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Genetics of β-Amyloid Precursor Protein in Alzheimer's Disease

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Alzheimer's disease (AD) is characterized neuropathologically by neuronal cell loss, extracellular neuritic plaques composed of β-amyloid (Aβ), and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein. Aβ is generated by proteolytic processing of the β-amyloid precursor protein (APP). Most individuals with Down syndrome (DS) have three copies of *APP,* leading to elevated APP expression, increased Aβ deposition, and characteristic AD neuropathology. Sequencing of *APP* in familial early-onset AD identified missense mutations that cause AD, while a recently discovered coding variant, APP A673T, reduces the risk for AD. Cellular and animal studies show that risk-associated mutations increase total Aβ levels, Aβ42 levels, or Aβ fibrillogenesis, while protective alleles reduce Aβ levels. Together, these studies provide compelling evidence for the Aβ hypothesis and suggest that therapeutics that reduces Aβ levels or Aβ fibrillogenesis should lower the risk for or prevent AD.

Alzheimer's disease (AD) exists as two genetically distinct forms: familial AD (fAD), which is usually characterized by the clinical onset before 60 years of age and Mendelian inheritance, and late-onset or sporadic AD (sAD) (Sadowski et al. 1999), which usually has a clinical onset after 60 years of age and exhibits no consistent pattern of inheritance (Bertram and Tanzi 2005). Early-onset fAD, which represents <1% of AD cases, is caused by rare and fully penetrant mutations in three different genes encoding β-amyloid precursor protein (APP) on chromosome 21, presenilin-1 (*PSEN1*) on chromosome 14, and presenilin-2 (*PSEN2*) on chromosome 1. (For additional details on the presenilin complexes, see Johnson et al. 2016.) In contrast, the most common form of the disease, late-onset or sAD, probably reflects the cumulative effects of both common and rare genetic risk factors and the environment. The ε4 allele of the *apolipoprotein E* (*APOE*) gene is the most common risk factor for AD and is associated with a dose-dependent increase in the risk of developing late-onset AD (a threefold increase for one copy of *APOE4* and a 10-fold increase for two copies of *APOE4*) and decrease in age at onset.

The human *APP* gene was first identified in 1987 using partial protein sequence information from purified β-amyloid (Aβ) to identify the corresponding cDNA (Kang et al. 1987). The gene was mapped to chromosome 21 (21q21.2-3) (Goldgaber et al. 1987; Tanzi et al. 1987). APP is a type I membrane protein with a large extracellular domain and a short cytoplas-

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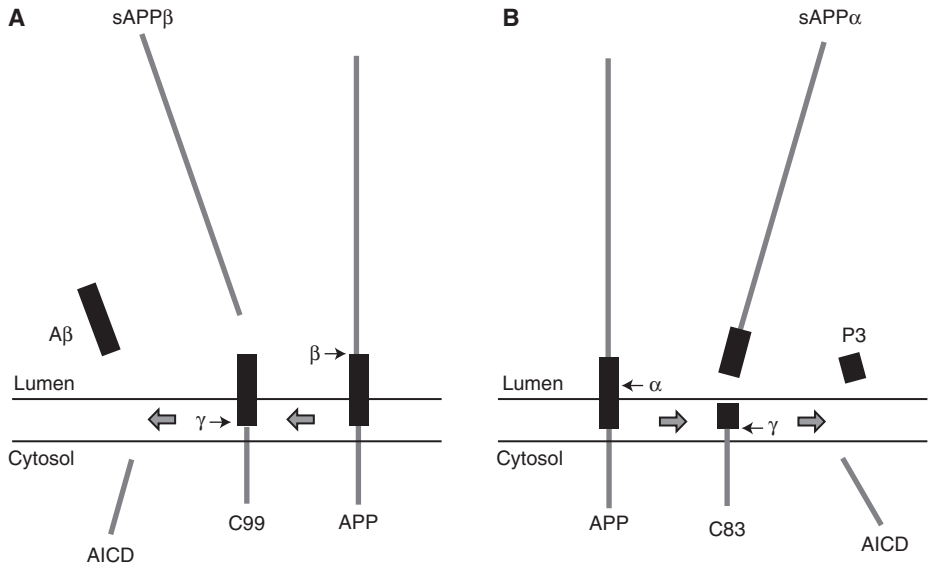

Figure 1. Processing of β-amyloid precursor protein (APP) by the secretases: (*A*) amyloidogenic processing and (*B*) nonamyloidogenic processing.

mic region. Two cleavage events, one in the extracellular domain (β-secretase cleavage) and one in the transmembrane region (γ-secretase cleavage), are necessary to release Aβ from APP (Fig. 1A). Several different APP proteins can be derived by alternative splicing from this single gene (695–770 amino acids). The major splice form in neurons is APP695 (Sandbrink et al. 1996).

The precursor proteins are proteolytically cleaved by two distinct pathways. In the nonamyloidogenic processing pathway, APP is cleaved within the Aβ domain by α-secretase, with the formation of a large soluble ectodomain (sAPPα) and an 83-residue membrane-associated C-terminal fragment (C83) (Fig. 1B). Several members of the ADAM family of proteases have α-secretase activity. Subsequent cleavage of C83 by γ-secretase leads to the formation of P3 and the APP intracellular domain (AICD) (Fig. 1B). In the amyloidogenic pathway, APP is cleaved at the N-terminus of the Aβ domain by β-secretase or BACE, a membrane-tethered protease, resulting in the generation of a soluble ectodomain (sAPPβ) and a 99-residue, membrane-retained C-terminal fragment (C99) (Fig. 1A). Subsequently, γ-secretase, a membrane-embedded complex with presenilin as the catalytic component, cleaves C99 to release Aβ peptides and AICD (Fig. 1A) (Haass 2004). Because the site of γ-secretase cleavage is promiscuous, it generates Aβ peptides with different C termini, including Aβ1–40 (Aβ40), Aβ1–42 (Aβ42), and other minor species (Ling et al. 2003; Kaminsky et al. 2010). Under normal physiological conditions, Aβ40 is the most abundant species generated in the amyloidogenic pathway, with Aβ42 representing only 10% of total Aβ (Wiltfang et al. 2002). However, Aβ42 is considered the harmful peptide because it is more prone to fibril formation and promotes Aβ aggregates, which are the key effectors of neurotoxicity (Wolfe and Guenette 2007; see also Prusiner 2016; Tycko 2016).

Although mutations in the *APP* gene explain only a small proportion of AD cases, these mutations, including duplication of *APP* and missense mutations, directly implicate Aβ generation as a causal factor in AD pathology. The mechanistic link between AD and *APP* has been further strengthened by studying Down syndrome (DS).

DOWN SYNDROME: TRISOMY OF CHROMOSOME 21

DS is the most common human aneuploidy, caused by trisomy of all or part of human chromosome 21 (HSA21) (Patterson 2009). This additional genetic material alters brain development and causes lifelong intellectual disability. Interestingly, AD pathology occurs at a high frequency in DS patients and progresses in an age-dependent manner. All individuals with DS caused by complete trisomy of HSA21 develop a neuropathology indistinguishable from AD by the age of 30–40 years (Burger and Vogel 1973; Oyama et al. 1994), and 67% develop an AD-type dementia by the age of 72 (Wisniewski et al. 1985; Mann and Esiri 1989; Zigman 2013). Multiple brain regions in individuals with DS undergo significant atrophy and loss of neurons with increasing age. The brains of older adults with DS show more than 40% of total volume loss and a 90% reduction in neuronal density in the entorhinal cortex. Additionally, like individuals with AD, individuals with DS display an age-dependent Aβ deposition, progression of neuroinflammation, neurofibrillary tangles, hyperphosphorylation of the microtubule-associated protein tau, and degeneration of basal forebrain cholinergic neurons (Hof et al. 1995; Sadowski et al. 1999). Aβ deposits begin to appear in individuals with DS as young as 10 years of age but are consistently found in the brains of DS individuals over 40 (Rumble et al. 1989). The abnormal accumulation of Aβ in the brains of both AD and DS patients induces cognitive decline through neural dysfunction. Because the *APP* gene is located on HSA21, it is present in three copies in DS individuals, leading to overexpression of APP and increased generation of Aβ. Even in the brains of fetuses with DS, the excess gene dosage of *APP* leads to early elevation of Aβ levels (Teller et al. 1996). It has been hypothesized that the triplication of *APP* in DS leads to AD symptoms early in life through overexpression of APP (Rumble et al. 1989), followed by deposition of Aβ and neurodegeneration (Wisniewski et al. 1985). Studies of DS, therefore, strongly support *APP* as a candidate causal gene in AD. However, a caveat to this is that many other genes are encoded by chromosome 21 and are overexpressed in DS (Hattori et al. 2000). Studies of the small percentage of DS cases that are caused by partial trisomy of HSA21 have helped to address this issue. Fine mapping in these individuals has shown that the so-called DS critical region (21q22.3), which is sufficient to produce the characteristic facies and developmental delay associated with DS, does not include the *APP* gene (Korenberg et al. 1990). However, AD neuropathology was not among the cardinal features considered for the DS phenotypes in this study. Other supportive evidence comes from the postmortem examination of a 78-yr-old woman with DS features because of a partial trisomy involving the distal 21q region. Despite her advanced age, she had no neuropathological evidence of AD (Prasher et al. 1998). Although a segment of HSA21 was triplicated, this region did not include the *APP* gene. Thus, the study of partial trisomies of HSA21 supports the hypothesis that triplication of *APP* is necessary for AD pathology in DS cases. These data also strongly suggest that DS and AD share pathogenic mechanisms and that the early onset of AD pathology in DS is in part a result of overexpression of the *APP* gene by gene–dosage imbalance (Salehi et al. 2006).

APP LOCUS DUPLICATION FAMILIES

Another feature of the mammalian genome that induces variation in gene expression is the presence of copy number variations (CNVs), which include both gene duplications and deletions. CNVs are regions of DNA, which can be variable in size, the copy number of which varies between individuals. Both common and rare de novo CNVs have been reported in the human genome (Zarrei et al. 2015). Much effort has been expended to identify and map CNVs in normal individuals and in disease (Henrichsen et al. 2009). Duplication of a region of HSA21 containing the *APP* gene has been reported to cause AD in several families with an autosomal-dominant form of the disease (Rovelet-Lecrux et al. 2006; Sleegers et al. 2006). Genomic duplications of small regions of HSA21, including

the *APP* locus, have been reported in nine families of different ethnic origins—French, Dutch, Japanese, and Swedish (Rovelet-Lecrux et al. 2006; Sleegers et al. 2006; Guyant-Marechal et al. 2008; Kasuga et al. 2009; Thonberg et al. 2011). These families have different overlapping duplications that each includes the *APP* locus. None of the families exhibited any clinical features suggestive of DS, other than progressive dementia of the AD type. Neuropathological examination of these brains showed abundant Aβ deposits and neurofibrillary tangles in the parenchyma and the induction of Aβ-related cerebral amyloid angiopathy (CAA) in the cerebral vasculature (Ellis et al. 1996; Pfeifer et al. 2002; Guyant-Marechal et al. 2008).

To address how far the duplications extend into the flanking chromosomal regions and investigate whether any neighboring genes were duplicated in addition to *APP*, copy-number assays targeting the genes around *APP* were analyzed in these families. Further fine mapping of the chromosomal region by array-comparative genome hybridization confirmed the presence of a genomic duplication of the *APP* gene and evaluated the size of the duplicated region. The measured size of duplicated segments, including the *APP* locus from five French families, ranged from 0.58 to 6.37 Mb and contained from five to 12 annotated genes, centromeric to the DS critical region (Rahmani et al. 1989). Compared to the French families, the duplicated genomic region in early-onset AD Dutch patients was much smaller (0.7 Mb) and included no other genes but *APP* (Sleegers et al. 2006). A real-time quantitative polymerase chain reaction of the *APP* promoter confirmed that the genomic duplication included *APP* and its promoter region but none of the adjacent genes. This suggests that a genomic duplication of *APP* is sufficient to cause the mixed phenotype of AD and CAA, without contribution from any of the adjacent genes. The size of the duplicated region in the Swedish cases was also small, 1.01–1.09 Mb, and included no flanking genes.

In the French families, a genomic duplication in the *APP* locus was observed in five out of 65 families (nearly 8%) with early-onset AD (Rovelet-Lecrux et al. 2006). In the Dutch population-based sample, *APP* duplications were detected at a frequency of one in 10 (10%) of the early-onset AD cases (Sleegers et al. 2006). While in the Swedish sample, one in 22 individuals (4.5%) diagnosed with clinical early-onset AD carried a duplication on HSA21, including the *APP* locus (Thonberg et al. 2011). Together with the data from partial trisomies of HSA21, these families provide compelling evidence that duplication of *APP*, resulting in overexpression of APP and elevated Aβ levels, is sufficient to cause AD pathology and CAA.

MISSENSE MUTATIONS IN THE *APP* GENE AND ITS NEUROPATHOLOGICAL PROFILE

Like the duplications in *APP*, most missense variants in *APP* are associated with autosomal-dominant inheritance of AD, usually with complete penetrance by age 60 (www.molgen.ua.ac.be/ADMutations). An autosomal-recessive mutation at codon 673 of *APP* was recently discovered and reported to be associated with AD (Giaccone et al. 2010). The *APP* gene is encoded by 18 exons that are alternatively spliced to produce proteins ranging in size from 695 to 770 amino acids. The Aβ peptide is encoded by parts of exons 16 and 17 (Yoshikai et al. 1990). To date, 26 pathogenic missense mutations have been reported within the *APP* gene (Table 1). These mutations are located within or immediately flanking the Aβ sequence. Aβ is generated from APP by the sequential cleavage of two enzymes, β-secretase and γ-secretase (Figs. 1A and 2). We will discuss further the mechanisms by which these mutations act and display their neuropathological hallmarks.

Table 1. Number of pathogenic mutations in *APP* gene in each type and domain

Type	Domain	Number of mutations
APP duplication	Entire sequence	26 (50.0%)
APP missense mutation	N terminal	1 (1.9%)
	Aβ sequence	11 (21.2%)
	C terminal	14 (26.9%)
Total		52

Autosomal-Dominant Mutations in *APP* and Their Associated Neuropathological Profiles

Mutations in the N-Terminal Aβ Domain and Their Effects on Aβ Formation

A double mutation in exon 16 of *APP* at codons 670 and 671 (using the numbering associated with the longest transcript of *APP*, APP770) was identified in a Swedish fAD pedigree (Mullan et al. 1992). These mutations result in a lysine-to-asparagine substitution at codon 670 and a methionine-to-leucine substitution at codon 671 (K670N/M671L) in the APP protein (Fig. 2; Table 1). These mutations are located at the N terminus of Aβ at the β-secretase cleavage site within the extracellular domain of APP. The K670N/M671L mutation appears to augment the production of total Aβ, resulting in higher levels of both Aβ40 and Aβ42 in vitro (Busciglio et al. 1993; Citron et al. 1994). In addition, a significant threefold increase in Aβ release is detected in peripheral fibroblasts from individuals carrying the Swedish mutation.

Mutations within the Aβ Domain and Their Effects on Aβ Formation

Ten pathogenic mutations have been reported within the Aβ sequence: D678N, E682K (Leuven mutation), A692G (Flemish mutation), E693Q (Dutch mutation, HCHWA-D), E693K (Italian mutation), E693G (Arctic mutation), E693del, D694N (Iowa mutation), L705V, and A713T (Fig. 2; Table 1). The first pathogenic mutation to be reported in the *APP* gene was APP E693Q, reported in a Dutch family with an inherited cerebral hemorrhage with amyloidosis (HCHWA-D) in which the amyloidosis is caused by Aβ (Levy et al. 1990). In vitro analysis of the full-length Aβ peptide or fragments containing the E693Q mutation has revealed that the mutated peptide aggregates and forms amyloid-like fibrils much faster than the wild-type (WT) Aβ sequence (van Duinen et al. 1987; Wisniewski et al. 1991).

Mutations within the Aβ domain can have complicated effects on APP processing, including impaired α-secretase cleavage and increased hydrophobicity of secreted Aβ species, thereby enhancing aggregation of Aβ into amyloid fibrils (Wisniewski et al. 1991; Haass et al. 1994). For example, E693G enhances Aβ protofibril formation (Nilsberth et al. 2001). In contrast to other fAD cases with predominantly Aβ42 plaques in the brain, it has been shown that AD patients carrying the A692G mutation predominantly deposit Aβ40, particularly in the vascular walls (Hendriks et al. 1992). Mutations associated with elevated Aβ40 species are associated with significant CAA and hemorrhagic stroke.

Mutations in the C-Terminal Aβ Domain and Their Effects on Aβ Formation

The first mutation to be associated with fAD was V717l in exon 17 of *APP* in a family of British origin with an age at onset in the mid-50s (Goate et al. 1991). Since this report, many other families have been reported carrying this same mutation as well as several other mutations at the same amino acid (V717G, V717L, and V717F). Early-onset AD mutations close to the γ-secretase cleavage site within the transmembrane domain of APP are located at APP714–717 and near the ε-secretase cleavage site at APP723–724. Fourteen mutations have been reported in the C-terminal Aβ domain, including T714l/A, V715M/A, V716V/T/F/M, and V717l/G/L/F at codons 714–717, and L723P and K724N at codons 723–724 (Fig. 2; Table 1). These mutations influence the activity of their respective secretases, resulting in aberrant processing of APP. Indeed, these *APP* mutations near the C-terminal region of the Aβ sequence lead to a selective increase in the production of longer Aβ peptides, especially those ending at residue 42 (Aβ42), which is prone to more rapid aggregation (Hardy 1997).

Neuropathological Profile of Autosomal-Dominant Mutations

Autosomal-dominant *APP* mutations close to the sites of β- or γ-secretase cleavage and flanking the Aβ sequence cause either elevated levels of total Aβ production or a specific increase in Aβ42 peptides, which are more hydrophobic

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Figure 2. Scheme depicting the structure of the *APP* sequence (*top*) and the location of pathogenic substitutions in *APP* (*bottom*).

and thus more prone to fibrillogenesis (Suzuki et al. 1994; Tamaoka et al. 1994; Younkin 1994). However, those that alter amino acids within the Aβ domain facilitate rapid aggregation and fibrillization (Levy et al. 2006). Mutations that cause an increase in Aβ42 peptides are associated with the classical clinical and neuropathological features of AD, including progressive dementia and senile plaques, neurofibrillary tangles, and neuronal cell loss (Rocchi et al. 2003). In contrast, mutations that are associated with an increase in total Aβ also have prominent Aβ deposition in cerebral vessels (CAA) and can be clinically associated with cerebral hemorrhages and stroke (Kumar-Singh et al. 2002; Castellani et al. 2004). Although both CAA and senile plaques are observed in the brains of patients with DS, HCHWA-D, and AD, mutations within the Aβ sequence are predominantly vasculotropic and lack neurofibrillary tangles (Obici et al. 2005; Bugiani et al. 2010). These mutations are also associated with more severe CAA, which is widely distributed throughout the brain (van Duinen et al. 1987; Hendriks et al. 1992; Rossi et al. 2004; Rovelet-Lecrux et al. 2006).

Autosomal-Recessive Mutation and Its Neuropathological Profile

The A673V Mutation and Its Effects on Aβ Formation

A novel *APP* mutation, an alanine-to-valine substitution at codon 673 (A673V), has been observed as a homozygous recessive mutation in a single Italian pedigree (Fig. 2) (Giaccone et al. 2010). Patients with A673V have early-onset AD with behavioral abnormalities at the onset and neurological deficits in later stages. On the basis of the formal neuropsychological assessment, individuals heterozygous for this mutation (aged between 21 and 88 years) had no signs of cognitive decline even at an advanced age.

The A673V*APP* variant shifts APP processing toward the amyloidogenic pathway, with increased production of Aβ peptides, and markedly enhances the aggregation and fibrillogenic properties of both Aβ40 and Aβ42 (Giaccone et al. 2010). Aβ40 is markedly increased in the insoluble fraction and is predominant over Aβ42, suggesting that this mutation strongly enhances the formation of Aβ40 aggregates (Di Fede et al. 2012). However, in vitro studies with synthetic peptides revealed that co-incubation of Aβ containing the A673V variant and WT Aβ species resulted in reduced amyloidogenesis and neurotoxicity of Aβ, consistent with the observation that heterozygous carriers do not develop the disease. These opposite effects of the A673V mutation on amyloidogenesis, in the homozygous and heterozygous states, likely account for the autosomal-recessive pattern of inheritance.

Neuropathological Profile of the A673V Mutation in the Homozygous State

Patients homozygous for the A673V mutation are characterized neuropathologically by the presence of both plaques and tangles but show several distinctive features compared with AD patients carrying dominant mutations (Giaccone et al. 2010). Patients displayed abundant Aβ deposits and CAA in all areas of the cerebral cortex, but the Aβ deposits were unusually large (up to 120 mm in diameter), and the localization of Aβ deposits was consistently perivascular.

PROTECTIVE RECOMBINANT A673T

At the same position in the Aβ peptide, APP673, where the recessive mutation was found (Fig. 2), three homozygous carriers of an alanine-to-threonine substitution (A673T) were discovered by whole-genome sequencing in Icelandic samples (Jonsson et al. 2012). None of these homozygous carriers, whose ages ranged from 67 to 88 years, had a history of dementia. This substitution is adjacent to the β-secretase cleavage site in the *APP* gene and results in an ~40% reduction in the formation of Aβ peptides in vitro (Jonsson et al. 2012). This variant was found to be significantly more common in the aged control group than in AD cases (0.62% vs. 0.13%; odds ratio = 5.29; $P = 4.78 \times 10^{-7}$), suggesting that A673T reduces the risk for AD.

A673T is a rare variant, with a reported frequency of 0.2%, 0.4%, 0.45%, and 0.5% in the Norwegian, Swedish, Icelandic, and Finnish general populations, respectively, whereas it has not been observed in elderly individuals of Asian descent and is rarely seen in the U.S. population (Jonsson et al. 2012; Kero et al. 2013; Liu et al. 2014).

In vitro studies show that A673T results in a reduced production of sAPPβ and ~40% less Aβ40 and Aβ42 compared with WT APP, suggesting that A673T reduces BACE1 (β-secretase) cleavage of APP. To further confirm the protective effect of A673T against AD, an in vitro BACE1 cleavage assay was performed with a WT synthetic APP peptide and a peptide bearing the A673T substitution. The A673T APP peptide was processed ~50% less efficiently than the WT substrate, suggesting that A673T carriers impaired BACE1 cleavage of APP (Jonsson et al. 2012). It has been shown that the A673T substitution decreases the catalytic turnover rate of APP by BACE1, thereby reducing Aβ aggregation (Maloney et al. 2014). Thus, this mutation prevents Aβ aggregation by inhibiting the generation of Aβ peptide from APP. The observation of this rare protective variant that decreases Aβ production and reduces the risk of AD provides compelling evidence in support of the amyloid hypothesis: mutations that increase Aβ levels increase the risk for AD, whereas those that decrease Aβ levels reduce the risk for the disease (Fig. 3) (Hardy and Selkoe 2002).

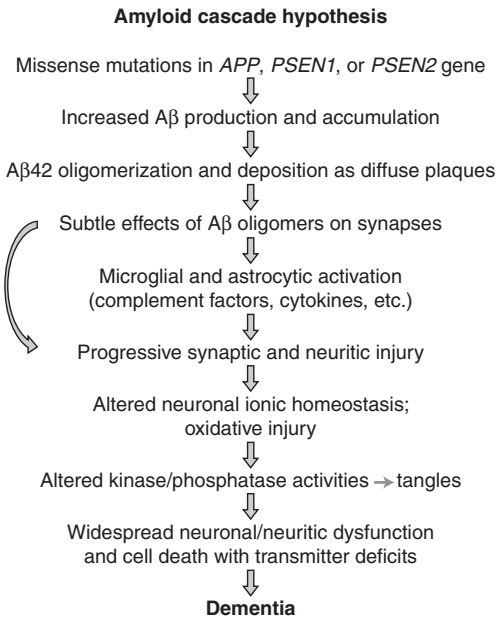

Figure 3. Proposed sequence of pathogenic events leading to Alzheimer's disease.

CONCLUDING REMARKS

The discovery of mutations in *APP* that increase or decrease Aβ production and the risk for AD provides strong support for the amyloid cascade hypothesis, which posits that accumulation of Aβ is the primary effector of AD pathogenesis (Fig. 3) (Hardy and Selkoe 2002). The amyloid hypothesis proposes that AD is caused by altered APP expression or *APP*-mutation-induced Aβ aggregation, following an imbalance between Aβ production and Aβ clearance.

Genetics and genomic studies of *APP* have identified 52 pathogenic mutations in *APP* that can lead to Aβ deposition in the brain parenchyma and in cerebral blood vessels. Studies of these families have conclusively shown that overexpression of the normal *APP* sequence (trisomy 21 or *APP* duplication) or mutations that lead to elevated total Aβ, elevated Aβ42, or increased Aβ aggregation lead to dementia and AD neuropathology. In the presence of elevated total Aβ, there is also widespread CAA and hemorrhagic stroke. In contrast, mutations that decrease Aβ levels substantially reduce the risk of developing AD.

Introduction of missense mutations in human *APP* into transgenic mice has enabled the recapitulation of at least some aspects of AD. These mice show an age-dependent deposition of Aβ in the brain. As is observed in human patients, mice carrying the Swedish mutation (Tg2576) develop diffuse and dense-cored plaques in the brain as well as severe CAA (Hsiao et al. 1996), whereas mice expressing mutations at the C terminus of Aβ, such as the V717F mutation, develop plaques but not CAA (Games et al. 1995). These animals do not exhibit neurofibrillary tangles or widespread neuronal loss, but they do exhibit some synapse

loss. Furthermore, mice unable to generate Aβ, such as the $BACE^{-/-}$ mouse, show no neuronal loss and improved cognitive function (Ohno et al. 2004). Together, the human and animal studies strongly support the notion that a therapeutic strategy aimed at reducing Aβ levels by either inhibiting production or increasing clearance could be useful, particularly if applied early enough during the course of disease.

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