD, which maintains that the accumulation of Aβ, as determined by its generation versus clearance in the brain, is the primary driver of AD-related pathogenesis, including neurofibrillary tangle formation, synapse loss, and neuronal cell death. The Aβ sequence published by Glenner and Wong (1984), together with one found later in β-amyloid isolated from senile plaques (Masters et al., 1985), was subsequently employed by four different groups in 1986 to isolate the gene encoding the β-amyloid precursor protein (Goldgaber et al., 1987; Kang et al., 1987; Robakis et al., 1987; Tanzi et al., 1987). As predicted by Glenner, the *APP* gene mapped to chromosome 21 (reviewed in Price et al., 1998). Concurrent with the cloning of APP, genetic linkage of AD to chromosome 21 was reported for four large early-onset familial Alzheimer's disease (EOFAD) pedigrees (St George-Hyslop et al., 1987). Ironically, while these four families would ultimately be found to be negative for APP mutations and instead be shown to be tightly linked to a different EOFAD locus on chromosome 14, the original

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Table 1. Overview of *Established* AD Genes Influencing the Aβ Life Cycle

Gene (Location [Mb])[a]	Genetic Mechanism	Biochemical Phenotype
APOE (19q13 [50 Mb])	LOAD: risk association (ϵ4-allele)	a) ↑ Aβ aggregation b) ↓ Aβ clearance
APP (21q21 [26 Mb])	EOFAD: AA-change *(n = 16 mutations*[b]*)* LOAD: mostly neg. association findings	a) ↑ $A\beta_{42}/A\beta_{40}$ ratio b) ↑ Aβ generation/Aβ aggregation
PSEN1 (14q24 [73 Mb])	EOFAD: AA-change *(n = 140 mutations*[b]*)* LOAD: pos./neg. association findings	↑ $A\beta_{42}/A\beta_{40}$ ratio
PSEN2 (1q42 [223 Mb])	EOFAD: AA-change *(n = 10 mutations*[b]*)* LOAD: mostly neg. association findings	↑ $A\beta_{42}/A\beta_{40}$ ratio

"Mb" = million base-pairs, "EOFAD" = early-onset familial AD, "LOAD" = late-onset AD.
[a] Location according to "UCSC Human Genome Browser," May 2004 assembly (URL: http://genome.ucsc.edu/cgi-bin/hgGateway).
[b] According to the "Alzheimer's Disease Mutation Database" (URL: http://molgen-www.uia.ac.be/ADMutations/).

report of their putative linkage to chromosome 21 did serve to motivate other laboratories to demonstrate genetic linkage of independent EOFAD kindreds to chromosome 21. These latter families would then lead the way to the identification of the first AD gene mutation.

In 1990, Frangione and colleagues reported that sequencing of exons 16 and 17 of *APP*, encoding the Aβ domain, revealed the first pathogenic mutation in APP (Levy et al., 1990), which caused hereditary cerebral hemorrhage with amyloidosis in a Dutch family linked to chromosome 21 (Van Broeckhoven et al., 1990). Subsequent sequencing of these same two APP exons (encoding the Aβ portion of the molecule) in EOFAD families that were truly linked to chromosome 21 led to the discovery of the first EOFAD mutation in 1991 (Goate et al., 1991). While additional mutations were subsequently found in *APP*, it would soon become apparent that APP mutations accounted only for a miniscule fraction of all EOFAD cases, and efforts turned toward identifying other EOFAD genes. In the summer of 1995, presenilin 1 and 2 (*PSEN1; PSEN2*) were reported as novel EOFAD genes on chromosomes 14 and 1, respectively (Levy-Lahad et al., 1995; Rogaev et al., 1995; Sherrington et al., 1995). The presenilins are serpentine proteins with eight transmembrane domains and large hydrophilic, cytoplasmic loops that undergo regulated endoproteolytic cleavage to produce N- and C-terminal fragments (Thinakaran et al., 1997). To date, a total of 16 rare, autosomal-dominant mutations have been found in *APP*, 140 in *PSEN1*, and 10 in *PSEN2* (AD mutation database; http://www.molgen.ua.ac.be/ADMutations/; Table 1).

In the same year that the first EOFAD mutation was found in APP, Pericak-Vance and colleagues reported significant genetic linkage of the more common late-onset form of AD (>65 years) to chromosome 19 (Pericak-Vance et al., 1991). Two years later, they found a common polymorphism, ϵ4, in the gene encoding apolipoprotein E (*APOE*) in the same chromosomal region associated with increased risk for late-onset AD (Schmechel et al., 1993; Strittmatter et al., 1993). This association has been corroborated in literally hundreds of independent studies worldwide, across a wide variety of ethnic groups and populations. A meta-analysis on *APOE* showed that the ϵ4-allele represents a major susceptibility factor for AD across all ages between 40 and 90 years, and in both men and women (Farrer et al., 1997). Despite these strong and robust effects, the ϵ4-allele is present in ~15% of the general population (and shows roughly twice this frequency in samples afflicted with AD), and carrying one or two copies of ϵ4 is neither necessary nor sufficient to actually *cause* AD. Rather, there is evidence that its presence reduces the age of onset for the disease (Blacker et al., 1997; Meyer et al., 1998). An up-to-date overview on the status of this and other potential AD candidate genes, including meta-analyses based on crude odds ratios calculated for each of the published case-control genetic association studies, can be found at the Alzheimer's Research Forum genetic database, "AlzGene" (http://www.alzgene.org).

Since *APOE*, *APP* and the presenilins have also been tested as late-onset AD susceptibility genes. In addition to the known EOFAD mutations in *APP* (see above and Table 1), evidence exists that the chromosomal region encompassing this locus on 21q21 may also harbor late-onset AD risk variants. A re-analysis of earlier genome screen data (Kehoe et al., 1999) using age-at-onset as a covariate revealed significant linkage in this area, particularly in older subjects lacking the *APOE* ϵ4-allele (Olson et al., 2001). This result agrees with a report from our group using an extended sample of the same NIMH AD family population that revealed the most pronounced linkage signal near 21q21 in families not included in the previous papers (Blacker et al., 2003). However, it currently remains unclear whether genetic variants in *APP* underlie these strong and consistent linkage signals, since thus far no study investigating this AD candidate as a risk gene has yielded more than marginal results (Athan et al., 2002; Li et al., 1998).

The first study aimed at investigating the presenilins as putative late-onset AD genes reported evidence for significant association between a single-nucleotide polymorphism (SNP) in intron 8 of the *PSEN1* gene and late-onset AD (Wragg et al., 1996). In that report, it was estimated that common variants in *PSEN1* could account for nearly half of the population-attributable risk for AD than that found for the *APOE* ϵ4-allele. Subsequently, nearly 50 studies investigated the putative association of *PSEN1* in independent late- and early-onset AD samples, most of them focusing on the original intron 8 polymorphism. Meta-analyses of these studies reveal a small (OR ~1.1) but significant risk effect of the more common T allele of the intron 8 SNP, which is mostly conferred by homozygous individuals (http://www.alzgene.org). However, it seems unlikely that

genetic variants in *PSEN1* play a major role in contributing to AD risk in general, and almost certainly the population-attributable risk is smaller than had been initially estimated by Wragg and colleagues (1996).

Another explanation for the overall positive association with the intron 8 SNP in *PSEN1* is that the detected effects may actually reflect linkage disequilibrium with rare, disease-causing mutations in individual subjects, which may have previously gone undetected due to incomplete or missing family histories. Along these lines, a recent report investigating a consecutive series of referral-based AD cases (i.e., not ascertained on the basis of positive family history or young onset age) found coding sequence mutations in 11% of the samples (Rogaeva et al., 2001), suggesting that *PSEN1* mutations may, indeed, be more frequent in the general population than had been previously assumed. Furthermore, recent reports have indicated that changes in the promoter region could lead to an altered expression pattern of the protein in neurons (Lambert et al., 2001; Theuns et al., 2003). Despite these potentially promising findings relating *PSEN1* to late-onset AD, no evidence for genetic association has yet been reported for common variants in the homologous *PSEN2* gene.

From AD Genetics to Function

While the mutations in APP and the presenilins account for less than 5% of all AD cases, they are fully penetrant and therefore guarantee onset of the disease. Thus, functional studies of these mutations and an elucidation of the biological pathways in which they operate have since become topics of intense investigation. To date, studies of these genes in cell- and animal-based model systems have lent strong support to the amyloid hypothesis first initiated by Glenner (Glenner and Wong, 1984) and further elucidated by many since. The amyloid hypothesis posits a central role for Aβ in initiating the AD pathogenic cascade and argues that the neurodegenerative disease process, including the development of neurofibrillary tangles, is a consequence of an imbalance between the generation and clearance of Aβ. The hypothesis is based on multiple findings from genetic, molecular, biochemical, and neuropathological studies (Hardy and Selkoe, 2002). However, the main support for the amyloid hypothesis derives from genetic studies and the fact that the vast majority of the EOFAD mutations confer a similar biochemical phenotype (Scheuner et al., 1996; Price et al., 1998): an increased ratio of cerebral Aβ ending at position 42 ($A\beta_{42}$) as opposed to position 40 ($A\beta_{40}$). $A\beta_{42}$ has been shown to be more prone to amyloid fibril formation and appears to be the more toxic form of the peptide (Jarrett et al., 1993). While it remains unknown as to exactly how these mutations alter the ratio of Aβ species generated from APP, it appears that they alter how APP is enzymatically cleaved (at the γ-secretase site) to produce the Aβ peptide (Price et al., 1998).

Interestingly, not all mutations in *PSEN1* lead to dementia via changes in Aβ production. More specifically, one recently reported mutation in *PSEN1*, Gly183Val, has been associated with Pick's disease in the absence of amyloid plaques (Dermaut et al., 2004). This mutation was associated with a frontal lobe dementia-like syndrome that is pathologically characterized by Pick disease-type tauopathy, which has also been found to be associated with the gene encoding the microtubule-associated protein tau (*MAPT*). This finding indicates that at least one putatively pathogenic mutation in the early-onset FAD gene, *PSEN1*, can lead to non-AD dementia in the absence of β-amyloid pathology.

A milestone finding in molecular and biochemical studies of EOFAD genes was showing that the presenilins are necessary for the generation of Aβ from APP (De Strooper et al., 1998). The bulk of APP is cleaved (see Figure 1) by α-secretase within the Aβ domain to produce the C-terminal fragment, C83, which can be further cleaved intramembranously by γ-secretase to produce the peptide P3 and the transcriptionally active, APP intracellular domain (AICD; Cao and Sudhof, 2001). Alternatively, APP can be sequentially cleaved to produce Aβ, which requires initial cleavage of APP by β-secretase, identified in 1999 as the aspartyl protease coined BACE (Vassar et al., 1999), followed by γ-secretase cleavage within the single-transmembrane domain. Additionally, in 1999, Wolfe et al. showed that the presenilins are not only necessary for γ-secretase cleavage but contain two aspartates in transmembrane domains 6 and 7 that may serve as the active sites for γ-cleavage (Wolfe et al., 1999). It should be noted, however, that, minimally, γ-secretase activity also requires three additional proteins that together with presenilin form the γ-secretase protein complex: nicastrin, aph-1, and pen-2 (Edbauer et al., 2003; Francis et al., 2002). From a genetic standpoint, the genes encoding the α-, β-, and γ-secretase molecules can all be considered biologically compelling candidate genes for early- and late-onset AD.

The Genetics of the AD-Associated Secretases

α-Secretase

Proteases with proposed α-secretase function belong to the ADAM ("a disintegrin and metalloproteinase domain") family of proteins and include ADAM 9 (*ADAM9*; on chromosome 8p11), ADAM 10 (*ADAM10*; 15q21), and ADAM 17 (*ADAM17* or *TACE*; 2p25). While the latter two map to chromosomal regions previously implied by full-genome screens to either show genetic linkage and/or association with microsatellite DNA markers which are usually located in noncoding regions (Hiltunen et al., 2001; Scott et al., 2003; reviewed in Bertram and Tanzi, 2004), none of these genes has yet been tested directly for association with AD (Table 2). Another gene in the general α-secretase category is BACE2 (*BACE2*; 21q22, at ~42 Mb), which likewise cleaves within the Aβ domain and abrogates amyloid formation. *BACE2* maps only ~15 Mb distal of *APP*, within the obligate DS region on chromosome 21 (Saunders et al., 1999; see above). The only two studies investigating polymorphisms in *BACE2* to date have both failed to produce any evidence for genetic association of this candidate with AD (Gold et al., 2003; Nowotny et al., 2001; Table 2).

β-Secretase

Cleavage at the β-secretase site is mediated by BACE1 (gene: *BACE1*; 11q23), which maps to a chromosomal region previously implicated in at least one AD linkage

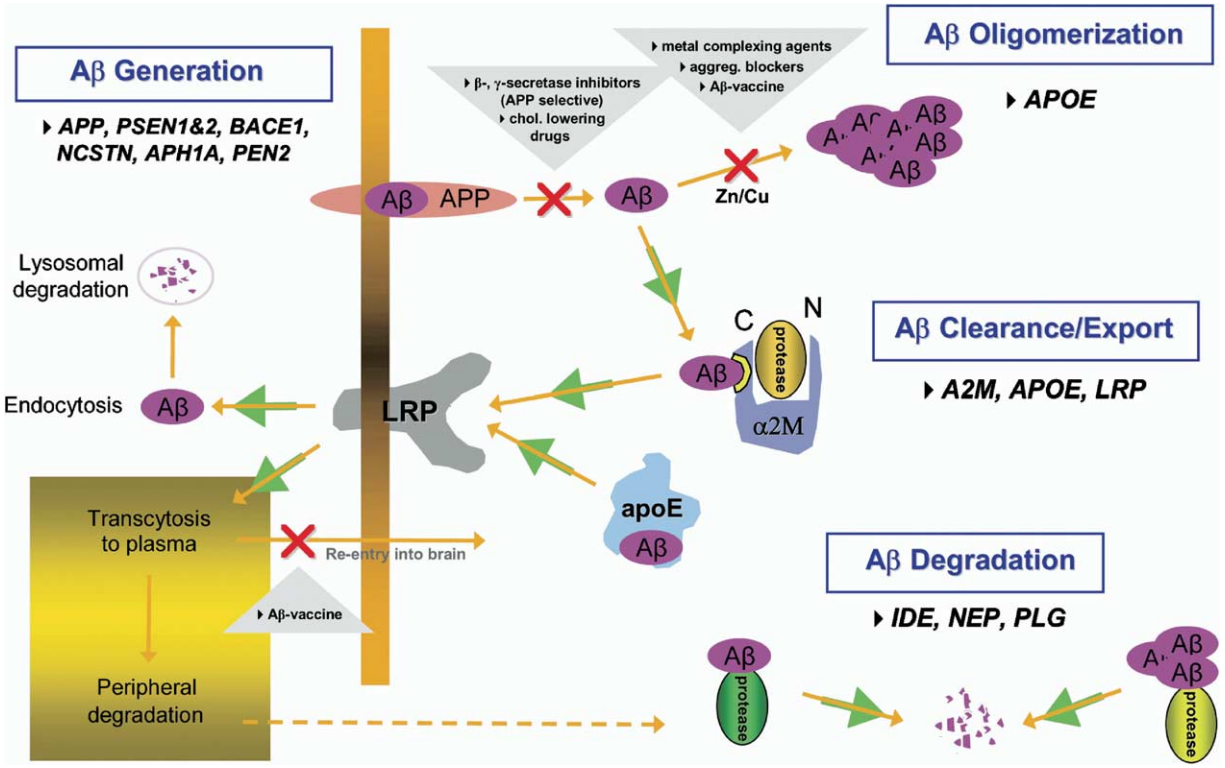

Figure 1. Genes Influencing the Aβ Life Cycle and Possible Points of Therapeutic Interventions

Aβ generation in brain is influenced by the known early-onset familial AD genes *APP*, *PSEN1*, and *PSEN2* along with the genes encoding *BACE* (β-secretase) and the three proteins, besides *PSEN1&2,* in the γ-secretase complex, *NCSTN, APH1A*, and *PEN2.* Therapeutic interventions in this pathway include β- and γ-secretase inhibitors (preferably selective for APP) and cholesterol-lowering drugs, e.g., statins. Once Aβ is secreted, metals such as zinc (Zn) and copper (Cu) and the established late-onset AD risk factor, *APOE*, can modulate Aβ oligomerization into fibrils. Either metal-complexing compounds or aggregation blockers that prevent β-pleated sheet formation can be used as therapeutic interventions for oligomerization. Aβ can also bind to apoE or α2M, which, in turn, can deliver the peptide to their common receptor, LRP. Once bound, the complex can undergo endocytosis and subsequent degradation in lysosomes. Alternatively, internalization by LRP at the blood brain barrier can lead to transcytosis of Aβ into the plasma where the peptide can either be delivered to sites of peripheral degradation, e.g., liver and kidney, or gain re-entry into the brain. As a potential therapy, the amyloid vaccine has been proposed to retain Aβ in the plasma precluding transport back into brain. Alternatively, anti-Aβ antibodies generated via the amyloid vaccine may also gain entry into brain and activate microglial digestion of Aβ. Finally, Aβ can undergo direct degradation by proteases such as IDE (which only cleaves monomeric peptide), neprilysin, and plasmin, either in brain or at peripheral degradation sites. Green arrows indicate steps in the pathway that might be potentiated as a means for preventing accumulation of cerebral Aβ, while red crosses indicate potential inhibition points.

study (Blacker et al., 2003). To date, the nine reports investigating a potential AD risk effect of *BACE1* have yielded only mixed results (Table 2). It is noteworthy, however, that all positive studies have observed their most significant results in individuals carrying at least one copy of the ϵ4-allele (reviewed in Bertram and Tanzi, 2004). Conceivably, this effect may have been missed by the reports that did not stratify by *APOE* and may be worth further investigation.

γ-Secretase

Besides *PSEN1*, the other essential members of the γ-secretase complex, which include aph-1a (*APH1A*; 1q21 and aph-1b [APH1B]; 15q22), pen-2 (*PEN2*; 19q13), and nicastrin (*NCSTN*; 1q22-23), also map close to chromosomal intervals implicated in full-genome linkage screens (reviewed in Bertram and Tanzi, 2004; Table 2). Nonetheless, comparatively few studies have investigated the potential role of these genes as susceptibility factors in either early- or late-onset AD. To date, *APH1A* and *PEN2* failed to show genetic association in one study each (Bertram et al., 2004; Poli et al., 2003). Meanwhile, genetic variants in *NCSTN* have been investigated more frequently. Of the six studies published to date, three noted a significantly elevated risk associated with specific haplotypes and AD (Dermaut et al., 2002; Confaloni et al., 2003; Helisalmi et al., 2004), while three studies did not see such an effect. Meta-analysis on all of these studies suggests a significant role for some of the investigated polymorphisms (e.g., in intron 16) but not for others (e.g., in intron 10, see "AlzGene" database for an up-to-date overview). Clearly, more detailed studies are needed on all three of these γ-secretase-related genes to more definitely elucidate their potential contribution to overall AD risk and to investigate the possibility of rare, disease-causing mutations in familial cases of AD.

The Genetics of Aβ Clearance

While elevated levels of cerebral Aβ are universally observed in AD and are caused by an increased generation of the peptide in the early-onset familial—and potentially also some late-onset forms of the disease—

Table 2. Overview of *Putative* AD Genes Influencing the Aβ Life Cycle

Gene (Location [Mb])[a]	Genome Screen Region[b]	Functional Relevance to AD	Association Results to Date		
			Positive	[c]	Negative
A2M (12p13 [9])	yes	Aβ clearance	yes	<	yes
ADAM9 (8p11 [39])	no	α-secretase	ND		ND
ADAM10 (15q21 [57])	yes	α-secretase	ND		ND
ADAM17 (2p25 [10])	yes	α-secretase	ND		ND
APH1A (1q21 [147])	yes	γ-secretase	no		yes
BACE1 (11q23 [117])	(yes)	β-secretase	yes	>	yes
BACE2 (21q22 [42])	yes	β-, α-secretase	no		yes
ECE2 (3q27 [185])	no	Aβ degradation	ND		no
IDE (10q23 [94])	yes	Aβ/AICD degradation	yes	>	yes
LRP1 (12q13 [56])	yes	Aβ clearance	yes	<	yes
NCSTN (1q23 [157])	yes	γ-secretase	yes	=	yes
NEP (3q25 [156])	no	Aβ degradation	yes	=	yes
PEN2 (19q13 [41])	yes	γ-secretase	no		yes
PLAT (8p11 [42])	no	Aβ degradation (via plasmin)	no		yes
PLAU (10q22 [75])	yes	Aβ degradation (via plasmin)	yes	<	yes
PLG (6q26 [161])	yes	Aβ degradation (via plasmin)	ND		ND

[a] Location according to "UCSC Human Genome Browser," May 2004 assembly (URL: http://genome.ucsc.edu/cgi-bin/hgGateway).
[b] Based on concordant linkage/association regions of all currently published full-genome screens (for details see Table 1 [Bertram and Tanzi, 2004]), except for those in parentheses, which currently only show linkage/association in a single study.
[c] ">" indicates a larger number of positive than negative studies (and "<" vice versa) in the literature to date; "=" same number of positive and negative studies; "Mb" = million base pairs; "ND" = no data. For a more formal summary of studies for any specific gene, including meta-analysis, visit the "AlzGene" database (URL: http://www.alzgene.org).

decreased clearance and degradation of the Aβ peptide are possibly even more common causes of AD. Increasing evidence suggests that the low-density lipoprotein receptor-related protein (LRP) mediates the efflux of Aβ from the brain to the periphery (reviewed in Tanzi et al., 2004; Zlokovic, 2004). LRP is a multifunctional signaling and scavenger receptor that can bind a variety of ligands including apolipoprotein E (apoE), α2-macroglobulin (α2M), and APP (reviewed in Herz, 2003). LRP has been shown to play a key role in exporting Aβ. LRP antagonists have been shown to specifically reduce the efflux of Aβ from brain by up to 90% (Shibata et al., 2000). Additionally, cerebral amyloid load was doubled in receptor-associated protein (RAP) knockout mice with resulting low levels of LRP at the blood brain barrier (Van Uden et al., 2002). When undergoing LRP-mediated export form the brain, Aβ can form a complex with the LRP ligands, α2M or apoE, on the abluminal side of the endothelium. These complexes bind to LRP, are internalized to late endosomes, and are then either delivered to lysosomes where they are degraded or undergo transcytosis across the blood brain barrier into the plasma (reviewed in Herz, 2003; Figure 1). Alternatively, Aβ can be exported from the brain by directly binding LRP (Deane et al., 2004), although this route of export into the plasma appears to be limited to soluble forms of Aβ.

The genes encoding LRP (*LRP1*) and its receptor α2M (*A2M*) both map to a region of chromosome 12 that has been genetically linked to AD (reviewed in Bertram and Tanzi, 2004; Table 2) and tested for association in many studies. However, variants in neither gene are significantly associated with risk for AD in meta-analyses across studies using a case-control design (http://www.alzgene.org). Family-based studies, on the other hand, appear to support an association of *A2M* with AD; four reports present significant evidence for genetic association, while the two others studies showed marginally significant results for at least one of the two polymorphisms (reviewed in Saunders et al., 2003). Taken together, these findings indicate that *A2M* may predominantly be a risk factor of relatively small effect that is concentrated in late-onset AD cases with a family history. In contrast to these results, the only family-based study performed on *LRP1* to date does not support an association of this gene with AD (Bertram et al., 2000a).

The Genetics of Aβ Degradation

Over the past several years, increased emphasis has been placed on studies aimed at determining the proteolytic pathways by which cerebral Aβ is regulated (reviewed in Guenette, 2003; Mukherjee and Hersh, 2002; Selkoe, 2001). The proteases probably playing the most significant role in proteolyzing Aβ in vivo are neprilysin (gene: *NEP* or *MME*) and insulin-degrading enzyme (IDE; gene: *IDE*). Additionally, the plasminogen system has also been shown to participate in Aβ degradation (reviewed in Selkoe, 2001).

Chromosome 10 has been implicated to harbor one or more novel major AD susceptibility gene(s) based on reports from several groups. To date, two principal linkage regions have been described: one near 75 Mb on 10q22 (Ertekin-Taner et al., 2000; Myers et al., 2000) and a second more distal peak near 95 Mb on 10q24 (Bertram et al., 2000b; Li et al., 2002). It remains unclear, however, as to whether these signals represent the same or two independent underlying AD loci. Several candidate genes located within both linkage regions have been reported to be associated with AD. Two of these are involved in the extracellular degradation of Aβ. One is *PLAU*, which is located at 75 Mb on 10q22, and encodes the urokinase-type plasminogen activator that can activate the Aβ-degrading enzyme, plasmino-

gen (*PLG*, located on chromosome 6q26). To date, five studies have investigated a common nonsynonymous SNP leading to a proline to leucine amino acid change at codon 141, with overall insignificant results (http://www.alzgene.org; Table 2).

The second is the *IDE* gene (encoding insulin-degrading enzyme), which is located in the more distal linkage region on 10q24 and degrades a variety of different substrates with a propensity for adopting a β-pleated sheet conformation (e.g., insulin, amylin, and Aβ). Functionally, *IDE* has been shown to be able to proteolyze Aβ monomers, and recent studies of *IDE* knockout mice (Farris et al., 2003; Miller et al., 2003) and a rat mutant model (Farris et al., 2004) have confirmed that this enzyme plays a key role in modulating cerebral Aβ levels. Loss of IDE function in these animals leads to increased cerebral Aβ levels as well as features of type 2 diabetes. Interestingly, only a 2-fold increase of endogenous IDE levels has been shown to profoundly diminish cerebral Aβ deposition and instances of premature death (Leissring et al., 2003). Further support for its functional relevance in AD pathogenesis stems from the observations that IDE has also been shown to degrade the APP intracellular domain (AICD)—which is thought to play an important role in nuclear signaling and transcriptional regulation—in vitro (Edbauer et al., 2002) and in vivo (Farris et al., 2003).

Following the initial report of significant linkage and association in the immediate vicinity of the *IDE* gene in the NIMH family sample (Bertram et al., 2000b), two relatively small case-control studies failed to see evidence of association with variants in *IDE* (Abraham et al., 2001; Boussaha et al., 2002). Subsequently, however, four positive reports were published looking at a variety of independent samples (Bian et al., 2004; Blomqvist et al., 2004; Ertekin-Taner et al., 2004; Prince et al., 2003), while a study from Japan did not observe any significant effects (Sakai et al., 2004b).

Two of the other known Aβ-degrading enzymes are located on chromosome 3: *NEP* (or *MME*; 3q25), which encodes neprilysin, and *ECE2* (3q27), which encodes endothelin converting enzyme-2. Since none of the full-genome screens published to date have revealed overlapping signals in these regions of chromosome 3 (reviewed in Bertram and Tanzi, 2004), these genes must be considered *functional*, but not *positional* candidate genes. For *MME*, one study reported association with the 22-repeat allele of a dinucleotide repeat polymorphism in the promoter region of the gene (Sakai et al., 2004a), while another study found association with the major allele of a SNP in the 3′ UTR (Clarimon et al., 2003). While no study has yet directly assessed the genetic role of *ECE2*, recently a SNP in its homolog, *ECE1*, located on chromosome 1p36, has been found associated with protection from AD in a large case-control study from France (Funalot et al., 2004). Interestingly, in that same study the protective allele that is located in the *ECE1* promoter was also found to increase the transcription of mRNA in human brain samples, possibly indicating a genetic link between the Aβ-degrading activity of this protein and AD risk.

Of the two genes in the plasmin pathway that have been implicated to play roles in the degradation of Aβ (i.e., *PLG* encoding plasminogen and *PLAT* encoding the tissue-type plasminogen activator), only *PLG* maps to an AD linkage region found in at least two independent studies, i.e., on chromosome 6q26 (reviewed in Bertram and Tanzi, 2004). However, despite their potential and clinically relevant roles on AD neuropathogenesis in vivo, neither of these genes has to our knowledge yet been positively associated with AD in case-control or family-based samples (Table 2).

The Genetics of Aβ Toxicity and Inflammation

Implicit in the amyloid hypothesis is that the Aβ peptide harbors neurotoxic properties. Yet, the precise mechanism by which Aβ exerts these putative toxic effects on neurons remains unclear. Studies of synthetic Aβ in cell-based and animal models have suggested that neurotoxicity requires assembly of the peptide into oligomers. In 1998, Yankner and colleagues (Geula et al., 1998) reported that microinjection of plaque-equivalent concentrations of fibrillar, but not soluble, Aβ in the aged (but not young) rhesus monkey cerebral cortex caused neuronal loss, tau phosphorylation, and microglial proliferation. These results suggested that Aβ neurotoxicity involves a pathological response of the aging brain to fibrillar peptide assemblies. The mechanism of Aβ neurotoxicity appears to involve the induction of apoptosis most likely via the p53-Bax cell death pathway (Zhang et al., 2002). While the exact mechanism by which Aβ induces apoptosis is not known, the "channel hypothesis" maintains that certain fibrillar forms of the peptide cause neurodegeneration by forming ion channels that are generally large, voltage independent, and relatively poorly selective amongst physiologic ions (Kagan et al., 2002). Recently, a role for the mitochondria has been indicated in Aβ-induced apoptosis. The alcohol dehydrogenase, ABAD, has been reported to interact with Aβ in the mitochondria of AD patients and transgenic mice (Lustbader et al., 2004) and to potentiate Aβ-induced apoptosis and free-radical generation in neurons. Another way in which Aβ potentially evokes the generation of free radicals is by binding and reducing reactive metals such as copper, which engenders the production of hydroxyl radicals (Bush et al., 2003). Thus far, reported AD candidate genes have not fit neatly into these various hypotheses for Aβ-induced neurotoxicity.

The situation is different for another hypothesis of Aβ neurotoxicity, which is based on the presence of elevated levels of a diverse range of proinflammatory molecules in the AD brain produced principally by activated microglia clustered around senile plaques (reviewed in Bamberger and Landreth, 2001). Among the candidate genes that have been most frequently assessed in AD relating to a documented role for inflammation in the neurodegenerative process, cytokines rank among the very top. However, none of the various members of the interleukin cytokine family that have been reported to be associated with AD actually map to chromosomal regions with evidence of genetic linkage.

The genes encoding interleukin-1α (*IL1A*) and interleukin-1β (*IL1B*) both map within a gene cluster near 115 Mb on chromosome 2q12, together with the interleukin receptor antagonist (*IL1RN*) and other members of the interleukin family, while *IL6*, encoding in-

terleukin-6, maps to the short arm of chromosome 7 (~23 Mb). While a number of studies have reported significant risk effects of these genes in AD, the majority of studies have failed to replicate these findings, which is in agreement with the allele-specific meta-analyses reported on "AlzGene." These analyses revealed a protective effect of the 640-bp allele of a variable number of tandem repeat (VNTR) polymorphism in the 3′ UTR of the *IL6* gene, in agreement with a previous report (Papassotiropoulos et al., 1999), although this result is based on only three studies. Interestingly, the same allele was also found to be associated with reduced IL6 activity in humans (Murray et al., 1997), suggesting a possible functional relevance in AD pathogenesis. In contrast, none of the variants studied in *IL1B* or *IL1A* showed significant effects upon meta-analysis. It should be added, however, that several reports describing association with the –889 promoter SNP in *IL1A* actually found an age dependency of the effect, which appeared most pronounced in subjects <65 years of age (e.g., Grimaldi et al., 2000; Rebeck, 2000). Thus, although inflammation and the upregulation of inflammatory mediators like the interleukins are regularly observed in the AD brain, it appears unlikely that variation at the genomic level of these proteins makes a large contribution to AD risk in general.

The gene encoding tumor necrosis factor-α (*TNFA*) is located near a chromosomal region (~30 Mb on 6p21) that has shown genetic linkage and association with AD in a number of the full-genome screens in AD (Bertram and Tanzi, 2004). Furthermore, several other genes—some of which are also involved in inflammatory pathways—located in the same 5 Mb interval on 6p21 have been associated with AD in previous reports, including *HSPA1B* (heat shock 70 kDa protein 1B), *HFE* (hereditary haemochromatosis protein), and *HLA-A* (major histocompatibility complex, class I A). While at this time no formal meta-analyses are yet available on these candidates, it is noteworthy that of the five reports published on these genes in 2003 alone, three showed at least some degree of association (Bertram and Tanzi, 2004).

From Genetics to Novel Therapeutics

Figure 1 illustrates some of the genes known to influence the Aβ life cycle from generation to aggregation to clearance, along with possible therapeutic interventions that have been suggested by the pathways in which these genes participate. Aβ generation in brain is dependent on the activities of the known EOFAD genes *APP*, *PSEN1*, and *PSEN2* along with the genes encoding *BACE* (β-secretase) and the three proteins besides *PSEN1&2* in the γ-secretase complex, *NCSTN, APH1A*, and *PEN2.* Therapeutic interventions in this pathway have included β- and γ-secretase inhibitors, although problems have arisen with these approaches because these proteases also process other critical substrates, e.g., γ-secretase processes Notch (De Strooper and Konig, 1999; Saura et al., 2004) and Nectin (Kim et al., 2002). Thus, there is a need to identify inhibitors that are selective for APP. Candidate γ-secretase inhibitors of this type include nonsteroidal anti-inflammatory drugs (NSAIDs; Weggen et al., 2003) and cholesterol-lowering drugs that inhibit the enzyme acyl-coenzyme a: cholesterol acyltransferase 1 (ACAT1; Hutter-Paier et al., 2004).

Once Aβ is secreted, metals such as zinc (Zn) and copper (Cu) and the established late-onset AD risk factor, *APOE*, can modulate Aβ oligomerization into fibrils. Either metal-complexing compounds, (Ritchie et al., 2003) or aggregation blockers (reviewed in Citron, 2004; Tanzi et al., 2004), that prevent β-pleated sheet formation could be useful as therapeutic interventions for oligomerization. Another potential therapy, the amyloid vaccine, has been proposed to retain Aβ in the plasma, precluding transport back into brain and enhancing the possibility for delivery to peripheral sites of degradation such as the liver and kidney (DeMattos et al., 2004). Alternatively, anti-Aβ antibodies generated via the amyloid vaccine may gain entry into brain and activate microglial digestion of Aβ (reviewed in Citron, 2004; Tanzi et al., 2004). Finally, Aβ can undergo direct degradation by proteases such as IDE (which only cleaves monomeric peptide), neprilysin, and plasmin. This can presumably occur either in brain or at peripheral sites of degradation. Selective potentiation of these peptidolytic activities could be utilized to treat or prevent AD (reviewed in Tanzi et al., 2004).

In addition to therapies based on curbing the production of Aβ, or enhancing its clearance, another therapeutic strategy would be aimed at attenuating Aβ toxicity and neuroinflammation in AD brain. Perhaps, the most currently effective way to approach blocking Aβ toxicity would be to prevent the formation of neurotoxic Aβ protofibrils (Caughey and Lansbury, 2003) employing an anti-aggregation strategy. Alternatively, therapies aimed at decreasing inflammatory responses in brain, including those aimed at cytokine pathways, could also provide potential benefit for AD patients (Rogers and Lahiri, 2004).

Conclusion

From Alois Alzheimer's earliest experimental forays into the AD brain in 1906 to Glenner and Masters' seminal biochemical analyses of β-amyloid in 1984, the isolation of *APP* in 1987, and the identification of the first pathogenic mutations in *APP* in the early 1990s, the "amyloid hypothesis" has firmly taken root over the last two decades. While rare but fully penetrant autosomal-dominant mutations in the known EOFAD genes primarily affect the generation of Aβ (particularly, $A\beta_{42}$), the well-established late-onset risk factor, *APOE*-ε4, appears to influence cerebral Aβ oligomerization and clearance (e.g., via export out of brain). Biological studies have revealed several other proteins that appear to play key roles in Aβ production and clearance, including those involved in β- and γ-secretase activity (e.g., BACE, nicastrin, pen-2, and aph-1), proteins involved in Aβ peptidolytic degradation (e.g., neprilysin, IDE, and PLAU), as well as those involved in Aβ export from brain (e.g., α2M and LRP).

While *APP*, the presenilins, and *APOE* represent the only firmly established AD genes to date, the other genes described in this review remain at best functional and/or positional *candidates*. Their potential contributions and impact on AD risk remain to be firmly estab-

lished, especially since they have not yet been shown to harbor clearly pathogenic mutations or functionally active DNA variants that predispose to AD. However, some of these loci exhibit genetic linkage and/or association with AD across independent datasets and are thus worthy of further investigation at both the genetics and functional levels.

Continuing genetic analyses of putative AD loci will elucidate whether and how they factor into the complex genetic matrix underlying the inheritance for developing AD. In studies of late-onset AD genetics carried out to date, only *APOE-ε4* has been universally supported as a risk factor. Based on the results of multiple independent genome screens aimed at localizing novel AD loci, it may be unreasonable to expect another single gene with effects on AD risk that are similar to those of *APOE*, although simulations suggest that such genes do exist (Warwick Daw et al., 2000). The majority of the remaining genes are likely to exert only minor to moderate effects on AD risk, and many are likely to operate interactively with each other and/or nongenetic factors. It is also possible that common variants may play a lesser role in contributing to risk for complex diseases than previously assumed. In this case, at least some of the remaining late-onset and/or early-onset AD genes may lead to neurodegeneration via the action of rare and still elusive mutations, similar to those found in EOFAD, as opposed to the effects of more common susceptibility polymorphisms.

Clearly, the task of validating novel late-onset AD genes beyond *APOE* has and will remain immensely challenging. Fortunately, help is on the way. First, the advent of more sophisticated statistical methods for detecting bone fide genetic effects in family-based and case-control association analyses along with increasing numbers of SNPs saturating the genome and emerging genomic data regarding their organization into haplotype blocks will foster genetics analyses. Second, the ongoing collection of larger, more uniformly ascertained and evaluated AD samples will also facilitate such analyses. And, finally, the increasing ability to effectively predict and test the potential functional consequences of coding as well as noncoding SNPs should help accelerate studies aimed at demonstrating possible pathogenic consequences of disease-associated DNA variants.

Ultimately, the identification of the remaining genes involved in AD will enable investigators and clinicians to further delineate the pathobiological events leading to AD-related neurodegeneration. In addition, knowledge gained from these analyses should facilitate the development of effective strategies for the treatment and prevention of AD that are "personalized" to one's own genome and genetic risk factors. Although, achieving this goal is still relatively far off into the future, the hard-earned data garnered from the four well-established AD genes have suggested that the most effective means for preventing AD will likely involve either curbing production of Aβ (and particularly $A\beta_{42}$) or accelerating the clearance and degradation of this peptide in the brain. It is conceivable that at some point in this century, a genetically personalized medical approach—following strict legal safeguards—will exploit genetics-based findings to guide both prediction of disease risk and advanced drug development to effectively treat and prevent this formidable disease.

Acknowledgments

This work was sponsored by grants from the NIMH, NIA (ADRC), and the Alzheimer Association. L.B. was supported by the Deutsche Forschungsgemeinschaft (DFG), the Harvard Center for Neurodegeneration and Repair (HCNR), and the National Alliance for Research on Schizophrenia and Depression (NARSAD). We would also like to thank Dr. U.F. for his helpful comments and continuing inspiration.

Dr. Bertram is a consultant to Neurogenetics, Inc. and holds equity or stock options with Neurogenetics, Inc. and Prana Biotechnologies, Ltd. Dr. Tanzi is a consultant or serves on the scientific advisory board and Board of Directors of Neurogenetics, Inc. and Prana Biotechnology, Ltd. He holds equity or stock options with Neurogenetics, Inc., Prana Biotechnology, Ltd., and the Elan Corporation, Plc. He has received consulting or lecture fees from Novartis, Inc., Aventis Pharma, Inc., Eisai, Inc. and PureTech Ventures, LLC.

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