Coronary Artery Disease, Hypertension, ApoE, and Cholesterol: A Link to Alzheimer's Disease?

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ABSTRACT: The premature presence of senile plaques (SP) in coronary artery disease (CAD), and neurofibrillary tangles (NFT) as well as SP in hypertension (HyperT), suggest a neuropathologic link between CAD, HyperT, and AD. Previous MI, CAD and HyperT often occur in and may increase the risk of AD. Expression of Apo-E4 likely increases risk of CAD by elevating blood cholesterol and the risk of AD via proposed interactions with β-amyloid and/or free radicals (FRs). Any Apo-E4 effect is vague, but FRs probably mediate vascular damage in HyperT. Increasing FR content in the blood is related to increasing CAD severity, while the severity of elevated FR level correlates with how deep into a blood vessel there is activation of the FR scavenger enzyme, superoxide dismutase (SOD). The ApoE genotype and SP/NFT areal densities were determined in a large population of non-demented CAD, HyperT and non-heart disease (non-HD) control subjects, and compared to findings in a similar number of AD patients. ApoE immunoreactivity was determined in many individuals. Cholesterol content in cortex was determined by HPLC in a small, loosely age-matched group of Apo-E4 genotype-matched AD, CAD and non-HD subjects. SOD immunoreactivity was also assessed in a number of subjects. The Apo-E4 genotype frequency was increased in CAD, HyperT and AD compared to non-HD controls. Dose of Apo-E4 correlated with SP densities, but not NFT, and only in the non-demented groups. Essentially all SP in CAD, HyperT and non-HD subjects were ApoE-immunoreactive. Cortical cholesterol was increased in CAD and AD compared to controls. SOD immunoreactivity was similar in HyperT and AD; SP were immunodecorated in both. AD, CAD and HyperT may be linked, while CAD and HyperT subjects may die of heart disease before showing cognitive change.

INTRODUCTION

Alzheimer's disease (AD) and ischemic heart disease as hypertension and/or coronary artery disease may be disorders linked pathophysiologically by the influence of apolipoprotein E genotype expression and the resultant effect on cholesterol levels, free radical activity, and formation of neuropathologic lesions. Senile plaques (SP) and neurofibrillary tangles (NFT) are neuropathologic lesions characteristic of AD.[1] If a sufficient number of SP and NFT occur in the brain of a subject experiencing antemortem cognitive deterioration culminating in profound dementia, then this individual can be diagnosed, by convention, as having AD.[2] Therefore, by definition, all AD patients exhibit abundant cerebral SP and NFT.

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Interestingly, there is an increased prevalence of cerebral SP in cognitively intact individuals with hypertension and/or critical coronary artery disease (cCAD) compared to age-matched non-heart disease (non-HD) controls.[3-5] Such SP have been shown to be indistinguishable from those found in AD, and occur at ages substantially younger than they are demonstrable in normal aging.[3] Furthermore, non-demented individuals with hypertension also prematurely exhibit increased incidence of cerebral NFT compared to age-matched non-HD controls.[5] The number of SP and NFT identifiable in the brains of individuals with hypertension and cCAD often achieve levels diagnostic of AD.[2]

Heart disease is a prevalent finding in subjects with neuropathologically confirmed AD.[5-9] Previous silent myocardial infarction in women has been proposed as a risk factor for AD.[6] Most patients with neuropathologically confirmed AD at full autopsy are reported to have had significant coronary artery disease.[5] Recently, one study suggested a significant relationship between atherosclerotic disease and cognitive impairment in the general population.[7] Hypertension has been suggested as a risk factor for AD,[8] while many patients experience hypertension early in their course, which may become normal or reduced at the end-stages.[5] Recently, the presence of hypertension was reported to predict the likelihood and severity of later dementia diagnosed as AD.[10]

It is noteworthy that cognitive alterations have been associated with a variety of cardiac conditions. While dementia is the ultimate behavioral condition, depression has been noted as an early symptom in some AD subjects.[11] The level of depression in an individual may predict the subsequent severity of cognitive dysfunction later in life.[12] Depression is commonly manifest in cCAD patients and is often apparent prior to overt symptoms and subsequent clinical diagnosis of heart disease.[13] Many survivors of myocardial infarction exhibit a variety of mental disorders.[14,15] Personality changes in long-term survivors of cardiac arrest are now believed to be due to cerebral impairment.[16] A recent population study established a relationship between cognitive impairment and the presence of atherosclerotic disease.[7] Behavioral alterations in heart disease could be the consequence of AD-like neuropathology. In support of this a study has established that the postmortem incidence and density of β-amyloid deposits in the neuropil correlates with mild antemortem cognitive alterations among elderly controls maintaining psychometric indices at levels above values considered normal.[17]

Recently, inheritance of the Apo-E4 genotype has been linked to an increased risk of developing AD.[18-23] Apolipoprotein E is expressed as a pair of three possible encoded alleles, resulting in six main phenotypic combinations (E2/2, E2/3, E2/4, E3/3, E3/4 and E4/4).[24] Most studies suggest a relationship between AD age-of-onset and the dose of Apo-E4,[18,25-27] although this could be an illusion, caused by excluding an individual from a general population study in the AD age range because of premature death due to coronary heart disease.[26,28]

Presence and phenotypic expression of the apolipoprotein epsilon-4 (Apo-E4) genotype is known to increase the risk of ischemic heart disease in the form of coronary heart disease.[29-33] Expression of Apo-E4 has also been linked to the occurrence of hypertension, a prime cause of coronary heart disease.[34,35] After adjusting for lipid content and other risk factors, Apo-E genotype is the strongest genetic determinant for coronary heart disease in both men and women.[36] Operating through low-density lipoprotein (LDL) receptors and by affecting metabolism, Apo-E4 expression causes increased circulating levels of cholesterol as a proposed mechanism inducing heart disease demonstrable as critically stenotic atherosclerotic coronary artery disease (cCAD).[24,37-40] A prime risk factor for cCAD is increased circulating levels of cholesterol.[41-43] Increased circulating cholesterol concentrations

and apolipoprotein E4 genotype expression lead to elevated levels of oxidized LDL, which can cause both necrotic and apoptotic neuron death in culture.[44] Elevated levels of free radicals (FRs), well-known neurotoxic agents, accompany increased circulating concentrations of cholesterol, while increasing FR levels in the circulation have been correlated to increasing severity of coronary artery disease (stenosis) in humans.[45]

The activity of the FR scavenger enzyme, superoxide dismutase (SOD) in the vasculature, varies with the steady-state concentrations of FRs in the circulation.[46] At low circulating levels of FRs, SOD immunoreactivity is limited to the luminal surface. At higher levels of FRs the immunoreactivity becomes full-vessel thickness, and at the highest FR concentrations underlying tissue on the abluminal surface expressed SOD immunoreactivity.[46] This could suggest that there may be a flow of FRs from the blood across the vasculature and into the brain, and that FR could induce the onset of AD-like neuropathology in heart disease, and perhaps AD.

This premise gains support from studies identifying AD-like pathology in the brains of rabbits fed a 2% cholesterol diet.[47–49] Pathophysiology in both hypercholesterolemic rabbits and humans with cCAD likely involves a free radical (FR) mechanism.[50–53] The neuropathology induced in the cholesterol-fed rabbit included increasingly severe neuronal accumulation of β-amyloid immunoreactivity with later extracellular deposition and increased numbers of SOD-immunoreactive neurons with longer duration on the experimental diet.[47,49,54] This was coupled with increased SOD enzymatic activity in the brain and blood of the cholesterol-fed rabbits.[54] The importance of this dietary cholesterol effect comes from the report that altered memory coincides with increased β-amyloid concentration and extracellular SP-like deposits in the brains of mutated β-APP_{695} transgenic mice.[55]

Although it has been consistently shown that very little brain cholesterol originates from the circulation,[56] a small pool may exchange and vary with concentrations in the blood.[57–60] There are reports that circulating levels of cholesterol are marginally elevated in AD.[61,62]

In the brain, apolipoprotein E is a close "chaperone" of cholesterol, and likely facilitates its transportation.[30] Excluding the liver, the highest levels of mRNA for apolipoprotein E are found in the brain.[63] The proposed genetic link between Apo-E genotype and AD gains support from observations that apolipoprotein E avidly binds β-amyloid (a main component of SP),[64] and binds tau (a main component of NFT) in a polymorphic-dependent fashion.[65] Both SP and NFT express Apo-E antigenicity in AD brain.[66]

The synthesis of cholesterol in the brain is not sensitive to end-product (feedback) inhibition,[67] and *de novo* synthesis is the overwhelming source of brain cholesterol in a variety of mammals, including man.[56,68,69] The inability to appropriately synthesize cholesterol in the brain of developing humans is known to result in mental retardation and physical anomalies,[70,71] while membrane alterations observed in the brains of individuals with AD are thought to be mediated by cholesterol.[72]

Although less pronounced in nervous tissue, increasing membrane cholesterol content causes reduced fluidity.[73,74] Elevated membrane cholesterol can also undergo autoxidation to produce a peroxide byproduct, which can cause peroxidation of membrane fatty acids[75] and exaggerated neuronal calcium influx resulting in cell death.[76] Calcium is thought to mediate neuron death after exposure to β-amyloid,[77] while enhanced free radical activity, predominently that of 4-hydroxynonenal, accompanying cholesterol-induced membrane peroxidation, has been implicated in the pathogenesis of AD.[78–83] A pivotal role for free radicals in the cause and progression of heart disease has been substantiated in a variety of animal models.[84–86]

In the present study a large population of non-demented individuals and numerous AD patients were investigated for apolipoprotein E genotype, densities of SP in temporal and frontal cortex, and NFT in hippocampus and associated cortex. In most of these subjects immunohistochemical studies with apolipoprotein E antibody were performed. Immunoreactivity with Cu/Zn SOD antibody was assessed in many of these subjects, while the concentration of cholesterol in frontal cortex was determined in a limited number of Apo-E4 genotype-matched individuals in the non-HD, cCAD, and AD groups.

METHODS

General

Samples of brain were obtained at autopsy; fresh tissue was immediately frozen at −70° and adjacent blocks of tissue were immersion-fixed in buffered formaldehyde (8%). Tissues from patients with neuropathologically confirmed AD[2] were provided by the ADRC of the University of Kentucky. All other tissue samples were portions of those routinely saved as part of the coroner-authorized autopsy protocol performed by the Medical Examiner's Program. All analyses were performed blind with respect to subject age and group designation. The postmortem interval (PMI) was under 24 hours for all individuals. The death of each non-demented subject was sudden and unexpected. The age of disease onset could not be accurately determined for all individuals, and therefore could not be considered in the data analysis.

Subjects

We performed Apo-E genotyping on the frontal pole of 138 individuals. Pertinent neuropathologic differences between these populations has been previously published.[3–5] Among the individuals genotyped there were 39 non-HD controls (56.6 ± 3.1 yr), 21 hypertensive individuals without cCAD (HyperT; 66.5 ± 2.8 yr), and 53 cCAD subjects (62.9 ± 2.2 yr), compared to 25 AD patients (76.9 ± 1.8 yr). Subjects with concomitant cCAD and hypertension were not included in the study. There was no significant difference in age among the non-demented groups. The AD patients were significantly older ($p < 0.05$) than the non-HD and cCAD groups only.

Diagnosis of Cardiac Status

Most autopsies on subjects with suspected AD are limited to the head only. Cardiac status was not determined in these individuals. Among those individuals with AD receiving a full postmortem examination, coronary heart disease and hypertension were prevalent postmortem findings.

For all non-demented subjects, a complete autopsy with toxicologic analysis was conducted. The cause of death was attributed to either HyperT or cCAD in many of the subjects, or were incidental findings at autopsy. The criteria and methods of assessing the absence or presence of cCAD or HyperT have been previously published.[3–5] No cCAD subject was hypertensive and HyperT subjects did not have

cCAD. The duration of hypertension or CAD could not be determined for most of the decedents in that they never sought medical attention or were unaware of their condition. These factors also caused the need for autopsy by the Medical Examiner's Program to determine a cause of death.

Non-HD subjects may have had atherosclerotic plaques of the coronary arteries, but stenosis was much less than that defined as critical. Some subjects may have blood pressure above the normal range, but there was neither history nor sufficient anatomic evidence to assign a diagnosis of HyperT.

No subject was utilized in which significant cerebral atherosclerosis was present, defined as 25% or greater stenosis of any major cerebral artery. The degree of narrowing was determined by serial cross-sectional luminal inspection, grossly and, when necessary, microscopically, of arterial segments.

Cognitive Status

Each AD patient experienced progressive cognitive decline culminating in profound clinical dementia prior to death. The age of onset was not available in all AD patients. Each non-AD subject was determined to be non-demented by retrospective investigation.[3-5] This review might not have detected the presence of mild cognitive alterations, but did exclude the presence of overt dementia.

Apo-E Genotyping

Fresh frozen frontal pole from each individual was assigned an independent code from that used for microscopic evaluation, and the samples were forwarded to the AD Research Center at Duke University. DNA was extracted from each sample and the Apo-E genotype was determined by PCR utilizing Saunders'[23] modification of the methods of Hixson *et al.*[87]

Silver Staining and Quantification of SP and NFT

Paraffin sections (8 μm) of the hippocampal formation [parahippocampal gyrus (PHG), Ammon's horn (CA) and transition zone between CA and PHG (Tz)], and frontal pole (FP) were stained by the Bielschowsky method. The incidence of SP and NFT for each individual was calculated using these stained sections. If SP or at least two NFT were observed in any of the circumscribed areas, that subject was scored as positive for SP or NFT, respectively. Both argyrophilic plaques and tangles were quantified (number per mm^2) using three random fields. SP were counted in PHG and FP only, and NFT were counted in each region except FP. In contrast to our previous reports, the mean densities of SP and NFT were assessed using all individuals in a group. A portion of the data has been previously reported, where only those individuals exhibiting SP were included in the assessment of mean SP densities.[3,4] This accounts for the differences reported here and previously.

Immunocytochemistry

Apolipoprotein E immunocytochemistry was performed using standard ABC methods on 8-μ paraffin sections. All immunohistochemical incubations were per-

formed in a moist chamber. Sections were reacted with 1% H_2O_2 in 30% methanol to extinguish endogenous peroxidase activity. Sections were reacted with commercially obtained pepsin reagent for 1 hr at 37° C (Pepsin reagent M77; Biomeda Corp.). Sections were reacted with commercially available Apo-E antibody (clone 3D12, Monosan-Sanbio; 1 : 10 dilution) overnight at 4° C, and then standard ABC methods (Elite Vectastain Kit) were used. Immunoreactivity was visualized by reaction with diaminobenzidine (DAB) for 4 minutes. Negative controls were obtained by incubation with 20% normal goat serum instead of primary antibody.

SOD immunocytochemical studies were performed using standard ABC methods on 8-μ paraffin sections adjacent to those immunoreacted with Apo-E antibody. No pepsin incubation was performed. We used Cu/Zn SOD antibody characterized and provided by Dr. Naoyuki Taniguchi, Osaka University Medical School, Japan. The Cu/Zn SOD immunoreactivity was assessed for neuronal and plaque immunodecoration blind to group designation, but densities were not determined.

Cholesterol Determinations

Brain cholesterol levels were determined in the grey matter (trimmed of all white matter at autopsy) of frontal pole in 6 non-HD, 5 cCAD, and 5 AD patients matched for Apo-E4 genotype before age; Apo-E (3/4) individuals were utilized. This accounts for the wide SEM for age in each group. Cholesterol levels in frontal cortex were determined by high-pressure liquid chromatography exactly as detailed by Lui *et al.*[88] In brief, samples of brain were extracted with chloroform : methanol (2 : 1) overnight. After the protein was discarded, samples were dried under nitrogen and suspended in methylene chloride. Samples were injected onto the HPLC utilizing 95 : 5 hexane : isopropanol as the mobile phase. Detection was carried out by UV at 208 nm. Cholesterol was quantified per mg of wet brain tissue.

Statistical Analysis

We only assessed the effect of the Apo-E4 genotype and, as in Mayeux *et al.*,[19] individual genotype scoring was based on the three types: E4/E4, E4/−, and −/−, where the (−) could be either E3 or E2. Accordingly, the Apo-E4 genotype allele data was scored for each individual (i.e., 1.0 = E4/E4, 0.5 = E4/E3, and 0 = non-E4). Group allele frequencies were compared using a one-way ANOVA and a *t*-statistic (two-tailed) for *post hoc* analyses.

Mean numerical density of SP and NFT in each brain region was calculated for each individual. Individuals of the entire population were grouped according to the absence or presence of the APOE4 genotype. The subsequent differences in mean SP and NFT density in a brain region were compared by individual two-tailed *t*-tests. The entire population was then grouped according to the allele of APOE4; densities of SP and NFT were compared using a one-way ANOVA and a *t*-statistic (two-tailed) for *post hoc* analyses.

Subsequently the data was analyzed utilizing two-factor ANOVA (diagnosis/APOE4 incidence frequency vs. lesion density per region of brain); significant diagnosis correlation (A), APOE4 correlation (B), and significant A × B interaction was established. If a significant APOE4 correlation occurred for a lesion in a brain region, then (1) the significance of a mean density difference for a group (e.g., HyperT) was determined by individual *post hoc* two-tailed *t*-tests, and (2) a further two-factor ANOVA for APOE4 allele frequency was appropriate and established

(diagnosis/APOE4 allele frequency vs. lesion density). If an APOE4 allele correlation existed for any lesion densities, it was compared using a one-way ANOVA and a *t*-statistic (two-tailed) for *post hoc* analyses within a population.

Apo-E4 genotype was controlled for in the cholesterol studies. Mean FP grey-matter cholesterol concentrations for a group were compared using a two-way ANOVA and a *t*-statistic (two-tailed) for *post hoc* analyses. All statistical analyses were performed using the StatView 512 statistics program.

RESULTS

The allele frequency of APOE4 indicates the number of E4 alleles among the total number possible in a population. The APOE4 allele frequency was significantly increased ($p < 0.05$) in cCAD compared to non-HD (FIG. 1). A further significant increase in frequency occurred in both HyperT and AD. The APOE4 allele frequency in the non-demented HyperT population was not significantly different from the demented AD group (FIG. 1). Homozygotic E4 allele was found in 20% of the AD patients, 15% of the HyperT subjects, and only one cCAD individual.

By grouping the entire population according to Apo-E genotype, regardless of cognitive or cardiac status, the number of senile plaques in the frontal pole (FP) and parahippocampal gyrus (PHG) was significantly increased in individuals with an E4 allele compared to those without (FIG. 2). Likewise, the number of NFT in subjects with Apo-E4 genotype was increased in the PHG and the transition zone (Tz) between Ammon's horn (CA) and PHG; the increase in CA was not significant (FIG. 2).

Upon subgrouping the population by presence of dementia (AD) and cardiac staus (non-HD, cCAD, and HyperT) many of the Apo-E4 genotype-related differences in SP and NFT densities disappear. Among the non-HD subjects the number of SP was increased, but not significantly, in those individuals with an Apo-E4 allele

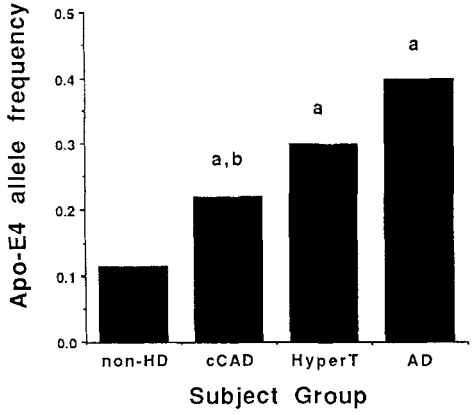

FIGURE 1. Allele frequency of Apo-E4 genotype in AD patients and in non-demented individuals with critical coronary artery disease, hypertension, or without heart disease. As previously reported,[19] Apo-E4 genotype was based on three types; E4/E4, E4/− and −/−, where the (−) could be either E2 or E3. The allele frequency for each individual was determined and scored for statistical analysis (i.e., 1.0 = E4/E4, 0.5 = E4/− and 0 = non-E4). Mean group frequencies were compared using a one-way ANOVA and a *t*-statistic (two-tailed) for *post hoc* analyses. non-HD = non-heart disease; HyperT = hypertensive without cCAD; cCAD = critical coronary artery disease; AD = Alzheimer's disease; a = $p < 0.05$ compared to non-HD; b = $p < 0.05$ compared to AD and HyperT.

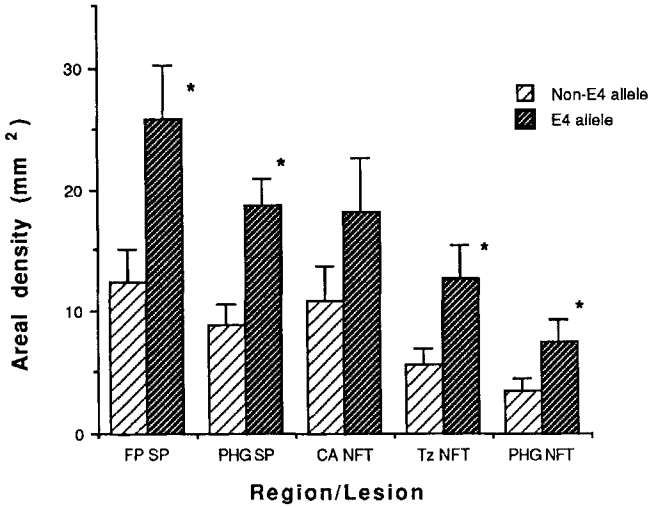

FIGURE 2. Densities of SP and NFT in brain regions of both demented and non-demented individuals grouped according to the absence or presence of an Apo-E4 allele irrespective of cardiac status. Values presented are means ± SEM per mm^2. * = $p < 0.05$ compared to non-E4 allele. SP = senile plaques; NFT = neurofibrillary tangles; FP = frontal pole; PHG = parahippocampal gyrus; CA = Ammon's horn; Tz = transition zone between PHG and CA.

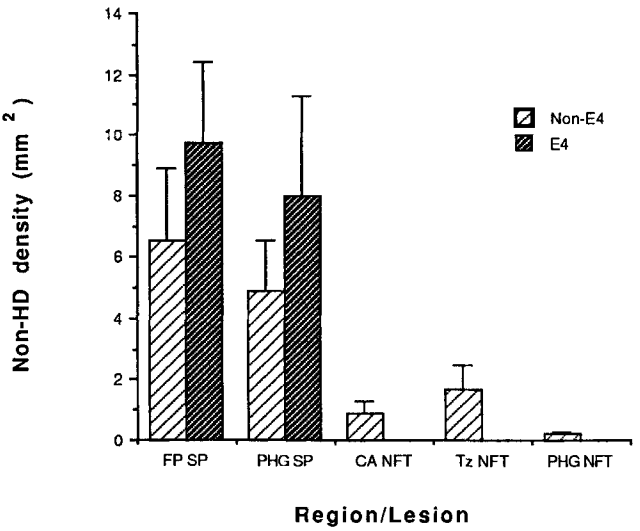

FIGURE 3. Densities of senile plaques (SP) and neurofibrillary tangles (NFT) in brain regions of non-demented individuals without heart disease (non-HD) grouped according to the absence or presence of the Apo-E4 allele. Values presented are means ± SEM per mm^2. FP = frontal pole; PHG = parahippocampal gyrus; CA = Ammon's horn; Tz = transition zone between PHG and CA.

compared to those without an E4 allele (FIG. 3). Neurofibrillary tangles were found in only those non-HD subjects without the Apo-E4 genotype (FIG. 3).

The density of SP was increased in the FP and PHG of non-demented subjects with cCAD with the Apo-E4 genotype (FIG. 4). The number of NFT was nonsignificantly increased in the PHG and Tz of non-Apo-E4 cCAD subjects (FIG. 4), while the increase in Ammon's horn (CA) was significant at the level of a trend ($0.05 < p < 0.1$). Similar to cCAD, non-demented hypertensive individuals (HyperT) with the Apo-E4 genotype displayed significantly increased number of SP in FP and PHG (FIG. 5). In contrast to cCAD, more NFT were observed in non-demented hypertensive individuals (HyperT) with the Apo-E4 genotype (FIG. 5). In the AD patients there was an increase in the number of SP and NFT if the Apo-E4 genotype was present (FIG. 6), but the difference from those patients without an E4 allele was not significant for any marker in any region of brain.

Essentially every senile plaque demonstrable by the Bielschowsky silver stain was immunoreactive with Apo-E antibody in adjacent sections from the same individual in the entire population, and therefore, densities are similar to those found for silver stained SP. Besides Apo-E-immunoreactive SP, neurons in the cortex, hilus, and granule cell layer were immunodecorated in cCAD (FIG. 7) and HyperT (not shown).

Immunolocalization of Cu/Zn superoxide dismutase (SOD) in human hippocampal formation suggested there was little SOD immunoreactivity in the hilus or granule cell layer or in hippocampal cortex, while numerous neurons were immunodecorated in CA_{3-4} of Ammon's horn in the non-demented non-heart disease (non-HD) control subjects (FIG. 8). In the hilus and granule cell layer of non-demented

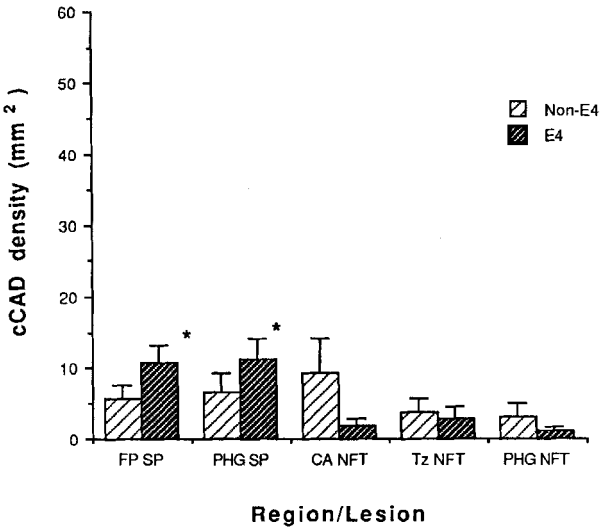

FIGURE 4. Densities of senile plaques (SP) and neurofibrillary tangles (NFT) in brain regions of non-demented individuals with critical coronary artery disease (cCAD) grouped according to the absence or presence of an Apo-E4 allele. Values presented are means ± SEM per mm^2. * = $p < 0.05$ compared to non-E4 allele. FP = frontal pole; PHG = parahippocampal gyrus; CA = Ammon's horn; Tz = transition zone between PHG and CA.

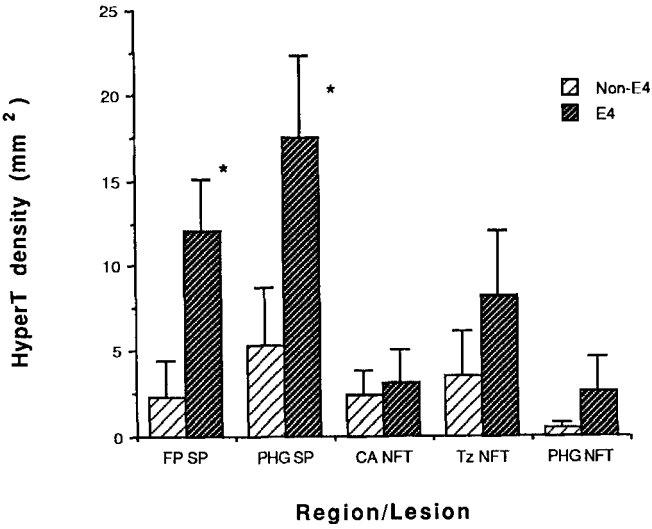

FIGURE 5. Densities of senile plaques (SP) and neurofibrillary tangles (NFT) in brain regions of non-demented hypertensive individuals without critical coronary artery disease (HyperT) grouped according to the absence or presence of an Apo-E4 allele. Values presented are means ± SEM per mm^2. * = $p < 0.05$ compared to non-E4 allele; FP = frontal pole; PHG = parahippocampal gyrus; CA = Ammon's horn; Tz = transition zone between PHG and CA.

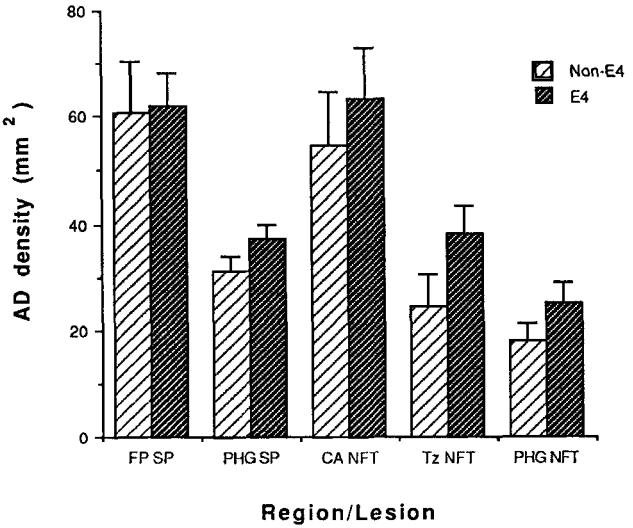

FIGURE 6. Densities of senile plaques (SP) and neurofibrillary tangles (NFT) in brain regions of demented Alzheimer's disease (AD) patients grouped according to the absence or presence of an Apo-E4 allele. Values presented are means ± SEM per mm^2. FP = frontal pole; PHG = parahippocampal gyrus; CA = Ammon's horn; Tz = transition zone between PHG and CA.

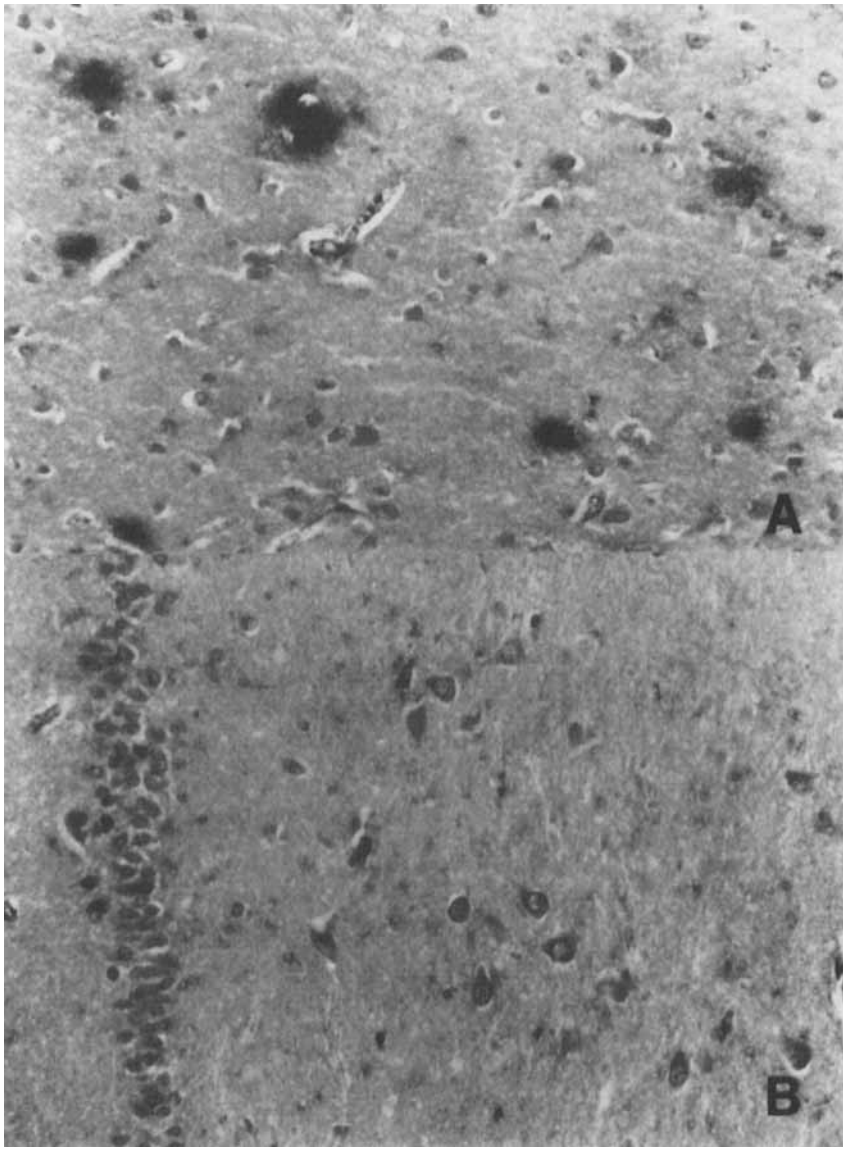

FIGURE 7. Apolipoprotein E–immunoreactive neurons and senile plaques (SP) in critical coronary artery disease (cCAD). Eight-micron paraffin sections are seen of hippocampal formation from a 67-year-old non-demented cCAD subject undergoing immunohistochemical analysis utilizing Apo-E antibody and standard ABC methods. Neurons and SP are Apo-E–immunoreactive in parahippocampal cortex (**A**). In the hilus and fascia dentata neurons were immunodecorated (**B**). Original magnification ×20.

hypertensives (FIG. 8) there was moderately intense neuronal SOD immunoreactivity, while neuronal immunoreactivity was pronounced in CA_{3-4} of Ammon's horn. In hippocampal cortex senile plaques were immunodecorated by SOD antibody and neurons were moderately stained (FIG. 8). In cCAD, SP were immunoreactive, but neuronal alterations were less severe than in hypertensive individuals (not shown).

In brains of persons with neuropathologically confirmed AD, SOD staining presented as intense punctate neuronal immunodecoration along with diffuse seas of immunoreactivity in the neuropil; the granule cell layer displayed little staining. In CA_{3-4} of Ammon's horn, somewhat shrunken neurons display intense SOD immunoreactivity in AD, while senile plaques are clearly immunodecorated in cortex of the parahippocampal gyrus (FIG. 8).

Conditions were established enabling baseline resolution of cholesterol, providing reproducible peaks the height of which was linearly related to known concentrations of pure cholesterol (representative chromatograph not shown). The efficiency of the cholesterol-extraction methods utilized in brain tissue was determined using pure cholesterol, and was found to be greater than 97%. Cholesterol levels were measured in cortical grey matter of frontal pole (FP) with all white matter dissected away. The concentration of cholesterol was significantly increased in the FP of both non-demented cCAD subjects and demented AD patients compared to Apo-E4 genotype-matched (E3/E4) non-HD controls of comparable age (TABLE 1). The AD group was significantly older that the cCAD group, but the age of neither group was different from that of the non-HD controls.

DISCUSSION

The data, coupled with previous observations, continue to suggest a significant overlap in the neuropathologic outcome of AD and heart disease. Alterations in individuals with confirmed AD and non-demented subjects experiencing antemortem hypertension and/or coronary artery disease are similar compared to age- and Apo-E4 genotype-matched non-demented non-HD controls. Once again senile plaques observed prematurely in heart disease (HyperT and/or cCAD) are shown to be identical to those found in AD; in each condition SP express Apo-E and SOD antigenicity. The frequency of the Apo-E4 genotype is shown to be significantly increased in cCAD, HyperT and AD compared to non-HD controls, while the presence of the Apo-E4 allele may be related to increased density of SP. The apparent graded effect on neuronal SOD immunoreactivity among the groups was similar to the graded increments of Apo-E4 allele frequency. Finally, similar increases in frontal cortex cholesterol levels of both AD and cCAD subjects compared to age- and Apo-E4 genotype-matched non-HD controls were disclosed.

The frequency of the Apo-E4 allele in the non-HD controls is not different from values previously reported for non-demented controls,[20-22,29,40] and it was no surprise that the allele frequency was significantly increased in cCAD[29,33,36] and AD.[19-22] The significant increase in the Apo-E4 allele frequency in autopsy-confirmed hypertensive subjects supports the previously reported observation that increased incidence of Apo-E4 is associated with increased systolic blood pressure in living subjects.[89]

The data clearly suggest a relationship between density of SP and Apo-E genotype if one accepts that SP formation and the Apo-E genetic influence occur early in the course of AD. A similar relationship can be asserted for NFT, if one accepts

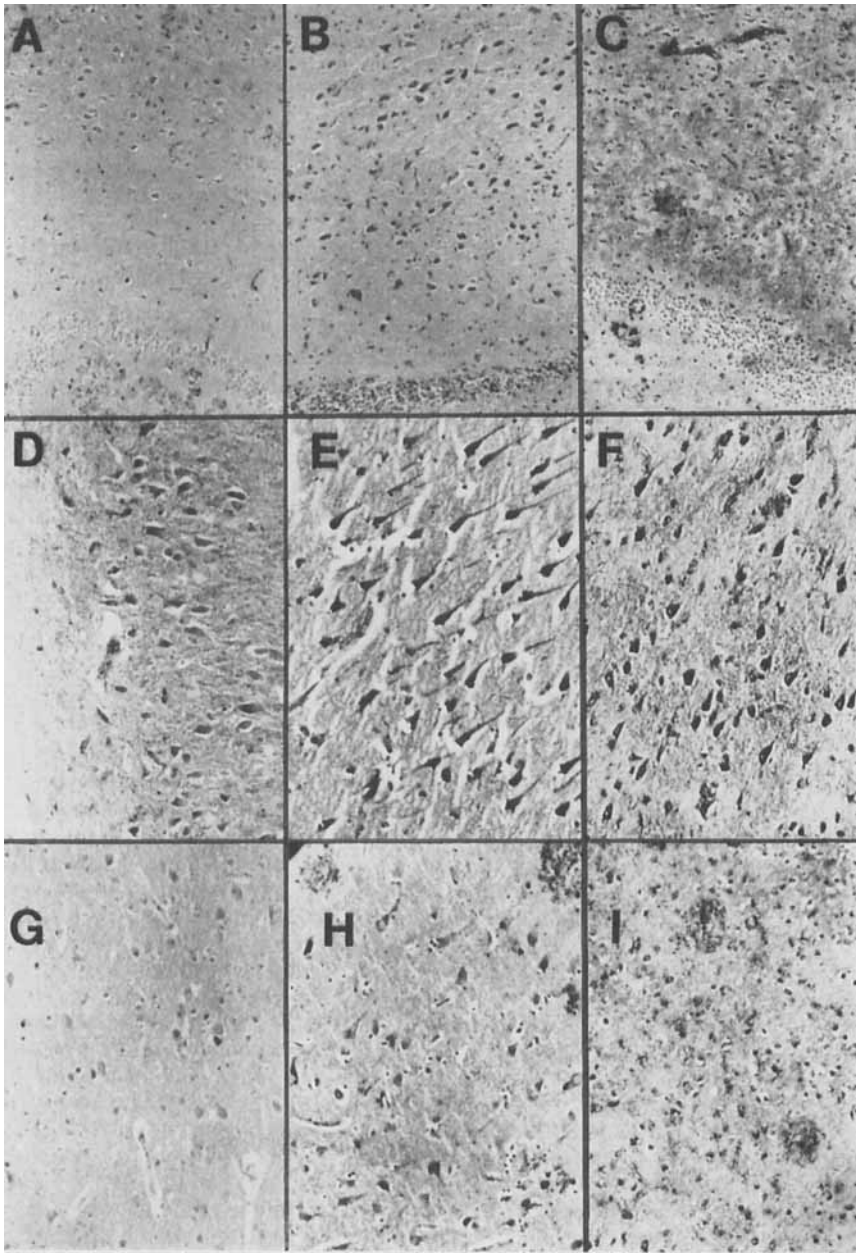
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TABLE 1. Levels of Cholesterol in Frontal Cortex Grey Matter from Age- and Apo-E4 Genotype-Matched Demented Patients with Neuropathologically Confirmed Alzheimer's Disease (AD), Non-Demented Individuals with Critical Coronary Artery Disease (cCAD), and Non-Demented Subjects without Heart Disease (non-HD)

Group	*N*	Age	Cholesterol
non-HD	6	69.5 ± 5.5	2.04 ± 0.18
cCAD	5	64.3 ± 4.3	2.59 ± 0.12*
AD	5	79.2 ± 7.3	2.65 ± 0.14*

NOTE: Values presented are means ± SEM in years for age and mg/g wet tissue weight for cholesterol.

* = $p < 0.05$ compared to non-HD

that two separate, unrelated mechanisms control SP formation and NFT formation. A nearly significant increase in number of SP was associated with presence of the Apo-E4 allele in non-HD controls. This relationship between SP and Apo-E genotype was significant in cCAD individuals and was highly significant in the HyperT subjects. The lack of difference in AD may be due to the substantial difference in time between initial formation of SP and Apo-E4 effects and the death of an individual with AD. In Down's syndrome, a dementing disorder analogous to AD, senile plaques are formed decades before NFT formation and demonstrable cognitive alterations.[90,91]

A subtle effect of Apo-E4 genotype on NFT formation can be supported by the data when keeping in mind that NFT incidence is significantly increased in HyperT and not cCAD.[5] Neurofibrillary tangles predominently occurred in non-HD and cCAD individuals without the Apo-E4 allele, who made up fewer than 10% of each population and were among the oldest subjects in each respective group. In contrast, a doubling of NFT density (nonsignificant) with the presence of the Apo-E4 allele occurred in hypertensive subjects, who constituted a full age-range population in which half of the subjects exibited NFT and some in the absence of identifiable SP. The observation of NFT-only HyperT subjects supports the premise of different mechanisms of SP and NFT formation. Again, the lack of

FIGURE 8. Immunolocalization of Cu/Zn superoxide dismutase (SOD) in human hippocampal formation. Photomicrographs are of the hilus and granule cell layer (**A–C**; 10×), CA_{3-4} of Ammon's horn (**D–F**; 20×) and the parahippocampal gyrus (**G–I**; 20×). In a 71-year-old male non-demented non-heart disease (non-HD) control subject there was little SOD immunoreactivity in the hilus or granule cell layer (**A**), numerous neurons were immunodecorated in CA_{3-4} of Ammon's horn (**D**) and faint neuronal SOD immunoreactivity was observed in hippocampal cortex (**G**). In the hilus and granule cell layer of another 67-year-old non-demented hypertensive male there was moderately intense neuronal SOD immunoreactivity (**B**), while neuronal immunoreactivity was pronounced in CA_{3-4} of Ammon's horn (**E**). In hippocampal cortex of this hypertensive individual senile plaques were immunodecorated by SOD antibody and neurons were moderately stained (**H**). In another subject, a 72-year-old male patient with neuropathologically confirmed AD, SOD staining presented as intense punctate neuronal immunodecoration along with diffuse seas of immunoreactivity in the neuropil; the granule cell layer displayed little staining (**C**). In CA_{3-4} of Ammon's horn from this AD subject, somewhat shrunken neurons display intense SOD immunoreactivity (**F**), while senile plaques are clearly immunodecorated in cortex of the parahippocampal gyrus (**I**).

significant difference between NFT density according to Apo-E genotype in AD may be related to a time differential between initial genetic influence and the neuropathologically observable end-point.

The rank-order relationships between the groups cannot be overlooked, since they tend to support the hypothesis of a relationship between heart disease and AD. Consistently observable increases in alteration severity for a population occur in order; non-HD < cCAD < HyperT < AD. This rank order can be suggested for behavior, has been reported for incidence of SP, density of SP, and incidence of NFT, and is shown here for Apo-E4 allele frequency and severity of neuronal SOD immunoreactivity. A stepwise increase in Apo-E4 allele frequency occurred in this order among the groups, although the further increase in HyperT compared to cCAD was not significantly different from AD. This may suggest a closer relationship between HyperT and AD than cCAD and AD. This is because subjects with both AD and HyperT exhibit disease-related SP, NFT, and similar increases in Apo-E4 allele frequency. A population order similar to Apo-E4 allele frequency was apparent for neuronal SOD immunoreactivity, in that the biggest gap in increased severity occurred between the non-HD/cCAD and HyperT/AD groups. These coupled observations may suggest a relationship between oxidative stress and neuron transformation into an NFT, because of differences in increased neuronal SOD immunoreactivity and incidence of NFT between populations of cCAD and hypertensive individuals.

The current state of investigation does not allow complete assessment of the relationship between brain cholesterol levels, Apo-E4 genotype, level of oxidative stress, and lesion incidence and density. It is of note that FP cortical grey-matter concentration of cholesterol is equally increased in cCAD and AD compared to Apo-E4 genotype-matched non-HD controls. This would suggest that the proposed effect of Apo-E4 expression on circulating cholesterol levels produces identical changes in cCAD and AD brain, which are different from findings in non-HD subjects with similar genetic influence. From these data it could be hypothesized that brain cholesterol level is more closely associated with SP formation than NFT formation, because only SP are prevalent in cCAD. Further studies of cholesterol concentrations in Apo-E4 genotype-matched subjects, including hypertensive individuals, especially those with NFT and without SP, may clarify and test this speculation.

The hypothesized causal relationship between heart disease and AD clearly relies on the premise that conditions produced by cCAD and/or HyperT leading to AD-like neuropathologic lesion formation are less severe than in AD or occur in an individual dying of their heart disease before achieving lesion densities associated with cognitive derangements. This would further suggest that if individuals can withstand the rigors of heart disease they may survive long enough to develop sufficient neuropathology and cognitive alteration. This may be supported by the reported clinical correlation between hypertension and eventual dementia, coupled with increased incidence of NFT in autopsy-confirmed HyperT individuals. Although HyperT is a risk factor for cCAD, the explanation for the difference in neuropathologic consequences of cCAD and HyperT may be that cCAD is more lethal than HyperT or that an individual with HyperT may survive longer if cardiac dysfunction (CAD) never becomes life-threatening, and therefore, the subject succumbs to some other disease much later in life (i.e., renal dysfunction or stroke).

One could inexorably be drawn to conclude (speculate) that there may be a vascular component and/or blood-borne agent involved in the production of AD-like neuropathology in heart disease. Although individuals with grossly observable atherosclerotic cerebrovascular disease were excluded from our studies, vascular

disease in cCAD and HyperT is well established. Because the sole means of direct communication between the heart and the brain is via the circulation mediated by the cerebrovasculature, agents in the blood could be mechanistically involved in the induction of neuropathology in non-demented individuals with heart disease. Circulating cholesterol and free radicals that can directly effect or navigate the cerebrovascular endothelia are likely candidates.[54,92] Once in the brain, cholesterol can undergo auto-oxidation, causing membrane peroxidation and formation of the highly toxic free radical byproduct, 4-hydroxynonenal. Because heart disease is prevalent in AD, it may be that a similar mechanism participates in the initiation of neuropathology in both disorders.

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