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Lipid metabolism parameters in patients with Alzheimer's disease and their first degree relatives

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Abstract. Recently, it was suggested that the presence of total cholesterol (TC), age and sex interaction in Alzheimer's type dementia (AD) is linked with the apolipoprotein E (*APOE*) genotype. Our objective was to determine whether the serum lipid profile in AD patients and their first degree non-demented relatives of a certain age (NDR) was dependent on *APOE* genotype. We included 28 mild to moderate AD and 30 NDR according to DSM-III-R and NINCDS-ADRDA criteria. NDR individuals were investigated in an age group similar to the AD group (brother-sister relationship) and in a group including younger individuals (AD patients-children relationship). Our data indicate significant differences between decreased total cholesterol and low density lipoprotein cholesterol ratio in the group of AD patients versus NDR individuals of similar age, independent of *APOE* genotype, and an increased total cholesterol and low density lipoprotein cholesterol ratio in a group of AD patients *versus* their children of the same genotype. There was no significant correlation between triglycerides and high density lipoprotein levels with *APOE* genotype in any of the tested groups. In conclusion, there was a decreased selected lipid serum profile parameters in AD compared to age matched non demented first degree relatives.

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Key words: dementia, Alzheimer's disease, serum lipid profile, *APOE* genotypes, first degree relatives

INTRODUCTION

The onset of Alzheimer's Disease (AD) can be influenced by various genetic and environmental factors. The major genetic risk factor for late onset AD is a presence of one or two copies of the apolipoprotein E- *APOE*ε*4 allele (Strittmatter et al. 1995). However, the presence of the ε*4 allele alone is neither necessary nor sufficient for pathogenesis of AD and other factors may participate independently or in concert with ApoE to determine the overall risk for the expression of AD (Richey et al. 1995).

There is evidence of a synergistic interaction of the ε*4 allele and the severity of atherosclerosis for the risk of dementia, including AD (Hofman et al. 1997). The inheritance of one or two copies of the *APOE*ε*4 allele also confers an increased risk of the negative outcome from vascular based brain injury (Mayeux et al. 1995). Elevated total serum cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) levels, risk factors for cardiovascular disease, are also associated with AD (Pedro-Botet et al. 1992). This suggests that cholesterol level reduction may diminish or delay the risk of development of AD pathology.

Among many roles proposed for ApoE, it mediates the remodeling of the cytoarchitecture and neural connections by its ability to bind and transport cholesterol-rich lipids into cells via the interaction with its endocytosis receptors. In addition, supportive evidence for the role of ApoE receptors in the pathogenesis of AD was confirmed by the discovery of the association of low density lipoprotein receptor related protein gene /LRP/ with late onset AD (Kang et al. 1997).

Our objective was to determine whether the serum lipid profile in AD patients and their first degree relatives (NDR) of certain ages was dependent on *APOE* genotype.

METHODS

AD patients and their family relatives selected for this study were from the outpatient clinic registry. We included 28 mild to moderate AD subjects and 30 first degree nondemented relatives of AD (NDR), according to DSM-III-R and NINCDS-ADRDA criteria. The NDR group consisted of 19 young NDR individuals (Y-NDR) with mean age 44.1 ± 4.8 years and 11 old NDR individuals (O-NDR) (mean age 67.3 ± 5.8). The mean age for AD patients was 71.3 ± 5.1 years. The Y-NDR group was formed from children while O- NDR individuals were selected from nondemented brothers and sisters of AD patients. This results in two comparison groups: 11 AD with O-NDR and 19 AD with Y-NDR.

APOE genotyping was performed by using PCR-restriction isotyping (Chapman et al. 1996). DNA was extracted from white blood cells (Hixson et al. 1988). Leukocyte DNA was amplified by PCR in a DNA Thermal Cycler (Biometra) using oligonucleotide primers: downstream primer, 23-mer: (5'-TCCAAGGAGC TGCAGGCGGCGCA-3') and upstream primer, 31-mer: (5'-ACAGAATTCGCCCCGGCCTGGTACACT GCCA3').

Each amplification reaction contained 600 ng of leukocyte DNA, 100 pmols of each primer, 5 µl of 100% dimethyl sulphoxide, 1U of Taq polymerase (Perkin Elmer Cetus), as well as nucleotide components in a 50 µl final volume of buffer. Each reaction mixture was heated at 94°C for 5 min, followed by 40 cycles of annealing (65°C for 30 s), extension (70°C for 60 s), denaturation (94°C for 30 s) and final extension at 70°C for 10 min. A 227 bp product of PCR amplification was digested for 16 h at 37°C with 5 units of *AflIII* or 10 units of *HaeII* restriction enzymes in the appropriate buffers (New England Biolabs). Each reaction mixture was loaded on 8.4%, bis polyacrylamide nondenaturating gels and subjected to electrophoresis for 4 h at a constant voltage 80 V. After staining with ethidium bromide, the digestion products were visualized under UV light and their sizes compared to known markers.

Total serum cholesterol (TC) and total serum triglycerides (TG) were determined using enzymatic methods (CHOD- PAP, DIRECT, GPO- PAP, BioMerieux). High density lipoprotein - cholesterol (HDL-C) was determined by measuring cholesterol after precipitation of ApoE- containing apolipoproteins. Low density lipoprotein - cholesterol (LDL-C) was calculated according to the Friedewald formula. Statistical analysis was done using Duncan's test (GLM procedure). The level of significance was set at 5% limits.

RESULTS

The mean age of Y-NDR and O-NDR subjects used for this study was significantly different (44.7 ± 4.8 *versus* 67.3 ± 5.8) while the mean age of AD patients was similar to O-NDR individuals (71.3 ± 5.1 *versus* 67.3 ± 5.8). AD patients and NDR individuals possess exactly the same APOE genotype between the examined pairs

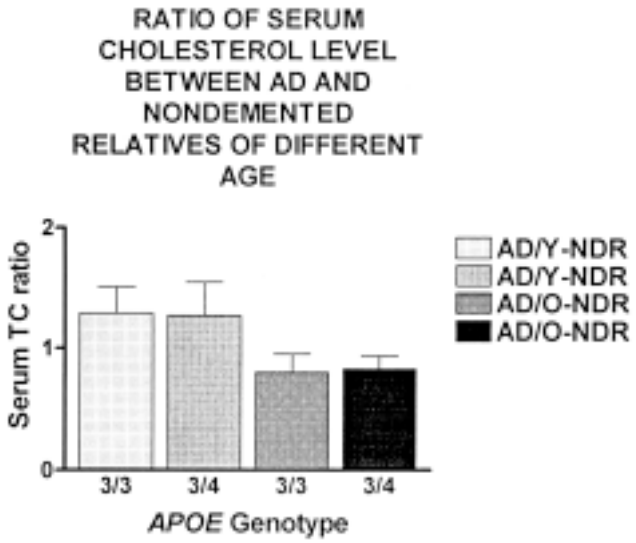

Fig. 1. Serum total cholesterol ratio for AD patients as compared to Y-NDR subjects ranged 1.29 ± 0.22 for *APOE*ε3/ε3 homozygotes and 1.30 ± 0.28 for *APOEe*3/ε4 heterozygotes. A significant difference was noted for AD patients compared to O-NDR subjects where serum total cholesterol ratio ranged 0.80 ± 0.15 for *APOE*ε3/ε3 homozygotes and 0.83 ± 0.11 for *APOE*ε3/ε4 heterozygotes ($P<0.05$).

and the distribution of frequencies ε3\3 and ε3\ 4 were 42% and 58%, respectively. Lipid parameters were presented as a ratio of TC, TG, HDL-C and LDL-C serum concentration between AD to Y-NDR or O-NDR subjects. These evaluations were performed separately for

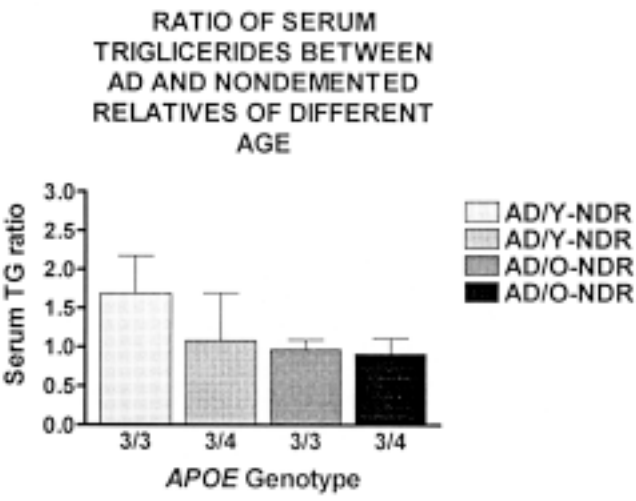

Fig. 2**.** Serum triglycerides ratio for AD patients as compared to O-NDR and Y-NDR subjects was found to be unaffected both in *APOE*ε3/ε3 homozygotes and *APOE*ε3/ε4 heterozygotes.

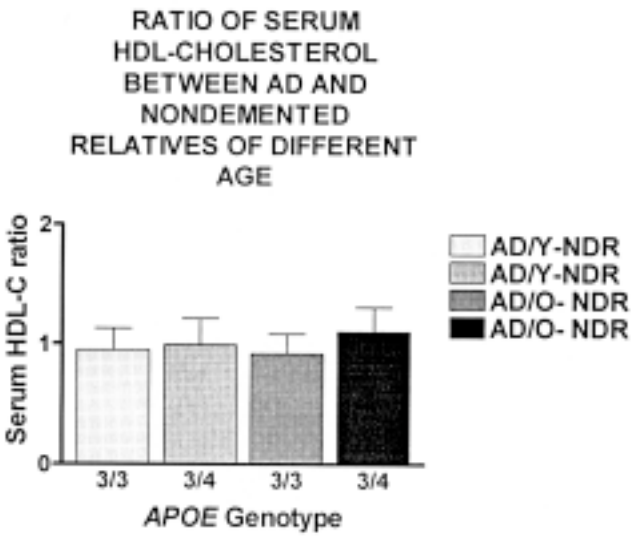

Fig. 3. Serum HDL-cholesterol ratios for AD patients as compared to O-NDR and Y-NDR subjects did not reach any statistical differences in *APOE*ε3/ε3 homozygotes and *APOE*ε3/ε4 heterozygotes.

each genotype. The serum TG ratio for AD patients compared to O-NDR and Y-NDR subjects was found to be unaffected both in APOE ε3\ε3 homozygotes and APOE ε3\ε4 heterozygotes. Statistically insignificant differences were noted for AD patients compared to Y-NDR subjects for APOEε3\ε3 homozygotes (ratio: 1.69 ± 0.49) and APOEε3\4 (ratio: 1.08 ± 0.62) heterozygotes (Fig. 2). We did not find any significant differences in HDL-C levels between AD patients with different *APOE* genotypes compared with NDR individuals (Fig. 3). Age factor did not have influence on these results. We found significant differences in TC and LDL-C levels (Figs. 1 and 4). The TC ratios of AD versus Y- NDR individuals was 1.29 ± 0.22 and 1.27 ± 0.28 in *APOE*ε3/ε3 homozygotes and *APOE*ε3/ε4 heterozygotes, respectively. The TC ratios of AD versus O-NDR individuals was 0.85 ± 0.15 in *APOE*ε3/ε3 homozygotes and 0.83 ± 0.11 in *APOE*ε3/ε4 heterozygotes ($P< 0.05$). In each investigated group, the *APOE* genotype did not influence the TC concentration. A similar tendency was observed for LDL-C (Fig. 4). The LDL-C serum concentration ratios of AD *versus* Y- NDR subjects was 1.42 ± 0.41 for *APOE*ε3/ε3 homozygotes while the *APOE*ε3/ε4 heterozygotes gave 1.53 ± 0.24. The ratios of AD *versus* O-NDR were 0.81 ± 0.05 (*APOE*ε3/ε3 homozygotes) and 0.71 ± 0.11 (for *APOE*ε3/ε4 heterozygotes). In conclusion, our data indicate a significant

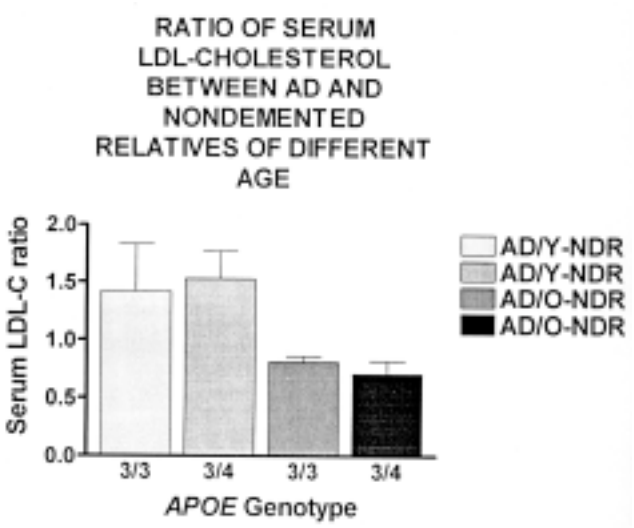

Fig. 4. LDL-cholesterol ratios for AD patients compared to Y-NDR subjects ranged 1.42 ± 0.41 for APOEε3/ε3 homozygotes and 1.53 ± 0.24 for *APOE*ε3/ε4 heterozygotes. A significant difference was noted for AD patients as compared to O-NDR subjects where LDL-cholesterol ratio ranged 0.81 ± 0.05 for *APOE*ε3/ε3 homozygotes and 0.71 ± 0.11 for *APOE*ε3/ε4 heterozygotes ($P<0.05$).

decreased serum TC and LDL-C level in the group of AD patients compared to age matched non demented first degree relatives, independent of *APOE* genotype.

DISCUSION

Several studies found a link between the *APOE*ε4* allele and coronary artery disease in the general population (Bockmeer et al. 1992, Wilson et al. 1994, Stengard et al. 1995). The Framingham Offspring study estimated the odds ratio for coronary heart disease prevalence to be 2.0 in persons with one or more *APOE*ε4 alleles (Wilson et al. 1994). This increased risk is thought to be due to the central role apolipoproteins play in cholesterol and lipid metabolism. ApoE4 differs from the apoE3 isoform only by a single amino acid substitution which increases its binding to very low density lipoprotein (VLDL) receptors and results in elevated total and low density lipoproteins (LDL) levels (Franceschini et al. 1996). Recently, it was concluded that high serum lipid levels, including total cholesterol, may be independent risk factors for AD (Wieringa et al. 1997, Natkola et al. 1998) and some of the effects of the *APOE*ε4* allele in AD may be mediated through its high serum levels of cholesterol (Notkola et al. 1998). The results presented here demonstrate that the age factor has implications in serum lipid profile and that is independent from *APOE* genotype status in AD patients and their nondemented relatives (NDR). Kotter et al. 1996 and Sygitowicz et al. 1996 found normal serum values of TC and TG in patients with AD and vascular types of dementia. The results presented here show no differences in the levels between TG and HDL-C between AD patients and their nondemented relatives of a different age in relation to *APOE* genotype (*APOE*ε3/ε3 homozygotes and *APOE*ε3/ε4 heterozygotes). Zeman et al. 1997 concluded in their studies on the Japanese population that *APOE*ε*2 carriers had lower levels of TC and a lower TC:HDL-C ratio, *APOE*ε*3 carriers had intermediate levels, while *APOE*ε*4 carriers had higher levels. These findings held whether sexes were analyzed separately or together. Pablos-Mendez et al. 1997, in their study on an elderly and multiethnic population concluded, however, that no significant independent effect was noted for any *APOE* genotype on HDL cholesterol. In their studies, plasma triglyceride levels were inversely correlated with the number of *APOE*ε*4 alleles and the observed effect increased with age. Jarvik et al.1997 concluded that individuals with the *APOE*ε2/ε3 genotype had higher TG and lower LDL-C and TC at each exam taken than were seen in those with the *APOE*ε3/ε3, although the differences in the values were not always statistically significant. On the other hand, Corzo et al. 1997 showed that there was a lack of association between serum cholesterol and TG levels with *APOE* genotype in the Spanish AD population. In Japanese patients with vascular dementia, LDL-cholesterol levels showed an association with the presence of the *APOE*ε4* allele whose higher frequency than in controls was suggested to cause a vascular dementia (Shimano et al. 1989). The results showed here clearly demonstrate the differences in TC and LDL-C levels (Figs. 1 and 4) among AD versus NDR individuals. The TC and LDL-C levels found in *APOE*ε3/ε3 homozygotes and *APOE*ε3/ε4 heterozygotes, were respectively higher in AD patients versus their Y-NDR counterparts and lower versus O-NDR individuals. In each investigated group, the *APOE* genotype did not influence the TC and LDL-C concentration. In a multivariate model presented by Jarvik et al. 1997, the presence of the *APOE*ε*4 allele did not significantly affect plasma lipid levels. In contrast, lowering effects on LDL cholesterol and total cholesterol/HDL ratio was associated with the presence of the *APOE*ε2* allele (Heng et al. 1995, Jarvik et al. 1997, Zaman et al. 1997).

Unfortunately, the results presented here on a selected group of AD patients and their NDR counterparts prevented us from drawing a conclusion on the role of the *APOE*ε2* allele on serum lipid metabolism. Recently, it was also suggested that TC, age and sex interact in AD patients according to their *APOE* genotype (Jarvick et al. 1995, 1997, Jarvick 1997, Zaman et al. 1997). It was suggested that the effects of individual genotypes may vary while grouped (Jarvick et al. 1997). The marked alterations in both ApoE and lipid constituents of ventricular fluid lipoproteins in AD, as compared to age-matched control patients, were also noted; however, these changes were not simply related to *APOE* genotype (Montine et al. 1997). These findings suggested that altered ventricular fluid, rather than serum lipoproteins metabolism, may be a component of AD pathogenesis independent of *APOE* genotype. In conclusion, in our studies the age factor has implications in the serum lipid profile and this seems to be independent from *APOE* genotype status in AD patients. The interactions between *APOE* genotype and a multiple number of factors are responsible for the effects which apolipoprotein E exerts in Alzheimer's disease.

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