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ABNORMAL CHOLESTEROL PROCESSING IN ALZHEIMER'S DISEASE PATIENT'S FIBROBLASTS

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Cholesterol has recently received attention as a potentially important factor in Alzheimer's disease etiology. Caveolin, which binds cholesterol, plays a prominent role in cellular cholesterol transport. Here, we found a higher level of cholesterol and caveolin in the caveolae-enriched fractions prepared from Alzheimer's disease patients' (AD) fibroblasts compared with age and sex matched controls (AC). Furthermore, the cross-linking activation of the prion protein, which is known to link to signal transduction of caveolin, is altered in AD fibroblasts. Our results suggest a dysregulation of cholesterol processing in AD fibroblasts which may contribute to the pathogenesis of AD.

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INTRODUCTION

Caveolae are vesicular invaginations of the plasma membrane involved in transcytosis, protocytosis, intracellular cholesterol transport and signal transduction.^{1,2} The main structural proteins of caveolae are the caveolins. Multiple isoforms have been isolated: caveolin-1 α , caveolin-1 β , -2, -3.³⁻⁶ Although they show general similarities in structure and function, they differ in tissue distribution. Caveolin-1 and -2 are coexpressed and form a hetero-oligomeric complex⁷ in many cell types,¹

whereas caveolin-3 seems to be expressed predominantly in muscles^{6,8} and astrocytes.^{9,10}

Beside its crucial structural role, caveolin undergoes determinant caveolae functions. Caveolae are membrane compartments for integration of signal-transduction pathways, and caveolin forms a scaffold to bind and concentrate several signaling molecules⁵ through its scaffolding domain.¹¹ The functional processing of caveolin (and caveolae) is dependent on the cellular level of cholesterol.¹²⁻¹⁶ The cholesterol itself regulates the expression of caveolin^{17,18} and binds it.¹⁹

Previously, biochemical, epidemiological and genetic characteristics have linked cholesterol levels to Alzheimer's disease.^{20,21,22ED+, 23ED+,24} In this study, we compared the expression of caveolin in fibroblasts isolated from Alzheimer's patients (AD) and a control population (AC). We have shown that caveolin expression and cholesterol concentration are increased in AD caveolae cellular fractions; these alterations seem to affect signal transduction processes. We propose a new hypothesis that includes a critical role for caveolin in regulating cholesterol in Alzheimer's disease.

MATERIALS AND METHODS

Cell culture. The present studies were carried out with human skin fibroblasts purchased from Coriell Cell Repositories (Camden, NJ, <http://locus.umdj.edu>), seeded and grown to confluence in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum in T-25 (or T-75 for caveolae isolation) culture flasks (Falcon).

Cell fractioning. *Preparation of Triton-soluble cell lysate.* Triton-soluble cell lysates were prepared from 6 AC cell lines (AG11020, AG09697, AG09603, AG09878, AG04461, AG13300; 72.5 ± 8.8 years, mean \pm SD) and 6 AD cell lines (AG05810, AG09908, AG06869, AG06844, AG06264, AG06265; 67 ± 10.1 , mean \pm SD). All the steps were carried out at 4°C. Each flask was washed twice with PBS, then cells were scraped into 0.8 ml of lysis buffer (10 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.5% NP40, 1% Triton X-100, 1% protease inhibitor cocktail, pH 7.4). The 6 flask contents were pooled and the cells were pelleted for 5 min, 1400 g (Sorvall RT7 centrifuge). The cells were resuspended in 0.5 ml of lysis buffer, then, passed through a 26^{1/2} needle 20 times, and sonicated 7 x 15 s on ice with 1 min rest in between times (total power, 50 J/W) with an Ultrasonic Homogenizer (4710 series, Cole-Parmer Instrument Co, IL). The sonicate was centrifuged for 10 min at 8000 g (Eppendorf 5417R centrifuge). The supernatant was collected and designated as the Triton-soluble cell lysate.

Preparation of membrane fractions. The following buffers were used: buffer 1 (10 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% protease inhibitor cocktail), buffer 2 (10 mM Tris HCl, 150 mM NaCl, 1 mM EDTA, 1mM EGTA, 0.5% NP40, 1% Triton X-100, 1% protease inhibitor cocktail, pH 7.4). To prepare AC and AD membrane fractions we used 6 AC cell lines: AG04461, AG11020, AG09697, AG11011, AG09603, AG09158 (68.8 ± 9.2 years old, mean \pm SD); and 6 AD cell lines: AG06263, AG04400, AG06844, AG06265, AG06869, AG06205 (62.5 ± 3.6 years old, mean \pm SD). A membrane fraction was prepared for each cell line. All steps were carried out at 4°C. Each flask was washed twice with PBS, then scraped into 0.8 ml of buffer 1. The suspension was sonicated 3 x 10 s on ice with 1 min rest in between times (total power, 50 J/W) with an Ultrasonic Homogenizer (4710 series, Cole-Parmer Instrument Co, IL). An aliquot of the sonicate was saved and designated as the whole cell fraction. The remaining sonicate was centrifuged for 1 h at 100,000 g (Beckman TL-100 ultracentrifuge). The pellet was resuspended in 100 μ l of buffer 2; then, the same centrifugation was repeated. The supernatant was collected and designated as the membrane fraction (plasma and intracellular membranes).

Membrane fractions were also prepared from another set of the same cell lines previously incubated for 1 h with polyclonal anti-PrP Ab-1 (Oncogene Research Products, Cambridge, MA), and diluted in DMEM (1/10, 000).

Caveolae isolation. The following buffers were used to prepare caveolae-enriched fractions: buffer A (0.25 M sucrose, 20 mM Tricine, 1 mM EDTA, pH 7.8), buffer B (0.25 M sucrose, 120 mM Tricine, 6 mM EDTA, pH 7.8), buffer C (50% OptiPrep in buffer B).

AC and AD caveolae-enriched fractions were purified at 4°C as previously described²⁵ with slight modifications. A plasma membrane fraction was prepared from 10 T-75 flasks of confluent AC or AD fibroblasts (AD cell lines: AG06265, AG06264, AG06844, AG09908, AG06869, 64.6 ± 9.2 years, mean \pm SD; age-matched AC cell lines: AG11020, AG07310, AG09697, AG04461, AG13300, AG09878 69 ± 9.0 years, mean \pm SD), and each cell line was grown in a separate flask. Each flask was washed twice with 5 ml of buffer A and then the cells were scraped into 3 ml of buffer A. The 10 AC (or AD) flask volumes were combined to pellet the cells by centrifugation for 5 min, 1400 g (Sorvall RT7 centrifuge). Cells were resuspended in 1 ml of buffer A, homogenized in a 2 ml tissue grinder (20 stokes), then passed through a 26^{1/2} needle 20 times. The suspension was transferred to a 1.5 ml Eppendorf tube and centrifuge for 10 min, 1000 g (Eppendorf 5417R centrifuge). The postnuclear supernatant fraction (PNS) was saved on ice, the pellet was resuspended in 1 ml of buffer A, then centrifuged again for 10 min at 1000 g. Both of the PNSs were combined and layered on the top of 23 ml of 30% Percoll in buffer A, and centrifuged for 30 min at 84,000 g (Beckman L8-55 ultracentrifuge, 70 Ti rotor, Beckman 355631 polycarbonate centrifuge tube). The plasma membrane fraction (PMF), a visible band at about 5 cm from the bottom of the tube, was collected and its volume was adjusted to 4 ml, before being sonicated with an Ultrasonic Homogenizer (4710 series, Cole-Parmer Instrument Co, IL). The PMF was sonicated 7 x 15 s on ice with 1 min rest in between times (total power, 50 J/W). The sonicate was mixed and homogenized with 3.68 ml of buffer C and 0.32 ml of buffer A in a Beckman 355631 polycarbonate centrifuge tube. A 20% to 10% linear OptiPrep gradient (12 ml, prepared by diluting buffer C with buffer A) was poured on the top of the sample, then centrifuged for 90 min at 52,000 g (Beckman L8-55 ultracentrifuge, SW28 swinging out bucket rotor). The top 10 ml of the gradient was collected and mixed with 8 ml of buffer C, then transferred to a Beckman 355631 polycarbonate centrifuge tube. The sample was overlaid with 4 ml of 5% OptiPrep (prepared by diluting buffer C with buffer A), and centrifuged for 90 min at 52,000 g (Beckman L8-55 ultracentrifuge, SW28 swinging out bucket rotor). The caveolae-enriched fraction was a visible

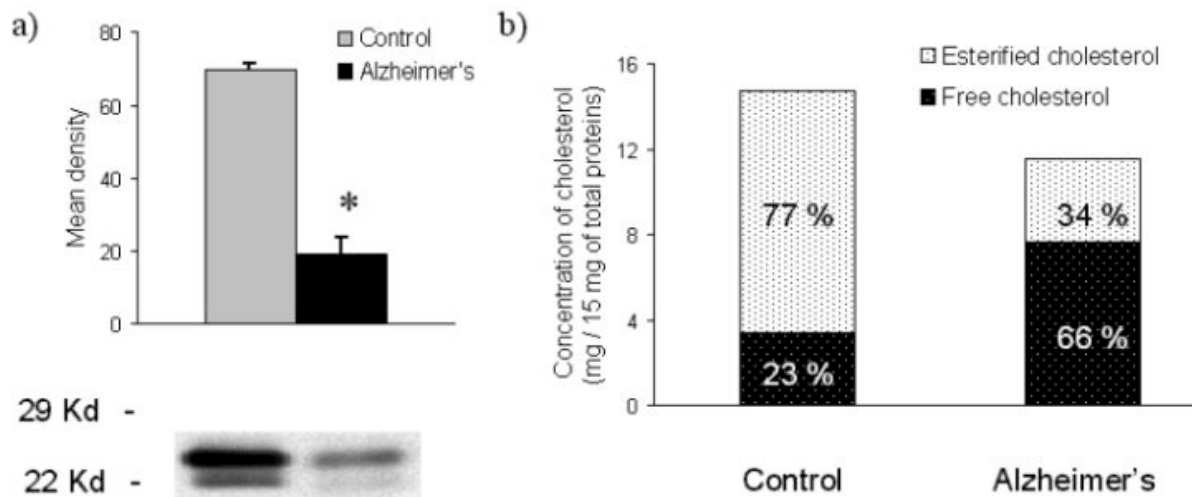


FIGURE 1. EXPRESSION OF CAVEOLIN (a) AND CHOLESTEROL CONCENTRATION (b) IN ALZHEIMERS AND CONTROL FIBROBLAST TRITON-SOLUBLE LYSATE.

Six Alzheimer's or 6 control cell lines were pooled to prepare the lysates as described in Materials and Methods. **a)** 2 μ g of total proteins were separated on SDS polyacrylamide gel, then transferred to a nitrocellulose membrane. The samples were immunoblotted with anti-caveolin, then with peroxidase-conjugated anti-rabbit IgG. The signal was detected by chemiluminescence and exposition to a film. Immunoblots were quantified by measuring the mean optical density on the film; the experiment was triplicated (* $p < 0.01$). **b)** cholesterol concentrations were measured in 15 μ g of total proteins by using a fluorometric method; the experiment was duplicated (* $p < 0.05$).

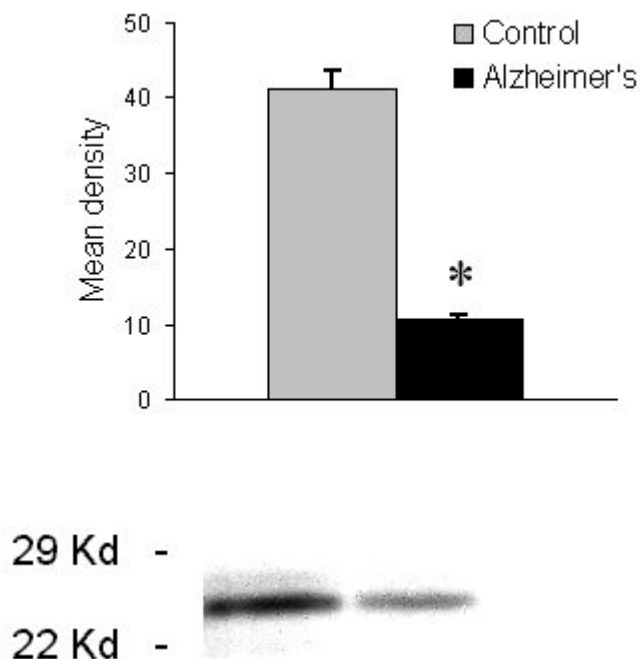


FIGURE 2. EXPRESSION OF CAVEOLIN IN ALZHEIMERS AND CONTROL FIBROBLAST PLASMA MEMBRANE FRACTIONS.

Six Alzheimer's or 6 control cell lines were pooled to prepare the plasma membrane fractions as described in Materials and Methods. 0.2 μ g of total proteins were separated on SDS polyacrylamide gel, then transferred to a nitrocellulose membrane. The samples were immunoblotted with anti-caveolin, then with peroxidase-conjugated anti-rabbit IgG. The signal was detected by chemiluminescence and exposition to a film. Immunoblots were quantified by measuring the mean optical density on the film; the experiment was triplicated (* $p < 0.001$).

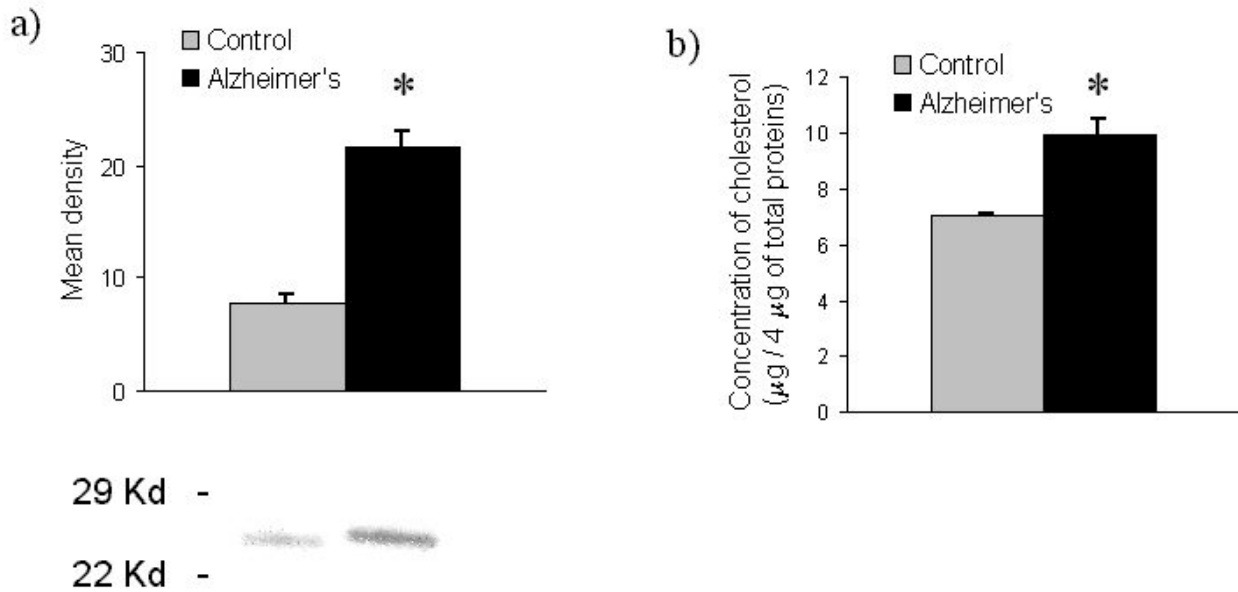


FIGURE 3. EXPRESSION OF CAVEOLIN (a) AND CHOLESTEROL CONCENTRATION (b) IN ALZHEIMERS AND CONTROL FIBROBLAST CAVEOLAE FRACTIONS.

Five Alzheimer's or 6 control cell lines were pooled to prepare the caveolae fractions as described in Materials and Methods. **a)** 0.2 μg of total proteins were separated on SDS polyacrylamide gel electrophoresis, then transferred to a nitrocellulose membrane. The samples were immunoblotted with anti-caveolin, then with peroxidase-conjugated anti-rabbit IgG. The signal was detected by chemiluminescence and exposition to a film. Immunoblots were quantified by measuring the mean optical density on the film; the experiment was triplicated (* $p < 0.01$). **b)** cholesterol concentrations were measured in 4 mg of total proteins by using a fluorometric method; the experiment was duplicated (* $p < 0.05$).

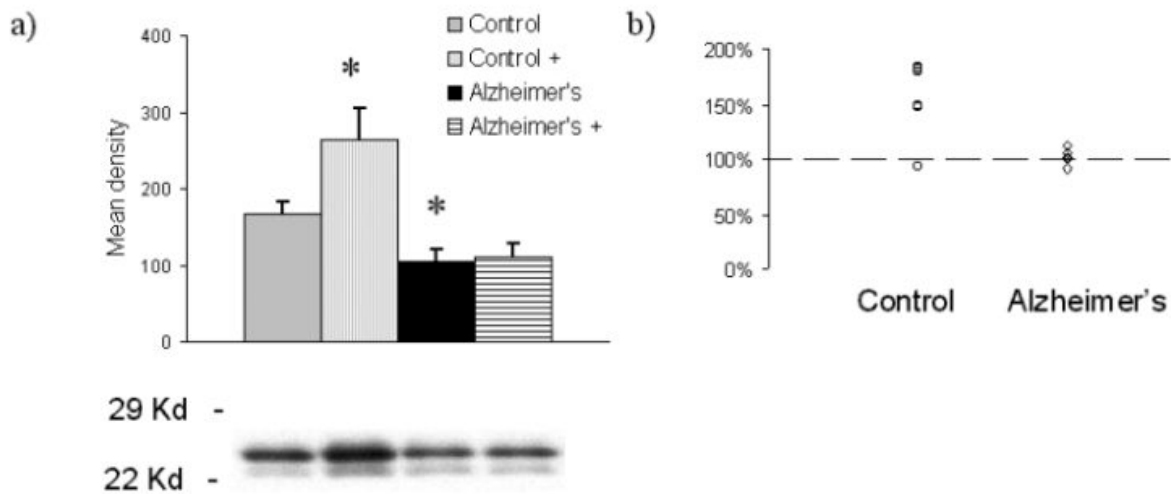


FIGURE 4: EFFECT OF ANTI-PRION CROSS-LINKING ON CAVEOLIN EXPRESSION IN ALZHEIMERS AND CONTROL FIBROBLAST MEMBRANE.

Six Alzheimer's or 6 control cell lines were incubated with anti-prion antibodies for 1 h, then a membrane fraction was prepared from each cell line as described in Materials and Methods. 1 mg of total proteins were separated on SDS polyacrylamide gel, then transferred to a nitrocellulose membrane. The samples were immunoblotted with anti-caveolin, then with peroxidase-conjugated anti-rabbit IgG. The signal was detected by chemiluminescence and exposition to a film. Immunoblots were quantified by measuring the mean optical density on the film (* $p < 0.05$). Figure 4a shows the expression of caveolin in Alzheimer's and control fibroblast membrane in basal conditions and after incubation with anti-prion (+). Figure 4b shows the distribution of anti-prion cross-linking effect on caveolin level in Alzheimer's and control fibroblast membrane fractions. Results are expressed in percentage of basal level.

band present in the 5% OptiPrep overlay about 1 cm under the surface.

Gel electrophoresis and immunoblots. The level of caveolin was quantified in each cell fraction by immunoblotting. All experiments were triplicated. Initially, the same amount of proteins from each sample was boiled for 10 min with an equal volume of 2X SDS sample buffer. The samples were loaded on a SDS polyacrylamide gel to separate the proteins, before transferring them to a nitrocellulose membrane. The samples were immunoblotted with anti-caveolin (BD Biosciences - Transduction Laboratories, Lexington, KY) at 4°C overnight. After several washes, the membranes were incubated with peroxidase-conjugated anti-rabbit IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) at room temperature for 1 h. Then, the signal was detected by ECL TM Western blotting detection reagents (Amersham Pharmacia Biotech Inc., NJ), and exposed to a Kodak Biomax MR Film (Eastman Kodak Company, Rochester, NY). Immunoblots were quantified by measuring the optical density with an appropriate software (UN-SCAN-IT, Silk Scientific Corporation).

Immunoprecipitation of phospho-caveolin. The level of caveolin phosphorylation was compared in AC and AD whole cell fractions, prepared from 5 AC (AG04461, AG11020, AG09697, AG11011, AG09603, 70.8 ± 8.7 years, mean ± SD) and 6 AD (AG06263, AG04400, AG06844, AG06265, AG06869, 61.6 ± 3.1 years, mean ± SD) cell lines, according to the protocol described above. Whole cell fractions were incubated with end-to-end rocking (4°C, overnight) with anti-phosphotyrosine antibodies (PY-20, Transduction Laboratories, Lexington, KY) in a buffer containing 50 mM Tris (pH 7.5), 150 mM NaCl, 1% protease inhibitor mixture (Sigma, St. Louis, MO), and 1% phosphatase inhibitor mixture (Sigma, St. Louis, MO). Protein A and G (1/3 ratio respectively) agarose beads were added to the mixture, and rocked at 4°C for 2 hours. After the wash, the precipitated proteins were separated by SDS-PAGE, and caveolin was detected in each sample with immunoblotting assays. Sample proteins were quantified to normalize the immunoblot optical density values.

Immunocytochemistry. AC (AG09697, AG09603, AG11020) and AD (AG06844, AG06869, AG06265) fibroblasts were grown on glass coverslips to reach confluence. Cells were washed twice in pre-chilled PBS, and fixed for 30 min at room temperature with 4% formaldehyde (wt/vol) in PBS. After fixation, cells were washed 3 times with PBS at room temperature for 10 min, then permeabilized with 0.1% Triton X-100, 10% normal horse serum, made in PBS, at room

temperature for 30 min. Cells were washed in PBS, incubated for 2 h at room temperature with anti-caveolin (BD Biosciences - Transduction Laboratories, Lexington, KY) diluted 1/500 in PBS, and washed again before the incubation with fluorescein anti-rabbit IgG diluted in PBS 1/400 (1.25 µg/ml) for 1 h at room temperature in the dark. Finally, cells were washed and coverslips were mounted with a drop of Vectashield mounting medium, and observed using a Nikon Eclipse E800 microscope (FITC filter). Immunofluorescence measurements were also carried out on the same cell lines previously incubated for 1 h with polyclonal anti-PrP Ab-1, prepared in DMEM (1/10,000).

Protein and cholesterol assays. Protein concentrations were determined by the Micro BCATM Protein Assay Reagent Kit (Pierce, Rockford, IL). Cholesterol concentrations were quantified by the Amplex® Red Cholesterol Assay Kit (Molecular Probes, Eugene, OR). All assays were duplicated.

RESULTS

Triton-soluble cell lysate and whole cell fractions. The amount of caveolin detected in Triton-soluble cell lysate was 72% lower in AD than AC (6 pooled cell lines, experiment triplicated, $p < 0.01$) (Figure 1a). Two bands were detected around 22 Kd, corresponding to previously described α (slowest band) and β (fastest band) isoforms of caveolin.²⁶ In the same preparation, the amount of cholesterol was 22% lower in AD than AC (6 pooled cell lines, experiment triplicated, $p < 0.05$) (Figure 1b). Moreover, in a preliminary study of the cholesterol detected in AC cell lysate, we measured that 77% was esterified, and 23% was free cholesterol. In AD, we found 34% esterified and 66% free cholesterol.

The amount of caveolin was the same in AC and AD whole cell fractions, and no difference was found in caveolin phosphorylation (data not shown).

Membrane fractions. Only the α -caveolin isoform was detected in the plasma membrane fractions. The level of caveolin was 74% lower in AD than in the AC plasma membrane fractions (Figure 2).

Caveolae fraction. In accordance with the findings of Smart and colleagues,²⁵ we found that caveolin was enriched in fraction 3 of the final Optiprep gradient. This fraction was called the caveolae fraction. Only the α -caveolin isoform was detected in this fraction. In this preparation, the caveolin concentration was 3 times higher in AD than AC (Figure 3a). The cholesterol concentration was also higher (+41%) in the AD caveolae fraction than in AC (Figure 3b).

Cross-linking with anti-PrP. Incubation of AC fibroblasts with a polyclonal anti-PrP increased the amount of caveolin found in the membrane fraction ($n=6$, $p<0.05$), (Figures 4a, 4b). The same incubation did not induce any increase of caveolin expression in AD fibroblast membranes (Figures 4a, 4b).

Immunocytochemistry. A qualitative localization of caveolin in fibroblasts was performed with immunofluorescence. In basal conditions, caveolin was mostly scattered through the cells in AC (Figure 5A), whereas it was more specifically located on the plasma membrane in AD fibroblasts (Figure 5C). Caveolin moved to membranes after anti-PrP incubation of AC fibroblasts (Figure 5B). This incubation did not change the pattern of caveolin distribution in AD fibroblasts (Figure 5D).

DISCUSSION

Alzheimer's patient fibroblasts. The use of skin fibroblasts is based on the hypothesis that an affliction of the human brain markers and pathological processes of Alzheimer's disease might also be expressed in peripheral tissues. A number of abnormalities in metabolic and biochemical processes described in AD brains have been found in cultured skin fibroblasts derived from AD patients.²⁷ Previous data have demonstrated a decrease in PKC activity,²⁸⁻³¹ alterations of calcium metabolism,³²⁻³⁴ dysfunctions of potassium channels³⁵ and MAP kinase signaling cascade³⁶ in AD fibroblasts. Thus, past findings suggest that fibroblasts might be useful to identify and test hypotheses on brain pathological mechanisms leading to Alzheimer's disease. Peripheral tissues do offer the potential of avoiding variables introduced by differences in the post-mortem state of brain tissues.

Specific pattern of caveolin distribution in AD fibroblasts. We measured the basal expression of caveolin-1 in AC and AD fibroblast cellular fractions. Two bands (around 22 Kd) were detected on western blots by incubating the membranes with rabbit polyclonal anti-caveolin-1 antibodies (C 13630 from Transduction Lab.), except in the plasma membrane and caveolae fractions. These bands correspond to the 2 isoforms of caveolin-1, a slower (α) and faster (β) migrating species.^{3,26} Moreover, Fujimoto *et al.* (2000, Ref. 3) have reported that C 13630 antibodies react better with the α than the β isoform. This could explain the weakest signal, or the absence of the caveolin β -isoform detected here. Because both α - and β -caveolin have different roles in caveolae formation,

it may be important to measure the isoform specific expression of caveolin in AC and AD cells. However, it has to be pointed out that in normal human skin fibroblasts, the α -isoform seems to be more efficient in and sufficient for forming functional caveolae.³

Because of their lipid-enriched content, caveolae are insoluble in Triton X-100 at 4°C.^{1,37,38} We used this specific property to compare the expression of caveolin in whole cell preparations with and without caveolae (i.e., Triton-soluble cell lysate). No differences were found between AC and AD whole cell fractions (data not shown). However, in Triton-soluble cell lysate, we found a higher caveolin expression in AC than AD fibroblasts. Moreover, in the plasma membrane fraction, caveolin expression was also higher in AC fibroblasts. This finding tends to argue that in AD, caveolin is mostly segregated to caveolae. Based on these data, we inferred that caveolin might be highly concentrated in caveolae of AD fibroblasts.

To test this possibility, a detergent-free method was used for purifying caveolae.²⁵ In these conditions, AD caveolae fractions were effectively richer in caveolin than AC. These results suggest an AD-specific distribution among cell compartments of caveolin in fibroblasts. The detection of caveolin in AC and AD fibroblasts by immunocytochemistry is in accordance with these biochemical results. Instead of being widely dispersed through out the internal and plasma membranes as was true for AC fibroblasts, caveolin was preferentially located in aggregates that appeared as dots in AD plasma membranes. Such aggregates may correspond to clustered caveolin in caveolae. Further studies with immunoelectron microscopy will be needed to clarify this issue. Nevertheless, considering that the expression of caveolin-1 led to de novo formation of caveolae,³⁹ a higher concentration of caveolin in a caveolae-enriched fraction could reflect a higher number of caveolae in AD fibroblasts.

The cellular localization of caveolin could depend on its phosphorylation status as previously shown.^{40,41} In the present study, however, we did not find any specific pattern of caveolin phosphorylation in AD. Thus, to understand the specific expression of caveolin in AD fibroblasts, we measured the concentration of cholesterol in Triton-soluble cell lysates and in caveolae fractions. Cholesterol plays an important role in caveolae structure and function.¹²⁻¹⁶ It binds caveolin¹⁹ and regulates its expression.^{17,18} In caveolae fractions, the amount of cholesterol was significantly higher in AD than in AC, consistent with the observed pattern of caveolin distribution. In a preliminary experiment, the level of cholesterol in Triton-soluble cell lysate was lower in AD. Interestingly,

we found that instead of 23% in AC fibroblasts, 66% of the total cholesterol was present as free cholesterol (FC) in AD. This observation could be critical for the final caveolin distribution, since the expression of caveolae, caveolin expression, and caveolin mRNA levels, are very sensitive to the FC content of the cell,^{17,42} and the newly formed FC is recovered mainly in caveolae.^{12,43} Thus, in AD fibroblasts, an abnormal level of FC, whatever its origin, could be responsible for the high level of caveolin sequestration in caveolae.

Signal transduction in AD fibroblasts. The subcellular distribution of several signaling molecules is restricted and regulated by their association with scaffolding proteins⁴⁴ of which caveolin is one^{1,11} (see Ref. 5 for review). Thus, we wondered if the overexpression of caveolin in caveolae fractions could affect cellular signal transduction in AD fibroblasts. Glycosylphosphatidylinositol (GPI)-anchored proteins are sequestered with caveolin in caveolae by antibody cross-linking.⁴⁵⁻⁴⁸ Prion protein, which is a GPI-anchored protein, has been localized in fibroblast caveolae after cross-linking.⁴⁹ We therefore tested the effect of prion cross-linking on caveolin expression in membrane fractions from AC and AD fibroblasts. In five AC fibroblast cell lines (6 tested), the incubation with a rabbit polyclonal anti-prion induced an increase of caveolin expression in membranes; no changes were observed in AD cell lines (6 tested). In AC fibroblasts, the same cross-linking stimulation changed the distribution of caveolin. In brief, prion cross-linking induced a caveolin shift from cytosol to membrane in most of the AC fibroblasts. This membrane localization of caveolin was quite similar to the caveolin localization in unstimulated AD fibroblasts. Such a concentration of caveolin in caveolae fractions might alter signal transduction in AD cells.

Previous studies have, in fact, shown abnormalities of signal transduction in AD fibroblasts.^{28,32,35,50-52} For instance, AD fibroblasts exhibit an enhanced response (calcium signaling) to bradykinin,³³ probably due to an enhanced IP3 production in response to this neuropeptide,⁵³ which is correlated with an up-regulation of bradykinin receptors.⁵³ Bradykinin receptors are G protein-coupled receptors, sequestered in caveolae after agonist-stimulation.^{54,55} Furthermore, the cellular bradykinin-stimulated calcium waves preferentially originate from caveolin-rich locations.⁵⁶ Considering these published data and the results presented in this study, the high expression of caveolin in AD caveolae fraction could participate in the enhancement of bradykinin-stimulated

calcium response by facilitating the recruitment of bradykinin receptors.

Cholesterol metabolism and Alzheimer's disease.

It is now well accepted that cholesterol may play a crucial role in pathological processes of Alzheimer's disease^{23ED+} (also see Related Articles in an online version of this article). Epidemiological studies showed that treatment with statins, cholesterol-lowering drugs initially prescribed for hypercholesterolemia, decreased the risk of developing Alzheimer's disease.^{57ED+,58ED+} In lab animals, a cholesterol-enriched diet induced amyloid burden in the brain.^{59ED+} APP, the amyloid precursor protein, can be processed either by α -secretase, and produce a non-amyloid A β sequence, or, by β - and γ -secretase, generating the A β amyloid peptide. The competition between these two alternative pathways is therefore crucial to the etiology of Alzheimer's disease. It has been suggested that at the cellular level, high cholesterol concentration could switch the secretase activities from the non-amyloidogenic to the amyloidogenic pathway. Cholesterol decreases the activity of the non-amyloidogenic α -secretase,⁶⁰ whereas it increases the activity of the amyloidogenic β -secretase.^{22ED+} Although an alteration of APP glycosylation,⁶¹ or a direct binding on α -secretase^{22ED+} by the cholesterol were proposed, no clear mechanism can yet explain the modulation of APP metabolism by cholesterol.

Here, we suggest an alternative hypothesis to understand the role of cholesterol in Alzheimer's disease (Scheme 1), in which caveolin would play a pivotal role. Caveolin upregulation activates β -secretase-mediated cleavage of APP in AD astrocytes.¹⁰ We suggest that the accumulation of (free?) cholesterol would induce an overexpression of caveolin in caveolae compartments. It was demonstrated that caveolin binds PKC,⁶² which is located in caveolae,⁶³⁻⁶⁵ and inhibits its activity.^{62, 66} PKC activity has been reported to be weaker in AD cells.³¹ An important role of α -secretase pathway activation has been attributed to PKC.^{67,68} In our hypothesis, the overexpression of caveolin would dramatically inhibit PKC. Such a process would result in an "underactivation" of α -secretase, and a consequent, progressive switch to the amyloidogenic pathways in Alzheimer's disease. PKC has been involved in memory processes,⁶⁹ and the initial inhibition of PKC proposed in our model could contribute to memory alteration which is shown in the early stages of Alzheimer's disease; this model would also explain the progressive formation of amyloid plaques. Further investigations are planned to test this hypothesis.

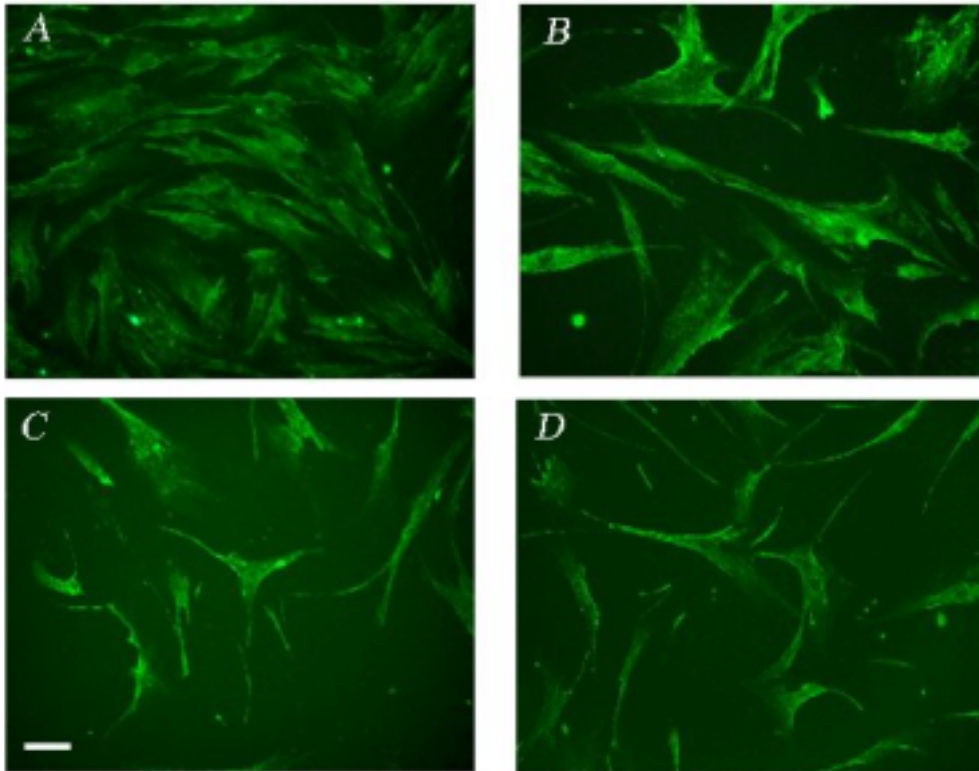
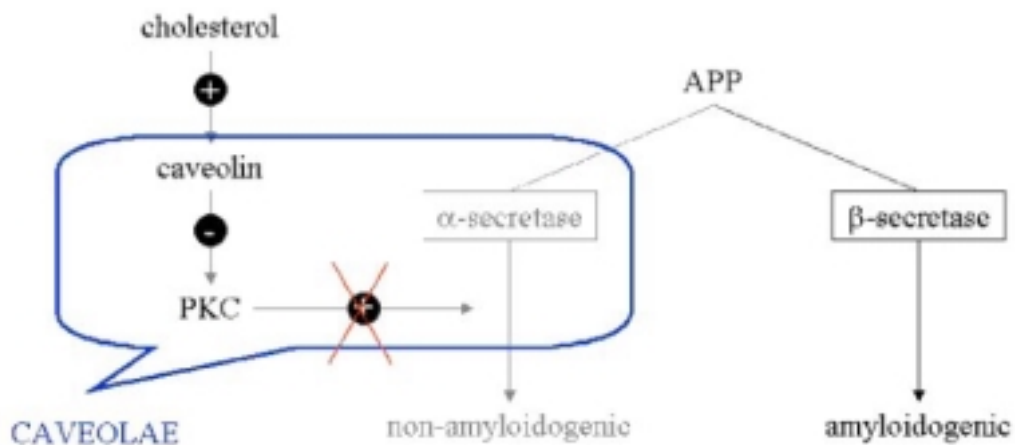


FIGURE 5. IMMUNOCYTOCHEMICAL LOCALIZATION OF CAVEOLIN IN CONTROL (A, B) AND ALZHEIMERS (C, D) FIBROBLASTS IN BASAL CONDITIONS (A, C) AND AFTER ANTI-PRION CROSS-LINKING (B, D).

Immunofluorescence localization of caveolin was performed on 3 Alzheimer's and 3 control fibroblast cell lines previously incubated for 1 h with anti-prion, or in basal conditions. Panels presented in Figure 5 are representative of the observed patterns (bar, 50 μ for all panels).



SCHEME 1. HYPOTHESIS INCLUDING A CRITICAL ROLE FOR CAVEOLIN IN REGULATING APP METABOLISM IN ALZHEIMER'S DISEASE.

The overexpression of caveolin would dramatically inhibit PKC, a well-known mechanism. Such a process would result in an "underactivation" of alpha-secretase pathway, and a consequent switch to the amyloidogenic pathways in a progressive process, as in Alzheimer's disease. PKC has been involved in memory processes, and the initial inhibition of PKC proposed in our model could contribute to memory alteration which is shown in the early stage of Alzheimer's disease; this model would also explain the progressive formation of amyloid plaques.

CONCLUSION

In conclusion, we found a caveolin enrichment in AD fibroblasts caveolae fraction. We suggest that this specificity could come from an alteration of cholesterol metabolism, and caveolin itself could play a pivotal role in the pathological process of Alzheimer's disease.

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