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Characterization of the Binding of Amyloid-β Peptide to Cell Culture-Derived Native Apolipoprotein E2, E3, and E4 Isoforms and to Isoforms from Human Plasma

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Abstract: The ε4 allele of apolipoprotein E (apoE, protein; APOE, gene) is a major risk factor for Alzheimer's disease (AD). Genetically, the frequency of the ε4 allele is enriched in early-onset sporadic, late-onset familial, and common late-onset sporadic AD. ApoE is found in the extracellular amyloid-β (Aβ) deposits that are characteristic features of AD. In this study, we examined the interaction between Aβ and apoE isoforms. The apoE isoforms used in this study were either produced by stably transfected Chinese hamster ovary cells (CHO) or were from human plasma. We report that when similar concentrations of the apoE isoforms were used, native nonpurified apoE3 from recombinant CHO-derived sources bound Aβ, but apoE4 did not. In fact, in our system, binding of recombinant apoE4 to Aβ was never detectable, even after incubation for 4 days. Furthermore, using the same assay conditions, native apoE2, like apoE3, binds Aβ avidly. Furthermore, when human plasma apoE isoforms are tested in Aβ binding experiments, apoE3 bound Aβ more avidly than apoE4, and a major apoE/Aβ complex (the 40-kDa form) was observed with plasma apoE3 but not apoE4. These data extend our understanding of apoE isoform-dependent binding of Aβ by associating apoE2 with efficient apoE/Aβ complex formation and demonstrate that native apoE3 (whether recombinant or derived from human plasma) forms sodium dodecyl sulfate–stable apoE/Aβ complexes more readily than native apoE4. The different Aβ-binding properties of native apoE4 versus native apoE3 provide insight into the molecular mechanisms by which the APOE ε4 allele exerts its risk factor effects in AD. **Key Words:** Alzheimer's disease—Apolipoprotein E isoforms—β-Amyloid—Human plasma—Apolipoprotein E/amyloid-β complexes.
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Apolipoprotein E protein (apoE) is involved in lipid transport and clearance by mediating the binding of lipid particles to specific lipoprotein receptors. There are three major isoforms of apoE, termed E2, E3, and E4. The apoE4 isoform has been linked with Alzheimer's disease (AD) owing to the increased frequency of the ε4 allele in AD (Strittmatter et al., 1993*a*). In addition, the cerebral amyloid plaque cores, a major neuropathological feature of AD that contain fibrils of amyloid-β (Aβ) protein, are also immunostained for apoE (Namba et al., 1991; Wisniewski and Frangione, 1992). Furthermore, in AD patients who are homozygous for the apoE gene (APOE) ε4, the amyloid burden in the brain is much greater than in AD patients homozygous for APOE ε3 (Rebeck et al., 1993).

The colocalization of apoE with Aβ in amyloid plaque cores suggests an interaction of the latter two molecules. Strittmatter et al. (1993*b*) demonstrated using purified delipidated human plasma apoE that the E4 isoform bound Aβ much more avidly than E3. Thus, it was suggested that E4 may contribute to the pathology of AD by promoting the deposition of Aβ. However, subsequent work by LaDu et al. (1994), using native human recombinant apoE expressed in HEK-293 cells, demonstrated that, in contrast to the previous report, native apoE3 bound Aβ more avidly than native E4. The latter authors attributed the different binding patterns observed between the two laboratories to differences in the apoE preparations, which may result in altered conformation of apoE (LaDu et al., 1995). These data on defective binding of native apoE4 to Aβ have recently been confirmed (Zhou et al., 1996).

The goal of this study was to extend the understand-

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Abbreviations used: Aβ, β-amyloid; AD, Alzheimer's disease; apoE, apolipoprotein E protein; APOE, apolipoprotein E gene; CHO, Chinese hamster ovary; DTT, dithiothreitol; SDS, sodium dodecyl sulfate.

ing of isoform-specific apoE/Aβ binding by characterizing the binding pattern of Aβ to native apoE isoforms secreted by transfected cell lines and also to native apoE from human plasma. We confirm that under nondenaturing conditions, the native recombinant apoE3 binds Aβ more avidly than E4, and we demonstrate that under similar conditions, apoE2 also binds Aβ more avidly than E4. Furthermore, like the apoE isoforms from conditioned media, human plasma native apoE3 bound Aβ more avidly than human plasma native apoE4 isoform. Thus, different sources of apoE, provided they are not denatured, may yield apoE of similar protein conformation, which, in turn, may result in similar abilities to form apoE isoform-specific complexes with Aβ.

MATERIALS AND METHODS

Materials

A$\beta^{1\text{-}40}$ was prepared at the Keck Foundation Protein Facility at Yale University (New Haven, CT, U.S.A.). The 10–20 or 10% sodium dodecyl sulfate (SDS)/tricine gels and 2× tricine SDS sample buffer were purchased from Novex (San Diego, CA, U.S.A.). Nitrocellulose membranes were from Bio-Rad (Hercules, CA, U.S.A.). Anti-human apoE antibody raised in a goat was purchased from INCSTAR (Stillwater, MN, U.S.A.). Mouse anti-Aβ antibody (6E10) was purchased from Dr. K. S. Kim (IBRDD, Staten Island, NY, U.S.A.). Biotinylated rabbit anti-goat or anti-mouse IgG was obtained from Dakopat (Copenhagen, Denmark). Streptavidin-alkaline phosphatase was purchased from Amersham (Buckinghamshire, U. K.), and Fast Red/naphthol and 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium were from Sigma (St. Louis, MO, U.S.A.).

Transfected cells

The human APOE ϵ4 expression vector was derived from a previously described genomic APOE 1 clone (Das et al., 1985). A *Hin*dIII linker was inserted into the first exon at the unique *Aat*II site at position +24 relative to the start of transcription. DNA extending from this artificial *Hin*dIII site to the *Eco*RI site 628 bp 3′ of the APOE polyadenylation site was cloned into pUC18. A 343-bp *Pvu*II–*Hin*dIII fragment of SV40, containing the viral early promoter and enhancer, was cloned into the artificial *Hin*dIII site of the APOE plasmid using *Hin*dIII linkers (Smith et al., 1988). APOE ϵ2 and APOE ϵ3 derivatives were generated by PCR mutagenesis of the APOE ϵ4 expression plasmid and confirmed by DNA sequencing. On day 0, Chinese hamster ovary (CHO) cells were plated in 60-mm-diameter dishes. On day 1, each plate of cells was transfected with 3 ml of serum-free Dulbecco's minimal essential medium containing 12 ml of Lipofectamine (GibcoBRL, Grand Island, NY, U.S.A.), 4 mg of an APOE expression plasmid, and 0.2 mg of pSV2neo (Southern and Berg, 1982). On day 2, the cells were aspirated and fed with Dulbecco's minimal essential medium containing 10% fetal bovine serum and 800 μg/ml geneticin (GibcoBRL). Two weeks later, geneticin-resistant colonies were picked, expanded, and assayed for human APOE DNA by PCR specific for the human APOE gene using primers E7 and E9 as previously described (Emi et al., 1988). Expression of human APOE mRNA was analyzed by an RNase protection assay (Smith et al., 1990).

Confirmation of APOE expression by the clonal cell lines was performed by immunohistochemistry. Immunohistochemistry for human apoE was performed on adherent cells fixed in methanol for 2 min; all incubations were performed at room temperature. Cells were treated with casein blocking buffer (Pierce, Rockford, IL, U.S.A.) for 10 min, followed by a 60-min incubation with the goat anti-human apoE antiserum (INCSTAR; 1:1,000) in blocking buffer. After washing, the cells were incubated with horseradish peroxidase-conjugated mouse anti-goat IgG (1:250) in blocking buffer for 30 min. Staining was performed with the 1-Step 4-chloronaphthol reagent (Pierce).

Preparation of conditioned media

ApoE isoform-transfected CHO cells were used to generate conditioned media containing each of the three apoE isoforms: E2, E3, or E4. Transfected CHO cells with an empty vector were used to generate empty vector conditioned media. Media were prepared using Dulbecco's minimal essential medium, which, in some cases, was supplemented with 0.2% (wt/vol) bovine serum albumin. Media were harvested after a 24-h conditioning period. ApoE isoform levels were determined by quantitative immunoblotting of conditioned media and a standard containing human apoE (Calbiochem, La Jolla, CA, U.S.A.).

APOE genotyping

Human plasma specimens from control and AD subjects were also used as a source of apoE. To determine the APOE genotype, DNA was extracted from white blood cells using a standard protocol (Hixson et al., 1988). PCR amplification was performed essentially as that described by Hixson and Vernier (1990) using the oligonucleotide primers 5′ TCCAAGGAGCTGCAGGCGGCGCA 3′ and 5′ ACAGATTCGCCCGGCCTGGTACACTGCCA 3′, as described by Wenham et al. (1991). Genotyping, using PCR followed by restriction enzyme digestion, revealed DNA fragments whose electrophoretic migration pattern was unique for each isotype (Wenham et al., 1991). Details of APOE genotype distribution of the Australian control and AD population have previously been reported (Martins et al., 1995).

ApoE/Aβ binding reactions

For standard binding reactions, recombinant apoE isoforms were each incubated with Aβ in phosphate-buffered saline (pH 7.4) in a final concentration of 44 μg/ml apoE and 500 μg/ml Aβ for 5 h at 37°C. The reaction was stopped by adding an equal volume of 2× tricine/SDS sample buffer. For the time course study of binding reactions, samples were aliquoted at different time points, added to an equal volume of 2× tricine/SDS sample buffer, and then frozen at −70°C. The apoE/Aβ binding studies on human plasma used 6 μl of plasma (plasma apoE at final concentrations ranging from 4 to 6 μg/ml) and 25 μg of Aβ in a total volume of 50 μl. Plasma apoE and Aβ were incubated for 5 h at 37°C.

Western blotting

Recombinant apoE samples were electrophoresed on 10–20% SDS/tricine gels, whereas plasma apoE sources were electrophoresed on 10% SDS/tricine gels and transferred to nitrocellulose membranes. The blotted membranes were incubated in primary antibody for 2 h at room temperature. Both anti-apoE antibody and the Aβ monoclonal antibody 6E10 were used at 1:1,000 dilution. Membranes were washed with Tris-buffered saline containing Tween 20 and then incubated with biotinylated secondary antibody

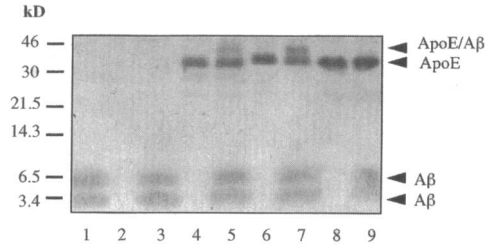

FIG. 1. Recombinant apoE isoform-specific binding to Aβ in an immunoblot of an apoE/Aβ binding reaction mixture containing 500 μg/ml Aβ and the apoE isoforms at 44 μg/ml incubated for 5 h at 37°C. Both Aβ and apoE were detected with a mixture of the Aβ-specific monoclonal antibody 6E10 and an anti-apoE antibody. Lane 1, Aβ only; lane 2, empty vector transfected CHO cell conditioned medium only; lane 3, empty vector transfected CHO cell conditioned medium and Aβ; lane 4, apoE2 only; lane 5, apoE2 and Aβ; lane 6, apoE3 only; lane 7, apoE3 and Aβ; lane 8, apoE4 only; and lane 9, apoE4 and Aβ.

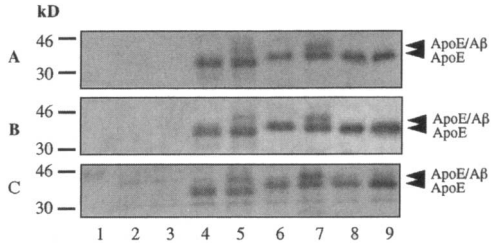

FIG. 3. Time course of recombinant apoE isoform-specific binding to Aβ. ApoE/Aβ binding reaction mixtures containing 500 μg/ml Aβ and 44 μg/ml apoE were incubated for (**A**) 1h, (**B**) 1 day, or (**C**) 4 days at 37°C, probed with an apoE antibody, and immunoblotted. Lane 1, Aβ only; lanes 2, 4, 6, and 8, empty vector transfected CHO cell conditioned medium, apoE2, apoE3, and apoE4, respectively; and lanes 3, 5, 7, and 9, Aβ incubated with empty vector transfected CHO cell conditioned medium and apoE2, apoE3, and apoE4 conditioned media, respectively.

(1:2,000 dilution), followed by incubation with strepavidin-alkaline phosphatase (diluted 1:2,000). Visualization of specific proteins was achieved by using Fast Red/naphthol or 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium.

RESULTS

In an attempt to gain a better understanding of apoE–Aβ interactions, we have undertaken studies to assess the binding of Aβ to either native apoE isoforms obtained from transfected CHO cells or native apoE from human plasma. We first examined the binding of the three native recombinant apoE isoforms, obtained from transfected CHO cells, with Aβ. The apoE isoforms, E2, E3, and E4, migrated in SDS/tricine gel as a 34-kDa protein (Fig. 1, lanes 4, 6, and 8). When Aβ was incubated with these isoforms, both apoE2 and apoE3 formed complexes with Aβ as demonstrated by the appearance of a higher-molecular-mass protein (~38 kDa; Fig. 1, lanes 5 and 7). However, no complex was observed under similar conditions with apoE4 (Fig. 1, lane 9). Increases in apoE concentrations from 44 to 132 μg/ml did not result in the appearance of apoE4/Aβ complexes (Fig. 2A). Incubating apoE4 (132 μg/ml) with increasing Aβ concentrations (250 μg/ml to 2 mg/ml) did not result in the appearance of apoE4/Aβ complexes (Fig. 2B). The apoE4/Aβ complexes were not observed even after longer incubation periods ranging from 1 to 4 days (Fig. 3).

Formation of the apoE/Aβ complexes of apoE2 and apoE3 was inhibited by addition of the sulfhydryl reducing reagent dithiothreitol (DTT) to the apoE and Aβ incubation mixture (Fig. 4A, lanes 7 and 10) or by coincubation with the Aβ-specific antibody 4G8 (Fig. 4B, lanes 7 and 10).

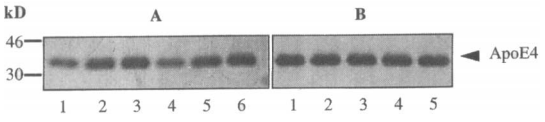

FIG. 2. Recombinant apoE4/Aβ binding reactions and effect of apoE dose (**A**) and of Aβ dose (**B**). A: An immunoblot of apoE4/Aβ binding reaction mixtures containing Aβ (500 μg/ml) with increasing concentrations of apoE4, incubated for 5 h at 37°C and detected with an apoE antibody. Lanes 1–3, 44, 88, and 132 μg/ml, respectively, of apoE4 only; lanes 4–6, apoE4 at 44, 88, and 132 μg/ml, respectively, together with 500 μg/ml Aβ. B: An immunoblot of apoE4/Aβ binding reaction mixtures containing apoE4 (132 μg/ml) with increasing concentrations of Aβ, incubated for 5 h at 37°C and detected with an anti-apoE antiserum. Lane 1, apoE4 only; lanes 2–5, apoE4 with Aβ at 250 μg/ml, 500 μg/ml, 1 mg/ml, and 2 mg/ml, respectively.

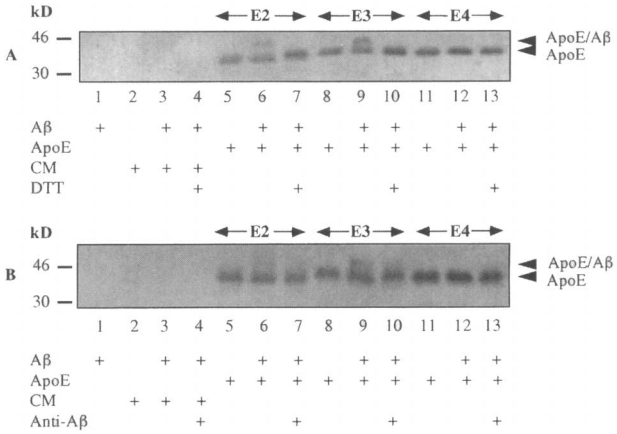

FIG. 4. Effect of DTT and Aβ antibody on apoE/Aβ complex formation in recombinant apoE isoform-specific binding to Aβ. ApoE/Aβ binding reaction mixtures containing 500 μg/ml Aβ and 44 μg/ml apoE were incubated for 5 h at 37°C and detected with an anti-apoE antibody. **A:** Effect of DTT on apoE/Aβ binding. **B:** Effect of the Aβ-specific antibody 4G8 on apoE/Aβ binding. Lane 1, Aβ only; lane 2, empty vector transfected CHO cell conditioned medium only (CM); lanes 3 and 4, empty vector transfected CHO cell conditioned medium with Aβ; lane 5, apoE2 only; lanes 6 and 7, apoE2 and Aβ; lane 8, apoE3 only; lanes 9 and 10, apoE3 and Aβ; lane 11, apoE4 only; and lanes 12 and 13, apoE4 and Aβ. Samples in lanes 4, 7, 10, and 13 in A were also incubated in the presence of 10 m*M* DTT. Samples in lanes 4, 7, 10, and 13 in B were incubated in the presence of Aβ monoclonal antibody 4G8 (5 μl in a total volume of 25 μl).

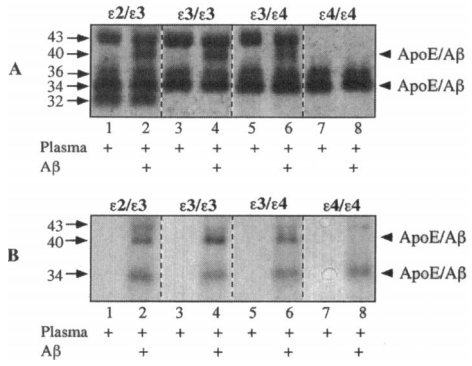

FIG. 5. Isoform-specific binding to Aβ of human plasma apoE. ApoE/Aβ binding reaction mixtures of Aβ (500 μg/ml) with different isoforms of human plasma apoE (ε2/ε3, ε3/ε3, ε3/ε4, and ε4/ε4; all isoforms were used at 120 μl of plasma/ml of reaction buffer) were incubated for 24 h at 37°C and immunoblotted. **A** and **B:** Paired blots detected with an anti-apoE antibody and a monoclonal Aβ antibody, respectively. Lanes 1, 3, 5, and 7, human plasma only; lanes 2, 4, 6, and 8, both human plasma and Aβ.

ApoE/Aβ complexes were also formed when human plasma was used as a source of apoE. The major 40-kDa complex was visualized with both the anti-apoE antibody and the Aβ-specific antibody 6E10 for three APOE genotypes, ε2/ε3, ε3/ε3, and ε3/ε4 (Fig. 5, lanes 2, 4, and 6), but not for the ε4/ε4 genotype (Fig. 5, lane 8). Again, complex formation could be inhibited with either DTT or by coincubating apoE and Aβ together with an apoE-specific antibody (data not shown). In addition to the 40-kDa complex, two other apoE/Aβ complexes were detected. A 34-kDa apoE/Aβ complex was observed with the two antibodies in all the above four genotypes studied (Fig. 5, lanes 2, 4, 6, and 8). A 43-kDa complex was detected with the 6E10 antibody in all the above four genotypes (Fig. 5B, lanes 2, 4, 6, and 8); it was also detected with the anti-apoE antibody in the ε2/ε3, ε3/ε3, and ε3/ε4 genotypes (Fig. 5A, lanes 2, 4, and 6) but not in the ε4/ε4 genotype (Fig. 5A, lane 8). This protein is unlikely to be an apoE/Aβ complex for the ε4/ε4 genotype because it was not detected by the anti-apoE antibody.

Time course studies revealed that apoE/Aβ complex formation was observed for apoE3 as early as 10 min following incubation at 37°C (Fig. 6A). On the other hand, apoE/Aβ complex formation was not observed until 1 h when plasma apoE4 was used as a source of apoE and then only for the lower-molecular-mass 34-kDa complex observed (Fig. 6B). After incubation of apoE4 and Aβ for 24 h, a 43-kDa protein was also detected with the Aβ antibody (Fig. 6B).

DISCUSSION

Our results obtained with apoE from conditioned media are in agreement with an earlier report of LaDu et al. (1994) in that native apoE3 binds Aβ avidly. These authors observed a 20-fold difference between the level of apoE3/Aβ complex and the apoE4/Aβ complex. In our study, we were unable to detect any apoE4/Aβ complex formation under various conditions using the CHO-derived native apoE4 isoform. This lack of detectable binding to Aβ by recombinant apoE4 is consistent with an earlier report by some of us using stably transfected RAW264 macrophage cells as a source of apoE (Zhou et al., 1996). We also report here that a 40-kDa human plasma apoE/Aβ complex was detected in all APOE genotypes except for APOE ε4 homozygotes. Taken together, our results confirm previous reports that the various sources of native recombinant apoE exhibit qualitatively similar isoform-specific Aβ binding patterns. Furthermore, this study extends these findings to demonstrate that these isoform-specific Aβ binding patterns are also observed with human plasma apoE where apoE4 is the only isoform that does not form a 40-kDa apoE/Aβ complex.

The density of Aβ deposition is directly related to inheritance of an ε4 allele (Schmechel et al., 1993). This increased Aβ deposition in AD brains for APOE ε4-containing individuals appears to contradict the in vitro data presented here where apoE4 binding to Aβ was markedly decreased or not detected at all under our conditions. However, this discrepancy may be explained by apoE3 efficiently binding Aβ and clearing it from the extracellular space; conversely, the inability of apoE4 to bind Aβ efficiently results in the accumulation of Aβ in the extracellular space leading to its deposition. Thus, the increased deposition of Aβ in brains of AD patients with an ε4 allele would suggest inefficient clearance of Aβ by this isoform of apoE.

It is possible, however, that apoE4 may behave differently in the CNS compared with its counterpart in plasma. The lipid associated with apoE in the CSF has not been thoroughly characterized but is thought to be different from the lipid associated with apoE in plasma.

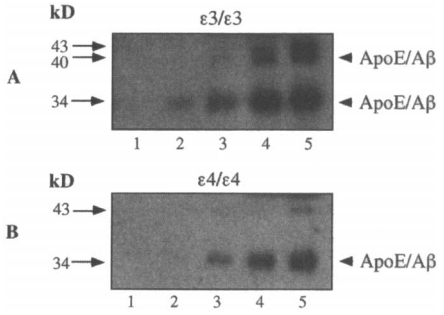

FIG. 6. Time course of human plasma apoE isoform-specific binding to Aβ. ApoE/Aβ binding reaction mixtures of Aβ (500 μg/ml) with human plasma apoE (ε3/ε3 and ε4/ε4 at 120 μl of plasma/ml) were incubated at 37°C for 2 min, 10 min, 1 h, 5 h, and 24 h (lanes 1–5, respectively) and immunoblotted. The apoE/Aβ complexes were detected with the Aβ-specific monoclonal antibody 6E10.

It is therefore important to investigate, in future studies, the CSF apoE isoform-specific binding pattern to Aβ to get a better understanding of the role of apoE4 in AD.

In summary, unpurified, native recombinant apoE showed marked isoform-specific differences with respect to binding Aβ, consistent with previous reports using apoE from transfected cells. In all these studies, including our own, apoE3 bound Aβ avidly, whereas apoE4 bound Aβ poorly or not at all. In addition, we report for the first time that apoE2 binds Aβ just as avidly as apoE3. These findings were consistent with subsequent experiments when human plasma was used as a source of native apoE. This is the first report, to our knowledge, demonstrating a 40-kDa apoE/Aβ complex in all genotypes except for APOE ε4 homozygotes. Furthermore, the slower formation of the 34-kDa plasma apoE/Aβ complexes in APOE ε4 homozygotes together with a complete lack of formation of the 40-kDa complex demonstrates that native human plasma apoE4 binds Aβ poorly under the conditions used in this study. Our results are consistent with the view that apoE4 may exert its risk factor effects in AD by inefficient clearance of Aβ. However, further studies are required to determine whether human CSF apoE forms similar apoE isoform-specific apoE/Aβ complexes as has been demonstrated in this study for both human plasma and recombinant apoE isoforms.

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REFERENCES

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

Emi M., Wu L. L., Robertson M. A., Myers R. L., Hegele R. A., Williams R. R., White R., and Lalouel J. M. (1988) Genotyping and sequence analysis of apolipoprotein E isoforms. *Genomics* **3,** 373–379.

Hixson J. E. and Vernier D. T. (1990) Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *Hhal*. *J. Lipid Res.* **31,** 545–548.

Hixson J. E., Borenstein L., Cox L. A., Rainwater D. L., and Vande-Berg J. L. (1988) The baboon gene for apolipoprotein A-I: characterization of a cDNA clone and identification of DNA polymorphisms for genetic studies of cholesterol metabolism. *Gene* **74,** 483–490.

LaDu M. J., Falduto M. T., Manelli A. M., Reardon C. A., Getz G. S., and Frail D. E. (1994) Isoform-specific binding of apolipoprotein E to beta-amyloid. *J. Biol. Chem.* **269,** 23403–23406.

LaDu M. J., Pederson T. M., Frail D. E., Reardon C. A., Getz G. A., and Falduto M. T. (1995) Purification of apolipoprotein E attenuates isoform-specific binding to β-amyloid. *J. Biol. Chem.* **270,** 9039–9042.

Martins R. N., Clarnette R., Fisher C., Broe G. A., Brooks W. S., Montgomery P., and Gandy S. (1995) ApoE genotypes in Australia: roles in early and late onset Alzheimer's disease and Down's syndrome. *Neuroreport* **6,** 1513–1516.

Namba Y., Tomonaga M., Kawasaki H., Otomo E., and Ikeda K. (1991) Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt–Jacob disease. *Brain Res.* **541,** 163–166.

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

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

Smith J. D., Melian A., Leff T., and Breslow J. L. (1988) Expression of the human apolipoprotein E gene is regulated by multiple positive and negative elements. *J. Biol. Chem.* **263,** 8300–8308.

Smith J. D., Plump A. S., Hayek T., Walsh A., and Breslow J. L. (1990) Accumulation of human apolipoprotein E in the plasma of transgenic mice. *J. Biol. Chem.* **265,** 14709–14712.

Southern P. J. and Berg P. (1982) Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. *J. Mol. Appl. Genet.* **1,** 327–341.

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

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

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

Wisniewski T. and Frangione B. (1992) Apolipoprotein E: a pathological chaperone protein in patients with cerebral and systemic amyloid. *Neurosci. Lett.* **135,** 235–238.

Zhou Z., Smith J. D., Greengard P., and Gandy S. (1996) Alzheimer amyloid-β peptide forms denaturant-resistant complex with type ε3 but not type ε4 isoform of native apolipoprotein E. *Mol. Med.* **2,** 175–180.