Sodium Dodecyl Sulfate-Resistant Complexes of Alzheimer's Amyloid β-Peptide with the N-Terminal, Receptor Binding Domain of Apolipoprotein E

Adam A. Golabek,*† Elizabeth Kida,*† Mariusz Walus,* Christian Perez,‡ Thomas Wisniewski,‡§ and Claudio Soto¶

*Department of Pathological Neurobiology, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York 10314 USA; †Department of Neuropathology, Medical Research Center, Polish Academy of Science, Warsaw 02-093, Poland; Departments of ‡Neurology and §Pathology, New York University Medical Center, New York, New York 10061 USA; and ¶Serono Research Institute, Geneva, Switzerland

ABSTRACT Immunocytochemical, biochemical, and molecular genetic studies indicate that apolipoprotein E (apoE) plays an important role in the process of amyloidogenesis-β. However, there is still no clear translation of these data into the pathogenesis of amyloidosis-β. Previous studies demonstrated sodium dodecyl sulfate (SDS)-resistant binding of apoE to the main component of Alzheimer's amyloid-Aβ and modulation of Aβ aggregation by apoE in vitro. To more closely characterize apoE-Aβ interactions, we have studied the binding of thrombolytic fragments of apoE3 to Aβ in vitro by using SDS-polyacrylamide gel electrophoresis and intrinsic fluorescence quenching. Here we demonstrate that SDS-resistant binding of Aβ is mediated by the receptor-binding, N-terminal domain of apoE3. Under native conditions, both the N- and C-terminal domains of apoE3 bind Aβ; however, the former does so with higher affinity. We propose that the modulation of Aβ binding to the N-terminal domain of apoE is a potential therapeutic target for the treatment of amyloidosis-β.

INTRODUCTION

The pathology of Alzheimer's disease (AD) brain involves synaptic alterations, neuronal loss, gliosis, and formation of two types of structures: neurofibrillary tangles (NFTs) and amyloid-β. Amyloid deposition is an invariant feature of AD, commonly believed to be responsible for a cascade of events leading to dementia, and finally death of AD patients (Selkoe, 1994). The major component of the amyloid accumulated in the leptomeningeal and cerebral vessels as amyloid angiopathy and in the neuropil as senile plaques (SPs) is a 39–43 amino acid hydrophobic peptide referred to as amyloid β-peptide (Aβ) (Glenner and Wong, 1984; Masters et al., 1985). Aβ is a proteolytic fragment of the larger transmembrane glycoprotein termed amyloid-β precursor protein (APP) (Goldgaber et al., 1987; Robakis et al., 1987; Tanzi et al., 1988). Proteolysis of APP by so-called β- and γ-seretases leads to the secretion of Aβ into the extracellular space by virtually all types of cells examined so far. This soluble form of Aβ (sAβ) is present in low nanomolar range in both plasma and cerebrospinal fluid (CSF) (Seubert et al., 1992; Shoji et al., 1992; Vigo-Pelfrey et al., 1993). Factors responsible for the exclusive deposition of Aβ only within the brain tissue remain elusive.

Recent studies suggest that the so-called amyloid-associated proteins such as apolipoproteins, serum amyloid P component, α_1-antichymotrypsin, or proteoglycans may contribute significantly to the progression of amyloidogenesis-β and Aβ toxicity (recent review: Wisniewski et al., 1997). One of these proteins, apolipoprotein E (apoE), has received special attention after discovery of the genetic association of apoE polymorphism with late-onset AD (Strittmatter et al., 1993a). ApoE is a 34-kDa glycoprotein whose primary role involves lipid transport and redistribution among different tissues (Mahley, 1988). ApoE is a major apolipoprotein in the brain and in CSF, and its expression is increased significantly after central and peripheral nerve injuries (Ignatius et al., 1986; Kida et al., 1995b). Studies in cell culture and in knockout mice indicate that apoE participates, in an isoform-specific manner, in tissue regeneration and remodeling (Nathan et al., 1994; Masliah et al., 1995).

The three major apoE isoforms in humans, apoE2, apoE3, and apoE4, are products of three alleles, ε2, ε3, and ε4, of a single apoE locus on chromosome 19 (Utermann et al., 1979). In the general population, the ε3 allele is the most common, accounting for ~78% of all apoE alleles (Hallman et al., 1991). The frequency of the ε4 allele is increased significantly in the population of late-onset sporadic and familial AD patients (Corder et al., 1993; Saunders et al., 1993). However, in Down's syndrome and AD associated with presenilin mutations, which typically develop early, and severe amyloidosis-β, apoE ε4 is not increased in comparison to the general population, although apoE deposition in amyloid-β is similar to that in sporadic AD (Van Gool et al., 1995; Levy-Lahad et al., 1995; Kida et al., 1995a; Gearing et al., 1995; Houlden et al., 1998). Despite intensive study, there is still no clear translation of genetic data into the pathogenesis of amyloidogenesis-β. Further-

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Address reprint requests to Dr. Adam A. Golabek, Department of Pathological Neurobiology, New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314. Tel.: 718-494-5202; Fax: 718-982-6346; E-mail: golabek@popmail.med.nyu.edu.

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more, the precise role of apoE in normal brain functioning and the molecular mechanisms underlying the physiological functions of apoE are also still poorly understood.

We and others have shown direct interactions between apoE and Aβ by demonstrating in vitro binding of Aβ to apoE (Wisniewski et al., 1993; Strittmatter et al., 1993a,b; LaDu et al., 1994), revealing the conformation-dependent manner of Aβ-apoE interactions (Golabek et al., 1996) and showing that apoE is capable of modulating the conformational and fibrillogenic properties of Aβ acting as a pathological chaperone (Wisniewski et al., 1994; Soto et al., 1996). The idea that apoE may be involved directly in Aβ fibrillogenesis was strengthened recently by data showing that platelet-derived growth factor-APP transgenic mice with apoE knockout show a prominent reduction of amyloid-β deposits, and especially of thioflavin-S-positive plaques, which are highly fibrillized (Bales et al., 1997). ApoE contains two functionally different domains: the N-terminal domain, which encompasses a receptor binding region, and the C-terminal domain, which is responsible for lipid binding (Mahley, 1988). Thus, to more closely characterize apoE-Aβ interactions, we have studied at present the binding of Aβ to both the N- and C-terminal fragments of apoE. Here we report that the strong, sodium dodecyl sulfate (SDS)-resistant Aβ binding to apoE3 is mediated by the N-terminal, receptor-binding domain of the apoE molecule.

MATERIALS AND METHODS

Synthetic peptide and proteins

Aβ 1–40 was custom synthesized by the W. M. Keck Foundation (New Haven, CT). Purity of the peptide was verified by analytical reversed-phase-high-performance liquid chromatography and mass spectrometry. A stock solution of Aβ was made at 2 mg/ml in 50% acetonitrile, 0.1% trifluoroacetic acid, quantitated by amino acid analysis on the Waters AccQ-Tag System (Millipore Corp., Milford, MA), and stored lyophilized in aliquots. Recombinant ApoE3 was obtained from BioTechnology General (Israel, lot 738-1) (Vogel et al., 1985).

ApoE cleavage by thrombin

To 10 mg of human recombinant apoE3 dissolved in 5 ml of 100 mM NH_4HCO_3, pH 8.5, thrombin was added at a weight ratio of 1:100 (thrombin:apoE) and incubated at room temperature for 2 h. After that, an additional portion of thrombin was added to a final concentration of 1:50, and the cleavage was continued for two more hours. After cleavage, the sample was lyophilized; dissolved in 2 ml of 6 M GuHCl, 100 mM Tris (pH 7.4), 2 mM EDTA, and 1% β-mercaptoethanol (buffer A) and incubated at room temperature for 10 min. ApoE fragments were isolated after gel exclusion chromatography on a Sephadex G-100 column (2 × 180 cm), equilibrated, and developed with buffer A. The flow rate was 6 ml/h, and absorbance was monitored at 280 nm. Absorbance peaks corresponding to ~10 and 22 kDa, respectively, were pooled, dialyzed against 50 mM NH_4HCO_3 (pH 8.5), lyophilized, dissolved in ddH_2O, and analyzed by SDS-polyacrylamide gel electrophoresis and N-terminal sequencing.

SDS-resistant binding analysis

Two micrograms of Aβ was mixed with 2 μg of human recombinant apoE3, 10 μg of human plasma very-low density lipoproteins (VLDL), or 2 μg of the N-terminal (N-apoE) or the C-terminal (C-apoE) fragments of apoE and incubated in 20 μl of 0.1 M Tris (pH 7.4) overnight at 37°C. Five microliters of 5× Laemmli sample buffer without reducing agent was then added; samples were incubated for 10 min at 37°C, loaded onto 15% SDS-polyacrylamide gel (Schägger and von Jagow, 1987), and electrophoresed at a constant current of 20 mA. After electrophoresis, gel was either stained with Coomassie blue or electrotransferred onto nitrocellulose membrane by the method of Towbin et al. (1979). After transfer, membrane was saturated with 5% nonfat dry milk (NDM) in phosphate-buffered saline (150 mM NaCl, 20 mM sodium phosphate, pH 7.4) and incubated with primary monoclonal antibody (mAb) 6E10, raised against amino acids (aa) 1–16 of Aβ (Kim et al., 1988), at 1:1000; mAb 3D12 (Biodesign, Kennebunk, ME), recognizing an epitope in the C-terminal domain of apoE, at 1:1000; or mAb 9H8 (Biodesign), recognizing an epitope in the N-terminal domain of apoE, at 1:500, in 0.5% NDM/0.5% Tween 20/phosphate-buffered saline. Then blots were washed, incubated with secondary antibody conjugated to horseradish peroxidase, and developed with an enhanced chemiluminescence (ECL) kit from Amersham Pharmacia (Piscataway, NJ). For amino acid sequencing, gels were transferred onto polyvinyl pyrrolidine fluoride membrane and stained with Coomassie blue, and the adequate bands were excised and sequenced.

Fluorescence quenching

To measure the affinity of Aβ for N-apoE and C-apoE, we have used a fluorescence quenching spectroscopy, taking advantage of the fact that Aβ does not contain any tryptophan residue, whereas apoE has six of them. ApoE3, N-apoE, and C-apoE were dissolved at 2, 10, and 4.5 μM, respectively, in 0.1 M Tris (pH 7.4), and their fluorescence spectra were recorded at 290–415 nm with an excitation wavelength of 285 nm on a Perkin-Elmer LS50B spectrofluorimeter (Perkin-Elmer, Norwalk, CT), with the slits set at 2.5 nm. Similarly, fluorescence spectra of apoE3, N-apoE, and C-apoE were recorded in the presence of increasing concentrations of Aβ (2–500 nM) after 20 min of equilibration and were corrected for residual fluorescence of the peptide itself. The fluorescence change at 340 nm was plotted versus Aβ concentration, and the resulting curves were analyzed by nonlinear regression fit with GraphPad Prism software.

Other methods

VLDLs from the fresh frozen human plasma were isolated by ultracentrifugation as described previously (Koudinov et al., 1994). Protein concentration was quantitated by the method of Bradford with bovine serum albumin (Pierce) as a standard. Amino-terminal sequencing was performed on a 477A protein sequencer, and the resulting phenylthiohydantoin derivatives were identified with the use of an on-line phenylthiohydantoin-derivative analyzer (Applied Biosystems, Foster City, CA).

RESULTS

SDS-resistant binding

First we analyzed the SDS-resistant properties of Aβ binding to the thrombolytic fragments of apoE3. Thrombin cleaves apoE at positions 196 and 215, giving rise to two fragments: the N-terminal fragment, with a molecular mass of ~22 kDa, and the C-terminal fragment, with a molecular mass of ~10 kDa (Fig. 1). Incubation of Aβ with N-apoE

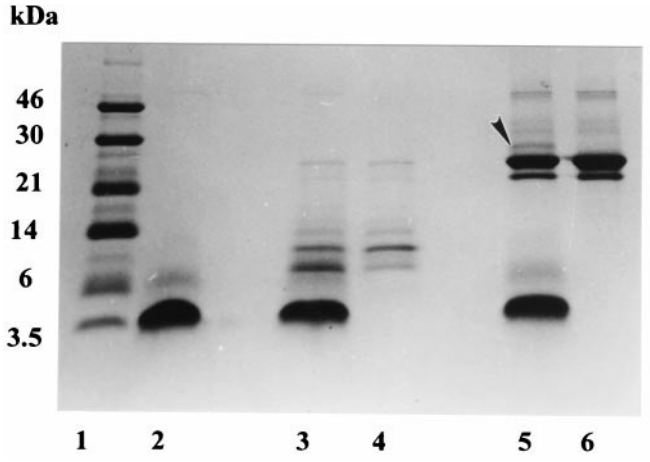

FIGURE 1 SDS-resistant binding of apoE fragments to Aβ. Incubation of Aβ with N-terminal and C- terminal domains of apoE3 led to formation of SDS-resistant complexes with the N-terminal fragment (*lane 5, arrowhead*) but not with the C-terminal fragment (*lane 3*). *Lane 1*: Molecular mass markers; *lane 2*: Aβ alone; *lane 4*: C-terminal fragment of apoE alone; *lane 6*: N-terminal fragment of apoE alone. Coomassie blue-stained gel.

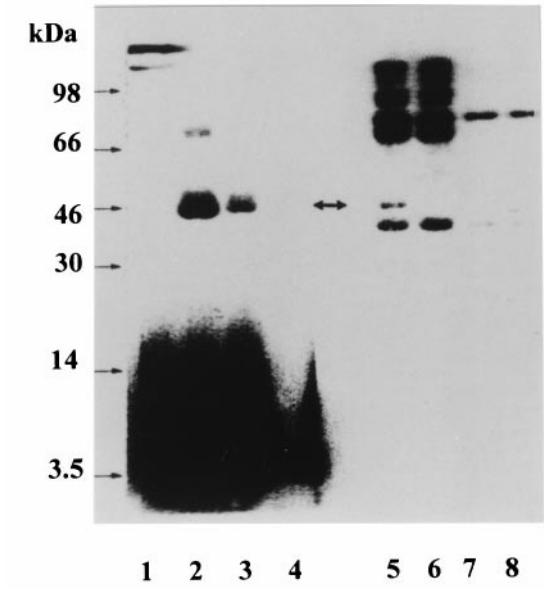

FIGURE 2 ApoE binding to Aβ is not inhibited by association of apoE with lipids. Incubation of the human plasma VLDL with Aβ led to the formation of an ~38-kDa complex, readily detected with anti-Aβ antibody (*lane 3*), similar to the complex of Aβ and purified apoE3 (*lane 2*). Because of the lower amount of apoE in VLDL and lower affinity of anti-apoE antibody than anti-Aβ antibody, the apoE/Aβ complex in lane 7 was detected only after prolonged exposure (not shown). *Lane 1*: Aβ alone; *lanes 2* and *5*: Aβ + apoE; *lanes 3* and *7*: Aβ + VLDL; *lanes 4* and *8*: VLDL alone; *lane 6*: apoE alone. *Lanes 1–4*: mAb 6E10, anti-Aβ; *lanes 5–8*: mAb 3D12, anti-apoE. ApoE/Aβ complexes are indicated by an arrow.

and C-apoE led to the formation of SDS-resistant complexes with the N-terminal fragment but not the C-terminal fragment (Fig. 1). This complex was readily visualized by Coomassie staining of the gel and by Western blotting, using monoclonal antibodies directed against Aβ (6E10) and apoE (9H8) (not shown). N-terminal sequencing of this N-apoE/Aβ ~26-kDa complex revealed the equimolar presence of apoE and Aβ, suggesting a 1:1 molar ratio in the complex. The sequences obtained were the following:

D A E F R (Aβ)

and

K V E Q A (apoE).

In vivo, most of the apoE is associated with lipid particles (lipoproteins) (Mahley, 1988). The binding of the protein to lipids is mediated by the C-terminal domain. Thus, in the lipoprotein environment, this apoE domain most likely will not be available for interaction with Aβ. To analyze whether the lipidated protein is able to bind Aβ, we performed SDS-resistant binding studies with the native apoE from human plasma in the form of purified VLDL particles. Incubation of the VLDL with Aβ, by a method similar to that used for purified apoE3, led to the formation of a ~38-kDa complex, readily detected with both anti-Aβ and anti-apoE antibodies (Fig. 2). These data indicate that SDS-resistant binding of Aβ to apoE is not inhibited by the association of apoE with lipids and therefore is not likely to be mediated by the C-terminal domain of apoE.

Monitoring apoE-Aβ interaction in solution by fluorescence spectroscopy

To estimate the parameters of Aβ binding to apoE3 and its fragments in solution, we used a method based on monitoring changes in the protein intrinsic fluorescence upon its binding to Aβ. Full-length apoE3 incubated alone when excited at 285 nm shows a fluorescence emission spectrum with a maximum at 345 nm (Fig. 3 *A*), which is typical of tryptophan residues in a protein environment. Incubation of apoE with increasing concentrations of Aβ caused a decline in its intrinsic fluorescence. When the changes in apoE fluorescence were plotted against Aβ concentration, we obtained a hyperbolic curve with a maximum at ~200 nM Aβ (Fig. 3 *B*). Nonlinear regression analysis of the data revealed an apparent dissociation constant for apoE3/Aβ complex of K_D = 11.65 nM, which is considered a high-affinity interaction and is very close to the value reported previously for a solid-phase binding assay (Golabek et al., 1996). Scatchard transformation of the fluorescence changes according to the Stern-Volmer equation $(-\Delta F/F_0/[A\beta] = KF_{max} - K(-\Delta F/F_0)$ resulted in a good fit to the two-binding-site populations model, with dissociation constants $K_{D1} \approx 2$ nM and $K_{D2} \approx 31$ nM (Fig. 3 *C*).

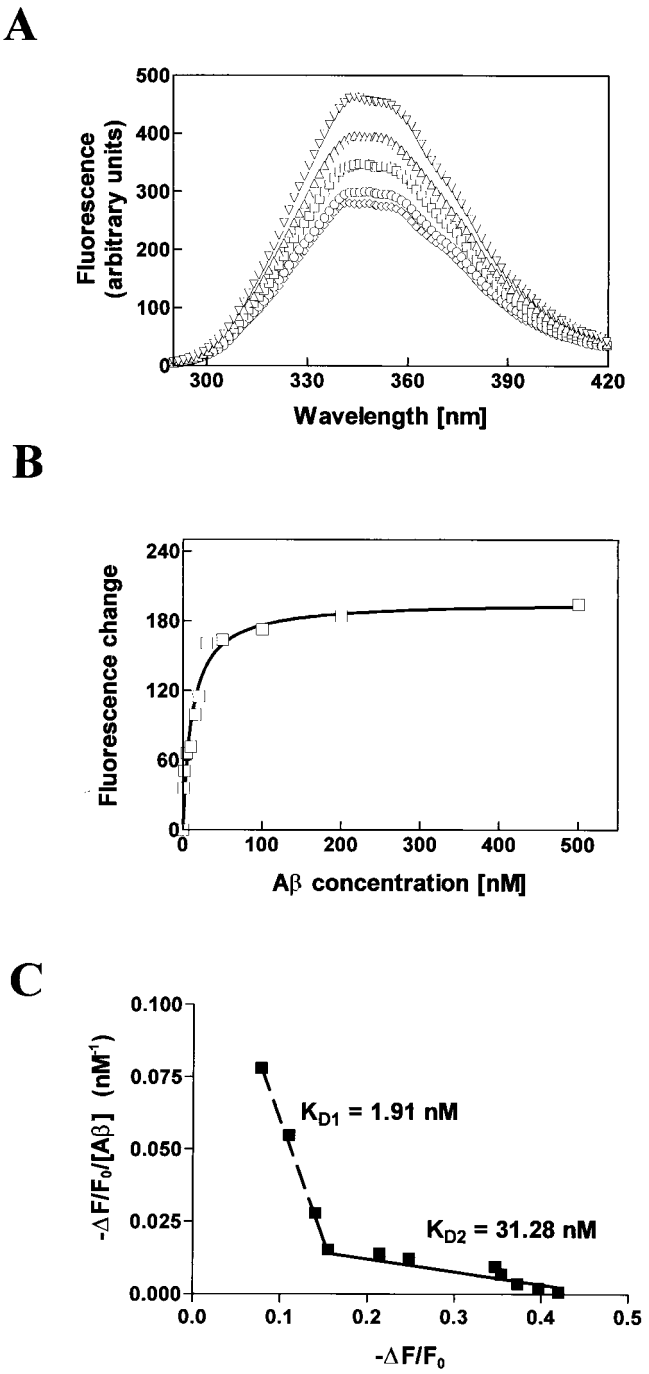

FIGURE 3 ApoE binding to Aβ under native conditions measured by using fluorescence quenching. (*A*) Decline of apoE3 intrinsic fluorescence (▽) after incubation with increasing concentrations of Aβ: △, 5 nM; △, 20 nM; ○, 50 nM; ◇, 200 nM. (*B*) Nonlinear regression analysis of apoE fluorescence changes revealed a saturable binding with B_{max} = 197 nM and a dissociation constant for the complex K_D = 11.65 nM. (*C*) Scatchard transformation of the fluorescence changes according to the equation $-\Delta F/F_0/[A\beta] = KF_{max} - K(-\Delta F/F_0$ resulted in a good fit to the two-binding-site model of interaction for the apoE/Aβ complex, with dissociation constants $K_{D1} \approx 2$ nM (– – –) and $K_{D2} \approx 31$ nM (——).

To determine where the two binding sites in the apoE molecule are located, binding of Aβ to the isolated domains was studied by fluorescence assay. The fluorescence spectra of N-apoE and C-apoE in the absence of Aβ were shifted slightly to the red (maxima at ~347 and ~350 nm, respectively), suggesting that the tryptophan residues were more exposed to the solvent than in the intact protein (Fig. 4, *A* and *B*). The presence of Aβ led to moderate changes in C-apoE fluorescence, whereas the changes in N-apoE fluorescence in the presence of increasing Aβ concentration were much higher and similar to the changes produced in the full-length apoE3 spectrum. Changes in fluorescence of apoE fragments were plotted against Aβ concentration, and apparent dissociation constants for binding of apoE fragments to Aβ were calculated with the help of curve-fitting software. The calculated K_D for the N-apoE/Aβ complex was ~11 nM, whereas the K_D for C-apoE/Aβ was ~66 nM (Fig. 4 *C*). Scatchard transformation of fluorescence data with the Stern-Volmer equation showed a simple population of binding sites for each apoE domain with dissociation constants of ~10 nM and ~ 45 nM for the N-apoE and C-apoE, respectively (Fig. 4, *D* and *E*).

DISCUSSION

Several lines of evidence suggest that apoE plays an important role in modulating amyloidosis-β. The aim of this study was to characterize apoE-Aβ interaction in terms of the protein regions implicated in the binding to the peptide. Our data show that delipidated apoE3 interacts with Aβ through two different binding sites, located in the N- and C-terminal functional domains of apoE. N-apoE binds Aβ with 4–10 times higher affinity than C-apoE. We have shown previously by using a solid-phase assay that the whole apoE3 molecule binds Aβ with a K_D of 20 nM (Golabek et al., 1996), and in the present study we obtained a K_D of ~11 nM by using a fluorescence binding assay in solution. A similar K_D value (10 nM) for apoE/Aβ heterodimer was obtained by others using surface plasmon resonance (Shuvaev and Siest, 1996). At present, we demonstrate that the SDS-resistant binding of Aβ to apoE can be attributed to the N-terminal domain of apoE and that the K_D of the complex formed by the N-terminal domain of apoE with Aβ 1–40 is similar to the dissociation constant of the complex formed by the whole apoE molecule with Aβ 1–40. Thus it is predominantly the N-terminal domain of apoE that is responsible for the stability of apoE/Aβ complexes.

These results differ from those published in the past by Strittmatter and colleagues (1993b) and Pillot et al. (1999), who found that residues 244–272 of apoE are critical for Aβ binding. The different results might be partially attributed to the distinct methodologies used. In addition, our earlier data indicate that these discrepancies might be caused by different Aβ peptides used for the experiments (Golabek et al., 1996). Strittmatter and colleagues per-

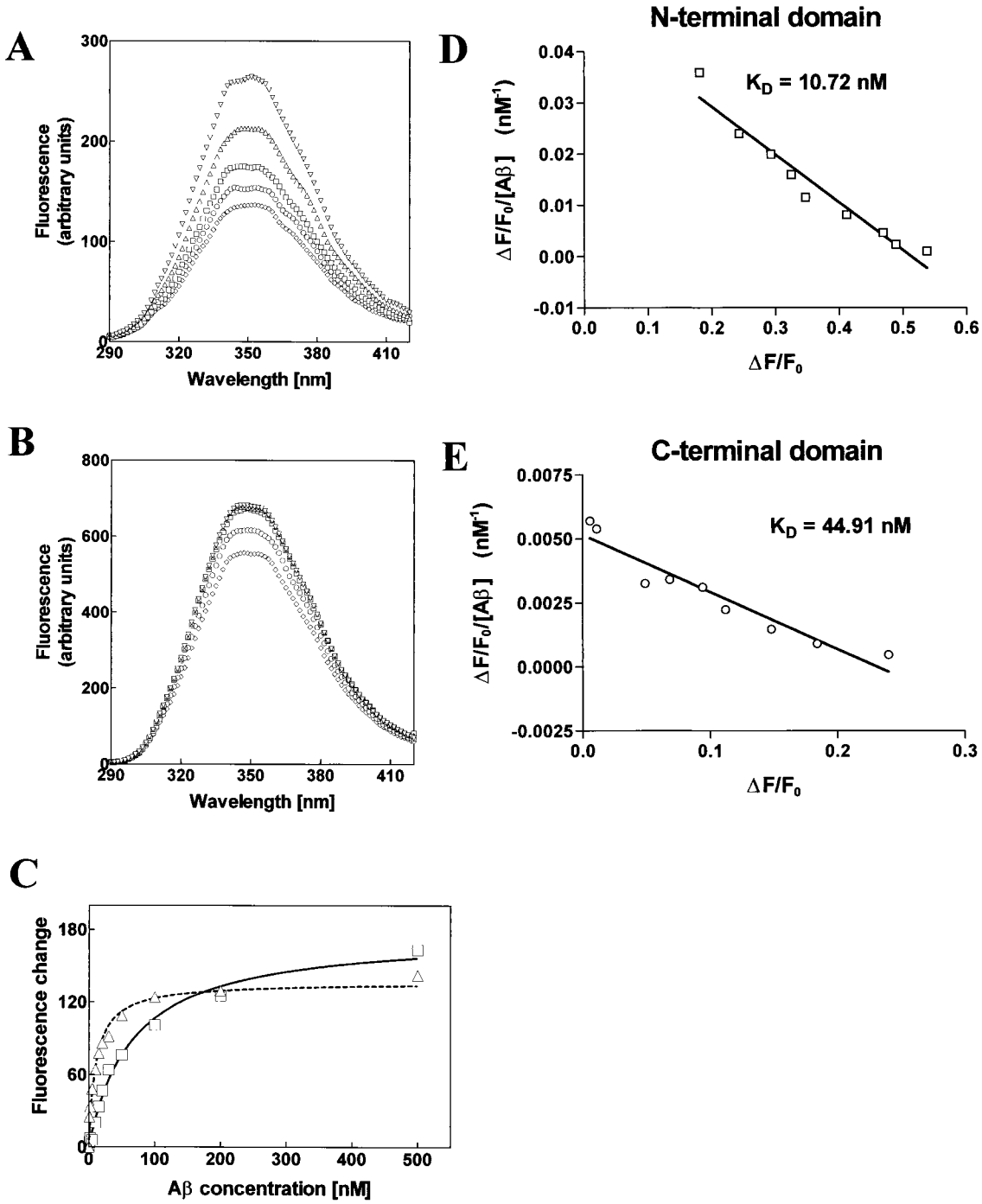

FIGURE 4 Native binding of apoE domains to Aβ. (*A* and *B*) N-terminal (*A*) and C-terminal (*B*) fragments of apoE3 were incubated with increasing concentrations of Aβ, and their fluorescence spectra were recorded: $\triangledown$, 0 nM of Aβ; $\triangle$, 5 nM; $\square$, 20 nM; $\bigcirc$, 50 nM; $\diamond$, 200 nM. (*C*) Both N-terminal (– – –) and C-terminal (——) domains of apoE3 saturably bound Aβ, resulting in dissociation constants K_D equal to ~11 nM and ~66 nM, respectively. (*D* and *E*) Scatchard transformation of the fluorescence changes induced by the interaction of Aβ with N-apoE (*D*) and C-apoE (*E*), fit with a single binding site population with $K_D \approx 11$ nM and 46 nM, respectively.

formed their experiments by using a shorter Aβ peptide, Aβ 1–28, of unknown conformation. Usually, this particular Aβ peptide shows an unordered structure at physiological pH (Barrow and Zagorski, 1991). On the other hand, studies of Pillot et al. (1999) utilized various fragments of Aβ peptides, excluding the most physiologically relevant full-length Aβ peptide (1–40 or 1–42). In contrast, we have chosen for our studies Aβ 1–40, a synthetic peptide with an

intact C terminus, the length of which corresponds to the main soluble Aβ species released by cells into physiological fluids. The Aβ1–40 selected for our experiments had a β-sheet content of 15–20%. According to our previous studies, the secondary structure of Aβ peptides is an important factor determining their ability to form complexes with apoE; peptides with high β-sheet content show higher affinity for apoE than Aβ peptides with low β-sheet content (Golabek et al., 1996). The binding of the N-terminal domain of apoE to Aβ also was observed by others; however, it appeared to be weaker than complexes formed by Aβ with full-length apoE (Chan et al., 1996).

The biological consequences of apoE/Aβ interactions are far from being elucidated. The N- and C-terminal domains of apoE molecule represent two independently folded structures connected by a hinge region (Aggerbeck et al., 1988; Wetterau et al., 1988; recent review: Weisgraber, 1994). The N-terminal domain of apoE has a stable globular structure, whereas the C-terminal domain is unstable in aqueous solvents and forms tetramers similar to the full-length, purified apoE. When each fragment is denatured with guanidine, its unfolding closely resembles the two transitions pattern characteristic of a full-length apoE molecule (Wetterau et al., 1988). On the basis of the observation that the thrombin-cleaved C-terminal fragment of apoE showed fibrillogenic properties in vitro, it was proposed that this fragment of apoE might constitute a fibrillar component of β-amyloid deposits in vivo (Wisniewski et al., 1995). Full-length apoE as well as both the N- and C-terminal fragments have indeed been isolated from amyloid deposits in AD brain (Wisniewski et al., 1995; Naslund et al., 1995). These results have also been confirmed by immunohistochemistry with the use of several antibodies directed to different regions of apoE (Namba et al., 1991; Wisniewski and Frangione, 1992; Aizawa et al., 1997).

Controversial studies have been published on the effect of apoE on Aβ fibrillogenesis. Several reports suggested that apoE might act as an inhibitor (Evans et al., 1995; Wood et al., 1996; Naiki et al., 1997) or as a promoter (Wisniewski et al., 1994; Ma et al., 1994; Castaño et al., 1995) of Aβ fibrillogenesis. Recent studies in apoE knockout mice with experimentally induced AA amyloidosis or Aβ amyloidosis strongly support the notion that apoE promotes, not inhibits, the process of amyloidogenesis. Amyloid-A deposition was clearly reduced in apoE knockout mice (Hoshii et al., 1997; Kindy and Rader, 1998). Of special importance is the observation that amyloid load can be restored in these animals after the expression of human *apoE3* gene (Kindy and Rader, 1998). Similarly, APP transgenic/apoE knockout mice demonstrate a dramatic decrease in amyloid load and lack of thioflavin-S-positive plaques in comparison with transgenes with a functional *apoE* gene (Bales et al., 1997, 1999). Seemingly opposite results were obtained recently by Holzman et al. (1999), who demonstrated the suppression of early amyloid deposition in human apoE3 and apoE4/APP transgenic mice on a mouse apoE null background. However, these authors used astrocyte-specific glial fibrillary acid protein promoter to drive the expression of human apoE in these animals. Unlike those of mice, human brain neuronal cells stain strongly for apoE (Han et al., 1994). Therefore, it is conceivable that the enhancement of amyloid deposition by apoE results from the specific interaction of neuronal apoE/neuronal Aβ, either within cells or upon their secretion, whereas apoE secreted by glial cells could participate in Aβ clearance, as suggested by Rebeck et al. (1993).

Considering that apoE genotype along with aging is a major risk factor for AD, the precise characterization of apoE/Aβ interaction may reveal novel therapeutic targets for the treatment of amyloidoses-β.

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REFERENCES

Aggerbeck, L. P., J. R. Wetterau, K. H. Weisgraber, C. S. Wu, and F. T. Lindgren. 1988. Human apolipoprotein E3 in aqueous solution. II. Properties of the amino- and carboxyl-terminal domains. *J. Biol. Chem.* 263:6249–6258

Aizawa, Y., R. Fukatsu, Y. Takamaru, K. Tsuzuki, H. Chiba, K. Kobayashi, N. Fujii, and N. Takahata. 1997. Amino-terminus truncated apolipoprotein E is the major species in amyloid deposits in Alzheimer's disease-affected brains: a possible role for apolipoprotein E in Alzheimer's disease. *Brain Res.* 768:208–214.

Bales, K. R., T. Verina, D. J. Cummins, Y. Du, R. C. Dodel, J. Saura, C. Fishman, C. A. DeLong, P. Piccardo, V. Petegnief, B. Ghetti, and S. M. Paul. 1999. Apolipoprotein E is essential for amyloid deposition in the APP^{V717F} transgenic mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA.* 96:15233–15238.

Bales, K. R., T. Verina, R. C. Dodel, Y. Du, L. Altstiel, M. Bender, P. Hyslop, E. M. Johnstone, S. P. Little, D. J. Cummins, P. Piccardo, B. Ghetti, and S. M. Paul. 1997. Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nature Genet.* 17:263–264.

Barrow, C. J., and M. G. Zagorski. 1991. Solution structures of beta peptide and its constituent fragments: relation to amyloid deposition. *Science.* 253:179–182.

Castaño, E. M., F. Prelli, T. Wisniewski, A. Golabek, R. A. Kumar, C. Soto, and B. Frangione. 1995. Fibrillogenesis in Alzheimer's disease of amyloid beta peptides and apolipoprotein E. *Biochem. J.* 306:599–604.

Chan, W., J. Fornwald, M. Brawner, and R. Wetzel. 1996. Native complex formation between apolipoprotein E isoforms and the Alzheimer's disease peptide Aβ. *Biochemistry.* 35:7123–7130.

Corder, E. H., A. M. Saunder, W. J. Strittmatter, D. E. Schmechel, P. C. Gaskell, G. W. Small, A. D. Roses, J. L. Haines, and M. A. Pericak-Vance. 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science.* 261:921–923.

Evans, K. C., E. P. Berger, C. G. Cho, K. H. Weisgraber, and P. T. Lansbury. 1995. Apolipoprotein E is a kinetic but not a thermodynamic inhibitor of amyloid formation: implications for the pathogenesis and treatment of Alzheimer disease. *Proc. Natl. Acad. Sci. USA.* 92:763–767.

Gearing, M., J. A. Schneider, R. S. Robbins, R. D. Hoillister, H. Mori, D. Games, B. T. Hyman, and S. S. Mirra. 1995. Regional variations in the distribution of apolipoprotein E and Aβ in Alzheimer's disease. *J. Neuropathol. Exp. Neurol.* 54:833–841.

Glenner, G. G., and C. W. Wong. 1984. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* 120:885–890.

Golabek, A. A., C. Soto, T. Vogel, and T. Wisniewski. 1996. The interaction between apolipoprotein E and Alzheimer's amyloid beta-peptide is dependent on beta-peptide conformation. *J. Biol. Chem.* 271: 10602–10606.

Goldgaber, D., M. I. Lerman, O. W. McBride, U. Saffiotti, and D. C. Gajdusek. 1987. Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. *Science.* 235: 877–880.

Hallman, D. M., E. Boerwinkle, N. Saha, N., C. Sandholzer, H. J. Menzel, A. Csázár, and G. Uterman. 1991. The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. *Am. J. Hum. Genet.* 49:338–349.

Han, S. H., G. Einstein, K. H. Weisgraber, W. J. Strittmatter, A. M. Saunders, M. Pericak Vance, A. D. Roses, and D. E. Schmechel. 1994. Apolipoprotein E is localized to the cytoplasm of human cortical neurons: a light and electron microscopic study. *J. Neuropathol. Exp. Neurol.* 53:535–344.

Holzman, D. M., K. R. Bales, S. Wu, P. Bhat, M. Parsasdanian, A. M. Fagan, L. K. Chang, Y. Sun, and S. M. Paul. 1999. Expression of human apolipoprotein E reduces amyloid-β deposition in a mouse model of Alzheimer's disease. *J. Clin. Invest.* 103:R15–R21.

Hoshii, Y., H. Kawano, D. Cui, T. Takeda, T. Gondo, M. Takahashi, K. Kogishi, K. Higuchi, and T. Ishihara. 1997. Amyloid A protein amyloidosis induced in apolipoprotein-E-deficient mice. *Am. J. Pathol.* 151:911–917.

Houlden, H., R. Crook, H. Backhovens, G. Prihar, M. Baker, M. Hutton, M. Rossor, J. J. Martin, C. Van Broeckhoven, and J. Hardy. 1998. ApoE genotype is a risk factor in nonpresenillin early-onset Alzheimer's disease families. *Am. J. Med. Genet.* 81:117–121.

Ignatius, M. J., P. J. Gebicke-Harter, J. H. Skene, J. W. Schilling, K. H. Weisgraber, and E. M. Shooter. 1986. Expression of apolipoprotein E during nerve degeneration and regeneration. *Proc. Natl. Acad. Sci. USA.* 83:1125–1129.

Kida, E., N-H. Choi-Miura, and K. E. Wisniewski. 1995a. Deposition of apolipoproteins E and J in senile plaques is topographically determined in both Alzheimer's disease and Down's syndrome brain. *Brain Res.* 685:211–216.

Kida, E., R. Pluta, A. S. Lossinsky, A. A. Golabek, N.-H. Choi-Miura, H. M. Wisniewski, and M. J. Mossakowski.1995b. Complete cerebral ischemia with short term survival in rat induced by cardiac arrest. II. Extracellular and intracellular accumulation of apolipoproteins E and J in the brain. *Brain Res.* 674:341–346.

Kim, K. S., D. L. Miller, V. J. Sapienza, C. M. J. Chen, C. Bai, I. Grundke-Iqbal, J. Currie, and H. M. Wisniewski. 1988. Production and characterization of monoclonal antibodies reactive to cerebrovascular amyloid peptide. *Neurosci. Res. Commun.* 2:121–130.

Kindy, M. S., and D. J. Rader. 1998. Reduction in amyloid A amyloid formation in apolipoprotein-E-deficient mice. *Am. J. Pathol.* 152: 1387–1395.

Koudinov, A., E. Matsubara, B. Frangione, and J. Ghiso. 1994. The soluble form of Alzheimer's amyloid beta protein is complexed to high density lipoprotein 3 and very high density lipoprotein in normal human plasma. *Biochem. Biophys. Res. Commun.* 205:1164–1171.

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

Levy-Lahad, E., A. Lahad, E. M. Wijsman, T. D. Bird, and G. D. Schellenberg. 1995. Apolipoprotein E genotypes and age at onset in early-onset familial Alzheimer's disease. *Ann. Neurol.* 38:678–680.

Ma, J., A. Yee, H. B. Brewer, S. Das, and H. Potter. 1994. Amyloid-associated proteins α1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer β-protein into filaments. *Nature.* 372:92–94.

Mahley, R. W. 1988. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science.* 240:622–630.

Masliah, E., M. Mallory, N. Ge, M. Alford, I. Veinbergs, and A. D. Roses. 1995. Neurodegeneration in the central nervous system of apoE-deficient mice. *Exp. Neurol.* 136:107–122.

Masters, C. L., G. Simms, N. A. Weinman, G. Multhaup, B. L. McDonald, and K. Beyreuther. 1985. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc. Natl. Acad. Sci. USA.* 82: 4245–4249.

Naiki, H., F. Gejyo, and K. Nakakuki. 1997. Concentration-dependent inhibitory effects of apolipoprotein E on Alzheimer's β-amyloid fibril formation in vitro. *Biochemistry.* 36:6243–6250.

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

Naslund, J., J. Thyberg, L. O. Tjernberg, C. Wernstedt, A. R. Karlstrom, N. Bogdanovic, S. E. Gandy, L. Lannfelt, L. Terenius, and C. Nordstedt. 1995. Characterization of stable complexes involving apolipoprotein and the amyloid peptide in Alzheimer's disease brain. *Neuron.* 15:219–228.

Nathan, B. P., S. Bellosta, D. A. Sanan, K. H. Weisgraber, R. W. Mahley, and R. E. Pitas. 1994. Differential effects of apolipoproteins E3 and E4 on neuronal growth in vitro. *Science.* 264:850–852.

Pillot, T., M. Goethals, J. Najib, C. Labeur, L. Lins, J. Chambaz, R. Brasseur, J. Vandekerckhove, and M. Rossenau. 1999. *J. Neurochem.* 72:230–237.

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

Robakis, N. K., N. Ramakrishna, G. Wolfe, and H. M. Wisniewski.1987. Molecular cloning and characterization of a cDNA encoding the cerebrovascular and the neuritic plaque amyloid peptides. *Proc. Natl. Acad. Sci. USA.* 84:4190–4194.

Saunders, A. M., W. J. Strittmatter, D. Schmechel, P. H. George-Hyslop, M. A. Pericak-Vance, S. H. Joo, B. L. Rosi, J. F. Gusella, D. R. Crapper-MacLachlan, and, M. J. Alberts.1993. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology.* 43:1467–1472.

Schägger, H., and G. von Jagow. 1987. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal. Biochem.* 166:368–379.

Selkoe, D. J. 1994. Alzheimer's disease: a central role for amyloid *J. Neuropathol. Exp. Neurol.* 53:438–447.

Seubert, P., C. Vigo-Pelfrey, F. Esch, M. Lee, H. Dovey, D. Davis, S. Sinha, M. Schlossmacher, J. Whaley, C. Swindlehurst, R. McCormack, R. Wolfert, D. J. Selkoe, I. Lieberburg, and D. B. Schenk. 1992. Isolation and quantification of soluble Alzheimer's β-peptide from biological fluids. *Nature.* 359:325–327.

Shoji, M., T. E. Golde, J. Ghiso, T. T. Cheung, S. Estus, L. M. Shaffer, X. D. Cai, D. M. McKay, R. Tintner, B. Frangione, and S. G. Younkin. 1992. Production of the Alzheimer amyloid β protein by normal proteolytic processing. *Science.* 258:126–129.

Shuvaev, V. V., and G. Siest. 1996. Interaction between human amphipathic apolipoproteins and amyloid β-peptide: surface plasmon resonance studies. *FEBS Lett.* 383:9–12.

Soto, C., A. Golabek, T. Wisniewski, and E. M. Castano. 1996. Alzheimer's β-amyloid peptide is conformationally modified by apolipoprotein E in vitro. *Neuroreport.* 7:721–725.

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

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

Tanzi, R. E., A. I. McClatchy, E. D. Lamperti, L. Villa-Kornaroff, J. F. Gusella, and R. L. Neve. 1988. Protease inhibitor domain encoded by an

amyloid protein precursor mRNA associated with Alzheimer's disease. *Nature.* 331:528–530.

Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350–4359.

Utermann, G., N. Pruin, and A. Steinmetz. 1979. Polymorphism of apolipoprotein E. III. Effect of a single polymorphic gene locus on plasma lipid levels in man. *Clin. Genet.* 15:63–72.

Van Gool, W. A., H. M. Evenhuis, and C. M. Duijn. 1995. A case-control study of apolipoprotein E genotype in Alzheimer's disease associated with Down's syndrome. *Ann. Neurol.* 38:225–230.

Vigo-Pelfrey, C., D. Lee, P. Keim, I. Lieberburg, and D. B. Schenk. 1993. Characterization of β-amyloid peptide from human cerebrospinal fluid. *J. Neurochem.* 61:1965–1968.

Vogel, T., K. H. Weisgraber, M. I. Zeevi, H. Ben-Artzi, A. Z. Levanon, S. C. Rall, T. L. Innerarity, D. Y. Hui, J. M. Taylor, D. Kanner, Z. Yavin, B. Arnit, H. Aviv, M. Gorecki, and R. Mahley. 1985. Human apolipoprotein E expression in *Escherichia coli*: structural and functional identity of the bacterially produced protein with plasma apolipoprotein E. *Proc. Natl. Acad. Sci. USA.* 82:8696–8700.

Weisgraber, K. H. 1994. Apolipoprotein E: structure-function relationships. *Adv. Protein Chem.* 45:249–302.

Wetterau, J. R., L. P. Aggerbeck, S. C. Rall, and K. H. Weisgraber. 1988. Human apolipoprotein E3 in aqueous solution. I. Evidence for two structural domains. *J. Biol. Chem.* 263:6240–6248.

Wisniewski, T., E. M. Castano, A. A. Golabek, T. Vogel, and B. Frangione. 1994. Acceleration of Alzheimer's fibril formation by apolipoprotein E in vitro. *Am. J. Pathol.* 145:1030–1035.

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

Wisniewski, T., J. Ghiso, and B. Frangione. 1997. Biology of Aβ amyloid in Alzheimer's disease. *Neurobiol. Dis.* 4:313–328.

Wisniewski, T., A. A. Golabek, E. Matsubara, J. Ghiso, and B. Frangione. 1993. Apolipoprotein E: binding to soluble Alzheimer's β-amyloid. *Biochem. Biophys. Res. Commun.* 192:359–365.

Wisniewski, T., M. Lalowski, A. A. Golabek, T. Vogel, and B. Frangione. 1995. Is Alzheimer's disease an apolipoprotein E amyloidosis? *Lancet.* 345:956–958.

Wood, S. J., W. Chan, and R. Wetzel. 1996. Seeding of Aβ fibril formation is inhibited by all three isotypes of apolipoprotein E. *Biochemistry.* 35:12623–12628.