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GENES IMPLICATED IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE

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1. ABSTRACT

Both the early and late-onset Alzheimer's disease affect millions of people throughout the world. A number of molecules have been implicated in the pathogenesis of Alzheimer's disease. These include presenilin 1 and 2 (PS1 and PS2), a β-amyloid peptide, and tau protein. Presenilin 1 and 2 genes implicated in the early-onset familial Alzheimer's disease have been cloned. Both PS1 and PS2 are integral membrane proteins and may function as receptors or channel proteins. Missense mutations in PS1 and PS2 genes have been found in families that cosegregate with early-onset Alzheimer's disease. Overexpression of the mutated PS1 gene produced amyloid plaques in the brain of *transgenic* mice. Secreted β-amyloid protein similar to that in the senile plaques of Alzheimer's disease was found to be elevated in fibroblast media from subjects with PS1 or PS2 mutations. *Transgenic* mice which carried the mutant form of the β-amyloid precursor protein gene expressed high concentrations of mutant copy of the gene and exhibited abundant amyloid plaques in the brain and memory loss. The mutated PS2 gene enhanced apoptotic activity. This enhanced apoptotic activity may accelerate the process of neurodegeneration leading to an earlier age in the onset of the disease. Identification of lesions in the molecules that are important in the Alzheimer's disease should allow developing therapeutic approaches for its treatment.

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2. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by a progressive decline in memory, judgment, ability to reason, and intellectual function and is accompanied by a wide range of neuropathologic features including extracellular amyloid plaques and intra-neuronal neurofibrillary tangles (1, 2). The cause of AD is unknown and as yet no cure for this disease is available. AD afflicts approximately 4 million American adults. More than 100,000 victims die annually of complications of AD making it the fourth leading cause of death in adults after heart disease, cancer, and stroke. The disease is caused by the degeneration of neurons in the brain. Complications associated with AD usually result in death within 10 years from the onset of the disease.

Although the etiology of this disease is complex, at least three genetic loci that confer inherited susceptibility to this disease have been identified. A locus (AD3) mapped by linkage studies to human chromosome 14q24.3 may account for 70% of early-onset (onset at age 30-50 years) autosomal dominant AD (3). Early-onset AD comprises up to 10% of all cases of AD (3-6). Mutations in the gene for the β-amyloid precursor protein (APP) have been found in a small number of families (<3% of cases) with a disease onset before 65 years of age (7, 8). The

apoE4 allele of the apolipoprotein E (apoE) is associated with a significant proportion of cases with late onset (>60 years) AD which comprises more than 90% of AD cases (9).

Recently two early onset AD genes (S182 and STM2) have been identified (4, 10, 11). These genes are called presenilin 1 and 2 (PS1 and PS2), respectively. PS1 and PS2 genes are mutated in about half of all inherited cases (5, 6, 10, 11). On the other hand, mutation of the gene encoding the β-amyloid peptide BA4 is seen in a few percent of patients with AD. Mutated forms of PS1 and PS2 genes cause increased production of β-amyloid peptide BA4 from its precursor and lead to formation of abundant amyloid plaques and memory loss (12, 13). Mutated PS2 gene can cause increased apoptotic activity and thus accelerate the process of neurodegenaration (14).

In the late onset AD, an increased frequency of type 4 apolipoprotein E (apoE4) allele has been described (9). In 42 families with late onset AD, with increasing apoE4 allele, the risk of AD increased from 20% to 90% and the mean age of onset of the disease decreased from 84 to 68 years (15). ApoE4 is found in senile plaques, in neurofibrillary tangles and at the sites of congophilic angiopathy in Alzheimer's disease and in the prion protein amyloid plaque of Creutzfeldt-Jacob's disease and scrapie (16-18). ApoE4 binds to beta amyloid peptide (BA4) *in vitro* (19). The localization of apoE4 in the pathological lesions of Alzheimer's disease suggests a functional pathogenic role for apoE4. Binding of apoE4 to BA4 peptide may be a requisite step in tagging these peptides for their intra and extracellular metabolism. Genetically, apoE4 allele is found to be associated with an increased susceptibility to Alzheimer's disease and appears to be a possible risk factor or susceptibility gene in the late-onset familial and sporadic Alzheimer's disease. The association of apoE4 with AD suggests that 1) the apoE transport system is involved in the pathophysiology of the disease or 2) the apoE4 allele is in genetic dysequilibrium with another gene that is involved in the development of the disease.

3. DISCUSSION

3.1. Genes for Early-Onset Familial Alzheimer's Disease.

Recently, two genes, one on human chromosome 1 and the other on chromosome 14, thought to be implicated in most types of early-onset, familial Alzheimer's disease, have been identified (4, 10). These two genes cause 70% to 80% of early-onset familial cases, which comprise up to 10% of all cases of the disease. Schellenberg *et al.* reported that a gene causing a high percentage of familial Alzheimer's disease is present on chromosome 14 (3). Specific DNA markers were identified in families prone to AD. These markers allowed to narrow the DNA region on chromosome 14 as a possible locus for the early onset AD gene. Using gene hopping technique, Sherrington *et al.* isolated a gene that was mutated in family members with Alzheimer's disease (4). This new gene is called presenilin 1 (PS1 or S182). In seven families five different mutations in the PS1 gene were found (4).

The second cloned gene, located on chromosome 1, also encodes an integral membrane protein STM2 (10, 11). STM2 is now called Presenilin 2 (PS2) gene. The amino acid sequence of PS2 is 67% homologous to PS1. This homology indicates that PS1 and PS2 may have similar function.

3.1.1. Hypothetical Function (s) of Presenilin 1 Gene.

From its sequence, the product of the PS1 gene appears to be a protein that is firmly embedded in one of the many membranes of the cell. Therefore, PS1 can be a receptor, achannel protein, or a structural membrane protein. The PS1 gene is expressed both in neuronal and non-neuronal tissues (4). The expression of the PS1 in non-neuronal tissues does not negate its role in AD, because genesis of the AD phenotype probably requires interactions of PS1 with other proteins like APP, apoE4, and tau (4). PS1 also has some sequence homology with the membrane protein, SPE-4, of the nematode C. elegans (4). Similarity between SPE-4 and PS1 gene products suggest that they may have similar functions. Studies of worms in which SPE-4 is mutated showed that the SPE-4 gene product was needed for transporting proteins between cellular compartments during the formation of sperm (4). The similarity between PS1 and its homologue SPE-4 raises the possibility that it may also be involved in protein transport within cells (4). It has been hypothesized that PS1 may be involved with the packaging of APP in a vesicle and its delivery to portions of the neurons where it is going to be processed normally (4). Defects in the PS1 gene may cause a snag in APP's travel through the cell, possibly detaining it in a spot where it is more likely to be cleaved to β-amyloid (4).

3.1.2. *Transgenic* Mice Expressing Presenilin 1 Gene.

Recently, *transgenic* mice overexpressing mutant form of the human PS1 gene have been developed (12). These mice show highly increased concentrations of BA4 in their brains. Since these mice are only 7 months old, they can not yet be tested for Alzheimer's related pathologies, such as the formation of amyloid plaques and neurofibrillary tangles, for another few months.

3.1.3. Possible Role of the PS1 Gene in the Development of Alzheimer's Disease.

Protein produced by the PS1 gene may be needed for the proper operation of a major developmental regulatory pathway known as Notch pathway (20). Notch protein transmits signals needed to make cell-fate decisions—such as whether to develop into nerve or muscle cells (20). PS1 may play an important role in helping bring Notch protein to its normal location, the external cell membrane. Disruption of both PS1 and Notch genes in knockout mice caused death of mice due to brain

hemorrhages which implies a functional link between these two genes (20).

A large body of evidence shows that mutations in presenilin 1 somehow alter the way the cells handle amyloid precursor protein (APP). When APP is processed in cells, it can release β-amyloid (BA) fragments of varying lengths, but BA4 variant particularly appears cytotoxic. BA4 is the predominant variant of amyloid protein in the plaques of Alzheimer's disease (2). BA4 is precisely the variant increased by PS1 mutations introduced in transgenic mice (12, 13). Therefore, It has been speculated that newly synthesized APP is folded and processed in the endoplasmic reticulum and Golgi for transport to the cell interior (12, 13). Ordinarily, proteins that fold improperly are destroyed (12, 13). Mutated PS1 may allow misfolded APP to accumulate in cells, whereas the wild type protein may destroy the misfolded APP (12, 13). Misfolding, in turn, might be what causes the APP to be cut in the wrong place, releasing the extra BA4 (12, 13).

3.1.4. Mutated PS2 Gene May Enhance Constitutive Programmed Cell Death in the Brain of Alzheimer's Patients.

Recently a mouse gene called apoptosis-linked gene-3 (ALG-3) has been cloned and found to have 100% sequence homology with human PS2 gene (21). Therefore, ALG-3 is the mouse homologue of PS2 (21). Transfection of cells with ALG-3 gene conferred resistance to T cell receptor-induced cell death (21). This finding raises the possibility that cell death plays an important role in the pathophysiology of Alzheimer's disease (21).

Overexpression of PS2 in nerve growth factor-differentiated PC12 cells increased apoptosis induced by β-amyloid (14). On the other hand, transfection of antisense PS2 conferred protection against apoptosis induced by amyloid precursor protein-expressing PC12 cells (14). Mutated PS2 gene in Alzheimer's disease was found to enhance constitutive apoptotic activity (14). This enhancement, due to mutation in PS2 gene, may accelerate the process of neurodegeneration in AD (14). Therefore, constitutive activation of PS2 by mutation increases the susceptibility of neurons to apoptotic stimuli that could sensitize neurons to the harmful insults of aging, such as free radical-mediated oxidation and neurotoxicity resulting from aggregated BA4 (14). The accelerated rate of neuronal cell death that occurs in the brains of Alzheimer's patients may, therefore, result from the additive effects of activated apoptosis, aging, and BA4 toxicity (14).

3.2. Role of β-Amyloid Protein in Alzheimer's Disease.

The senile plaques of AD consists of extracellular deposits of amyloid surrounded by glial cells and degenerating neurites. The amyloid core consists largely of a 4.2 kDa peptide (BA4) derived from a larger precursor called amyloid precursor protein (APP). The APP is normally present in the plasma membrane of neurites. BA4 is the main component of the neurite plaques which, together with the neurofibrillary tangles, constitute the neuropathological features that confirm AD (2). Mutations of the APP, from which BA4 originates, are present in a limited number of AD cases (1 to 5%) (22), and altered APP processing has been proposed to result in the generation of amyloidogenic protein fragments (23). In AD, stellate neurons in layer II of the entorhinal cortex are affected early in the disease process and sustain a heavy damage. Such selective neuronal vulnerability is a product of cell-specific expression of genes and interactions of gene products that are required in the maintenance of cellular homeostasis and differentiation, and all in the context of response to external stimuli.

3.2.1. A *Transgenic* Mouse Model with Mutated Amyloid Precursor Protein Gene of Alzheimer's Disease.

A *transgenic* mouse model has been developed which carries a mutant form of the β-amyloid precursor protein gene (24). This mouse expresses high concentrations of mutant copy of the gene which is linked to Alzheimer's disease, exhibits abundant amyloid plaques and shows evidence of memory loss (24). This new *transgenic* mouse may allow unraveling the complex processes of APP expression, plaque formation, neuronal degeneration, and memory loss associated with AD.

3.2.2. β-Amyloid Induces Alzheimer's Phenotype by Virtue of Altering Potassium Channels.

β-amyloid is the main constituent of neurite plaques and may play a role in the pathophysiology of Alzheimer's disease. Mechanisms by which soluble β-amyloid might produce early symptoms such as memory loss, before diffuse plaque deposition, have not been determined. Treatment of fibroblasts with β-amyloid (10 nM) induced the same potassium channel dysfunction previously shown to occur in fibroblasts of patients with Alzheimer's disease (25, 26). Therefore, β-amyloid may alter potassium channels in AD patients and by impairing the neuronal function lead to symptoms such as memory loss by means other than plaque formation. If K^+ channel dysfunction occurs in the central nervous system neurons, it might affect brain functions such as memory storage, which has been found to involve long-term changes of K^+ channels (25).

3.3. Alzheimer's Disease; the Zinc Connection.

BA4 is the main constituent of Alzheimer's plaques, and was recently shown to be present in a soluble form in the cerebrospinal fluid (2, 9). Bush *et al*. reported that from transition metals, zinc more effectively bound to BA4 and led to the formation of amyloid clumps (27). At low concentrations such as those found in the cerebrospinal fluid, zinc bound to BA4 without causing it to clump and precipitate out of solution. But at higher concentrations, zinc led to a rapid clumping of the peptide.

The clumps were similar in size to the naturally occurring amyloid plaques, and fluoresced under the polarized light. So it is conceivable that under certain conditions sufficient concentrations of zinc cause precipitation and clumping of BA4.

3.4. Role of ApoE4 in the Pathophysiology of Alzheimer's Disease.

Apolipoprotein E is a 299 amino acid sialoglycoprotein with an apparent molecular weight of 35-39 kDa (28). ApoE has three common alleles, apoE2, apoE3 and apoE4, which differ in cysteine-arginine interchanges (28). ApoE2 contains two cysteine residues one in each of positions 112 and 158. ApoE3 contains one arginine in place of cysteine at residue 158. ApoE4 contains arginines in place of cysteines in both the positions 112 and 158.

Apolipoprotein E is one of the eight well characterized apolipoproteins involved in the cholesterol transport.

An increased frequency of apoE4 allele in late onset AD has been described (9). As compared with 31% of control subjects, 80% familial and 64% of sporadic late onset AD have at least one apoE4 allele. Homozygosity for the apoE4 allele is sufficient to cause AD by the age of 80. ApoE genotyping can be easily achieved by PCR (29)

3.4.1. Binding Properties of ApoE4.

In addition to receptor-binding properties, apoE binds to a number of other substances including glycosaminoglycans (GAGs) (30), urate crystals (31), and amyloid including the Alzheimer β-amyloid peptide A4 (19). ApoE4 has been found to be present in the senile plaques in AD (32). *In vitro*, apoE in the cerebrospinal fluid binds to synthetic beta A4 peptide (the primary constituent of the senile plaque) with a high avidity (19). Both apoE3 and apoE4 bind to the synthetic BA4 (19). This binding requires residues 12-28 of BA4 and residues 244-272 of apoE. Complex formation is mediated by oxygen and occurs an order of magnitude faster with apoE4 than apoE3. It is not known whether the dependence of apoE:BA4 complex formation on oxygen results from oxidation of apoE or from oxidation of BA4. The involvement of oxygen in binding of apoE to BA4 lends support to the suggested involvement of oxidative stress in the pathogenesis of AD. In the presence of β-VLDL, apoE3 was found to increase neurite outgrowth, whereas apoE4 decreased outgrowth (33). This suggests the involvement of apoE4 in the degeneration of the neurons.

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In normal humans, apoE is localized to astrocytes and in microglial cells. At autopsy, AD patients who are homozygous for apoE4 allele (E4E4) exhibit more highly developed senile plaques than other AD patients (34). In patients with AD, apoE is found in vascular endothelial cells in senile plaques. Exaggerated apoE gene expression was associated with low LDL cholesterol and low total cholesterol in plasma due to the higher clearance of chylomicrons, VLDL and VLDL remnants (35). Conversely, apoE gene knockout in mice resulted in a higher plasma total cholesterol due to lack of clearance of above lipoproteins by their respective receptors (36, 37). However, enhanced expression of apoE (33) or knockout of apoE gene (36, 37) have not led to disturbed brain function.

ApoE gene is localized on human chromosome 19q13.2 within the region linked to the late-onset familial AD (38). Significant association of E4 with late onset form of familial AD further indicates the direct or indirect involvement of apoE gene in the pathogenesis of AD (9). Binding of BA4 to apoE in cerebrospinal fluid and presence of chylomicron remnant receptor (LRP) to senile plaques implicate apoE and LRP in the pathogenesis of AD (9, 39).

3.5. Role of Tau Protein in the Generation of Neurofibrillary Tangles in the Alzheimer's Disease.

There are two major pathological features of AD. One is the formation of senile plaques and the other is the generation of neurofibrillary tangles. Microtubule associated protein, tau, is a major component of neurofibrillary tangles and makes up the paired helical filaments that comprise the neurofibrillary tangles (40). By binding to microtubules, tau proteins leads to their stabilization. Evidence suggests that if tau becomes phosphorylated, it loses its ability to bind the microtubules. This, presumably, allows degeneration of microtubules and ultimately leads to the death of the neurons (40). The isoform specific effects of apoE3 and apoE4, and β-VLDL on neuronal growth and branching suggest that isoform-specific effects of the interaction of apoE with tau protein or other cellular proteins may play a role in the development of AD (33). According to a theory, apoE3 strongly binds to tau protein, but apoE4 does not bind to tau (33). ApoE3 binding to tau will protect tau from phosphorylation and prevent formation of

neurofibrillary tangles in AD patients (33). On the other hand, apoE4 does not bind to tau and thus could contribute to the phosphorylation of tau which may cause the formation of neurofibrillary tangles in AD patients (33).

Proline-directed kinases such as mitogen-activated protein (MAP) kinases, cyclin-dependent protein kinase 5 (CDK5) and glycogen synthase 3 (GSK3) have been implicated in the hyperphosphorylation of the tau protein in the Alzheimer's disease (41). Alzheimer's disease may result from the accumulated defects in the proline-directed kinases such that their inappropriate activation is sustained in the affected neurons (41). Also implicated in the pathogenesis of AD are phosphatases which are involved in the regulation of phosphorylation of tau (40).

4. REFERENCES

1. H. K. Das & H. Lal. Pathogenesis of Alzheimer's Disease: Current status on apolipoprotein E4 gene research. *Rev. Neurosciences* 7, 277-283 (1996)

2. R. Katzman. Medical progress, Alzheimer's disease. *N Eng J Med* 314, 964-967 (1986)

3. G. D. Schellenberg, T. D. Bird, E. M. Wijsman, H. T. Orr, L. Anderson, E. Nemens, J. A. White, L. Bonnycastle, J. L. Weber, M. E. Alonso, H. Potter, L. L. Heston & G. M. martin. Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. *Science* 258, 668-670 (1992)

4. R. Sherrington, E. I. Rogaev, Y. Liang, E. A. Rogaeva, G. Levesque, M. Ikeda, H. Chi, C. Lin, G. Li, K. Holman, T. Tsuda, L. Mar, J. -F. Foncin, A. C. Bruni, M. P. Montesi, S. Sorbi, I. Rainero, L. Pinessi, L. Nee, I. Chumakov, D. Pollen, A. Brookes, P. Sanseau, R. J. Polinsky, W. Wasco, H. A. R. Da Silva, J. L. Haines, M. A. Pericak-Vance, R. E. Tanzi, A. D. Roses, P. E. Fraser, J. M. Rommens & P. H. St George-Hyslop. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* 375, 754-760 (1995)

5. P. St. George-Hyslop, J. Haines, E. Rogaev, M. Mortilla, G. Vaula, M. Pericak-Vance, J. F. Foncin, M. Montesi, A. Bruni, S. Sorbi, I. Rainero, L. Pinessi, D. Pollen, R. Polinsky, L. Nee, J. Kennedy, F. Macciardi, E. Rogaeva, Y, Liang, N. Alexandrova, W. Lukiw, K. Schlumpf, R. Tanjzi, T. Tsuda, L. Farrer, J. M. Cantu, R. Duara, I. Amaducci, L. Bergamini, J. Gusella, A. Roses & D. Crapper McLachlan. Genetic evidence for a novel familial Alzheimer's disease locus on chromosome 14. *Nature Genetics* 2, 330-334 (1992)

6. C. Van Broeckhoven, H. Backhovens, M. Cruts, G. De Winter, M. Bruyland, P. Cras & J. J. Martinl. Mapping of a gene predisposing to early-onset Alzheimer's disease to chromosome 14q24.3. *Nature Genetics* 2, 335-339 (1992)

7. A. Goate, M. Chartier-Harlin, M. Mullan, J. Brown, F. Crawford, L. Fidani, L. Giuffra, A. Haynes, N. Irving, L. James, R. Mant, P. Newton, K. Rooke, P. Roques, C. Talbot, M. Pericak-Vance, A. Roses, R. Williamson, M. Rossor, M. Owen & J. Hardy. Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* 349, 704-706 (1991)

8. H. Karlinsky, G. Vaula, J. L. Haines, J. Ridgley, C. Bergeron, M. Mortilla, R. G. Tupler, M. E. Percy, Y. Robitaille, N. E. Nolde, T. C. K. Yip, R. E. Tanzi, J. F. Gusella, R. Becker, J. M. Berg, D. R. Crapper McLachlan & P. H. St. George-Hyslop. Molecular and prospective phenotypic characterization of a pedigree with familial Alzheimer's disease and missense mutation in the codon 717 of the β-amyloid precursor protein gene. *Neurology* 42, 1445-1453 (1992)

9. W. J. Strittmatter, A. M. Saunders, D. Schmechel, M. Pericak-Vance, J. Enghild, G. S. Salvesen & A. D. Roses. Apolipoprotein E: High-avidity binding to beta amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci USA* 90(5), 1977-1981 (1993)

10. E. Levy-Lahad, E. M. Wysman, E. Nemens, L, Anderson, K. A. B. Goddard, J. L. Weber, T. D. Bird & G. D. Schellenberg. Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* 269, 973-977 (1995)

11. E. I. Rogaev, R. Sherrington, E. A. Rogaeva, G. Levesque, M. Ikeda, Y. Liang, H. Chi, C. Lin, K. Holman, T. Tsuda, L. Mar, S. Sorbl, B. Nacmias, S. Placentini, L. Amaducci, I, Chumakov, D. Cohen, L. Lannfelt, P. E. Fraser, J. M. Rommens & P. H. St. George-Hyslop. Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* 376, 775-778 (1995)

12. K. Duff, C. Eckman, C. Zehr, X. Yu, C-M. Prada, J. Perez-tur, M. Hutton, L. Buee, Y. Harigaya, D. Yager, D. Morgan, M. N. Gordon, L. Holcomb, L. Refolo, B. Zenk, J. Hardy & S. Younkin. Increased amyloid-β42 (43) in brains of mice expressing mutant presenilin 1. *Nature*, 383, 710-712 (1996)

13. D. Scheuner, C. Eckman, M. Jensen, X. Song, M. Citron, N. Suzuki, T. D. Bird, J. Hardy, M. Hutton, W. Kukull, E. Larson, E. Levy-Lahad, M. Viitanen, E. Peskind, P. Poorkaj, G. Schellenberg, R. Tanzi, W. Wasco, L. Lannfelt, D. Seilkoe & S. Younkin. Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nature Med.* 2, 864-870 (1996)

14. B. Wolozin, K. Iwasaki, P. Vito, J. K. Ganjei, E. Lacana, T. Sunderland, B. Zhao, J. W. Kusiak, W. Wasco & L. D'Adamino. Participation of Presenilin 2 in apoptosis: enhanced basal activity conferred by an Alzheimer's mutation. *Science* 274, 1710-1713 (1996)
E. H. Corder, A. M. Saunders, W. J. Strittmatter, D. E. Schmechel, P. C. Gaskell, G. W. Small, A. D. Roses & J. L. Hainse. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* 261, 921-923 (1993)

15. J. F. Diedrich, H. Minnigan, R. I. Carp, J. N. Whitaker, R. Race, W. Frey & A. T. Haase. Neuropathological changes in scrapie and Alzheimer's disease are associated with increased expression of apolipoprotein E and cathepsin D in astrocytes. *J Virol* 65, 4759-4768 (1991)

16. Y. Namba, M. Tomonaga, H. Kawasaki, E. Otomo & K, Ikeda. Apolipoprotein E immuno-reactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and Kuru plaque amyloid in Creutzfeldt-Jakob disease. *Brain Res* 541, 163-166 (1991)

17. SB Prusiner, KK Hsiao, DE Bredesen & SJ DeArmond. *In: Handbook of Clinical Neurology* , Ed. RR McKendall. (Elsevier, Amsterdam). 12, 543-580 (1989)

18. W. J. Strittmatter, K. H. Weisgraber, D. Y. Huang, D. Li-Ming, G. S. Salvesen, M. Pericak-Vance, D. Schmechel, A. M. Saunders, D. Goldgaber & A. D. Roses. Binding of human apolipoprotein E to synthetic amyloid beta peptide: Isoform-specific effects and implications for late-onset Alzheimer's disease. *Proc Natl Acad Sci USA* 90, 8098-8102 (1993)

19. D. Levitan, T. G. Doyle, D. Brousseau, M. K. Lee, G. Thinkaran, H. H. Slunt, S. S. Sisodia & I. Greenwald. Assessment of normal and mutant presenilin function in Caenorhabditis elegans. *Proc Natl Acad Sci USA* 93, 14840-14944 (1996)

20. P. Vito, E. Lacana & L. D' Adamino. Interfering with apoptosis: Ca^{+2}- binding protein ALG-2 and Alzheimer's disease gene ALG-3. *Science* 271, 521-525 (1996)

21. R. E. Tanzi, G. Vaula, D. M. Romano, M. Mortilla, T. L. Huang, R. G. Tupler, W. Wasco, B. T. Hyman, J. L. Haines, B. J. Jenkins, M. Kalaitsidaki, A. C. Warren, M. C. McInnis, S. E. Antonarakis, H. Karlinsky, M. E. Percy, L. Connor, J. Growdon, D. R. Crapper-Mclachlan, J. F. Gusella & P. H. St George-Hyslop. Assessment of amyloid β-protein precursor gene mutations in a large set of familial and sporadic Alzheimer's disease cases. *Am J Hum Genet* 51, 273-282 (1992)

22. S. S. Sisodia, E. H. Koo, K. Beyreuther, A. Unterbeck & D. L. Price. Evidence that beta-amyloid protein in Alzheimer's disease is not derived by normal processing. *Science* 248, 492-495 (1990)

23. K. Hsiao, P. Chapman, S. Nilsen, C. Eckman, Y. Harigaya, S. Younkin, F. Yang & G. Cole. Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. *Science* 274, 99-102 (1996)

24.R. Etcheberrigaray, E. Ito, C. S. Kim & D. L. Alkon. Soluble beta-amyloid induction of Alzheimer's phenotype for human fibroblast K^+ channels. *Science* 264, 276-279 (1994)

25. R. Etcheberrigary, E. Ito, K. Oka, B. Tofel-Grehl & G. E. Gibson. Potassium channel dysfunction in fibroblasts identifies patients with Alzheimer's disease. *Proc Natl Acad Sci USA* 90, 8209-8213 (1994)

26. A. I. Bush, W. H. Pettingell, G. Multhaup, M. Paradis, J-P. Vonsattel, J. F. Gusella,Beyreuther, C. L. Masters & 27. R. E. Tanzi. Rapid induction of Alzheimer Aβ amyloid formation by zinc. *Science* 265, 1464-1467 (1994)

28. S. S. Ball & V. H. Mah. Apolipoprotein E4, mental vitality in youth and Alzheimer's disease in old age? *Age* 16, 136-149 (1993)

29. P. R. Wenham, W. H. Price & G. Blundell. Apolipoprotein E genotyping by one-stage PCR. *Lancet* 337, 1158-1159 (1991)

30. M. Lilly-Stauderman, T. L. Brown, A. Balasubramaniam & J. A. Harmony. Heparin releases newly synthesized cell surface-associated apolipoprotein E from HepG2 cells. *J Lipid Res* 34(2): 190-200 (1993)

31. R. A. Torkeltaub, C. A. Dyer, J. Martin & L. K. Curtiss. Apolipoprotein (apo) E inhibits the capacity of monosodium urate crystals to stimulate neutrophils. Characterization of intraarticular apoE and demonstration of apoE binding to urate crystals *in vivo*. *J Clin Invest* 87(1), 20-26.22 (1991)

32. T. Wisniewski, A. Golabek, E. Matsubara, J. Ghiso & B. Frangione. Apolipoprotein E: binding to soluble Alzheimer's beta-amyloid. *Biochem Biophys Res Commu* 192(2), 359-365 (1993)

33. B. P. Nathan, S. Bellosta, D. A. Sanan, K. H. Weisgraber, R. W. Mahley & R. E. Pitas Differential effects of apolipoprotein E3 and E4 on neuronal growth *in vitro*. *Science* 264, 850-852 (1994)

34. D. E. Schmechel, A. M. Saunders, W. J. Strittmatter, B. J. Crain, C. M. Hulette, S. H. Joo, M. A. Pericak-Vance, D. Goldbarger & A. D. Roses. Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer's disease. *Proc Natl Acd Sci USA* 90, 9649-9653 (1993)

35. H. Shimano, N. Yamada, M. Katsuki, M. Shimada, T. Gotoda, K. Harada, T. Murase, C. Fukazawa, F. Takaku & Y. Yazaki. Overexpression of apolipoprotein E in

transgenic mice: marked reduction in plasma lipoproteins except high density lipoprotein and resistance against diet-induced hypercholesterolemia. *Proc Natl Acad Sci USA* 89 (5), 1750-1754 (1992)

36. A. S. Pulp, J. D. Smith, T. Hayek, K. Aalto-Setala, A. Walsh, J. G. Verstuyft, E. M. Rubin & J. L. Breslow. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell* 71 (2), 343-353 (1992)

37. S. H. Zhang, R. L. Reddick, J. A. Piedrahita & N. Maeda. Spontaneous hypercholesteroemia and arterial lesions in mice lacking apolipoprotein E. *Science* 258 (5081), 468-471 (1992)

38. H. K. Das, J. McPherson, G. A. Bruns, S. K. Karathanasis & J. L. Breslow. Isolation, characterization, and mapping to chromosome 19 of the human apolipoprotein E gene. *J Biol Chem* 260, 6240-6247 (1985)

39. G. W. Rebeck, J. S. Reiter, D. K. Strickland & B. T. Hyman. Apolipoprotein E in sporadic Alzheimer's disease: Allelic variation and receptor interactions. *Neuron* 11, 575-580 (1993)

40. W. Roush. Protein studies try to puzzle out Alzheimer's Tangles. *Science* 267, 793-794 (1995)

41. S. L. Peleeh. Networking with proline-directed protein kinases implicated in tau phosphorylation. *Neurobiol Aging* 16 (3), 247-256 (1995)