Decreased plasma C-reactive protein levels in APOE ε4 allele carriers

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Abstract
Objective: Apolipoprotein E (APOE) ε4 allele is a well-established risk factor in Alzheimer’s disease (AD). Here, we assessed the effects of APOE polymorphism on cardiovascular, metabolic, and inflammation-related parameters in population-based cohorts. Methods: Association of cardiovascular, metabolic, and inflammation-related parameters with the APOE polymorphism in a large Finnish Metabolic Syndrome in Men (METSIM) cohort and Finnish Geriatric Intervention study to prevent cognitive impairment and disability (FINGER) were investigated. Brain-specific effects were addressed in postmortem brain samples. Results: Individuals carrying the APOE ε4 allele displayed significantly elevated serum/plasma LDL cholesterol and apolipoprotein B levels. APOE ε3ε4 and ε4ε4 significantly associated with lower levels of plasma high-sensitivity C-reactive protein (hs-CRP). Plasma amyloid-β 42 (Aβ42) and reduced hs-CRP levels showed an association independently of the APOE status. Interpretation: These data suggest that the APOE ε4 allele associates with lower levels of hs-CRP in individuals without dementia. Moreover, Aβ42 may encompass anti-inflammatory effects reflected by reduced hs-CRP levels.
Introduction

Alzheimer’s disease (AD) is the leading cause of dementia, affecting an increasing number of people each year. AD has a strong genetic component with apolipoprotein E (APOE) being established as the strongest genetic risk factor for the late onset form of AD.\(^1\) Three alleles in the APOE gene, e2, e3, and e4 differ in two single-nucleotide polymorphisms (SNPs), rs7412 and rs429358, and encode different apolipoprotein E (ApoE) protein isoforms.\(^2\) The presence of the APOE e4 allele considerably increases the risk and lowers the age of onset of AD, while the APOE e2 allele significantly decreases the AD risk.\(^3\) ApoE is a multifunctional protein that has an important role in modulating plasma lipid and lipoprotein levels as well as inflammatory responses in the brain and periphery.\(^3,4\) Binding of ApoE to low-density lipoprotein (LDL) receptors in the liver mediates the clearance of chylomicrons and LDL from the bloodstream. Amino acid substitutions directly affect the function of the protein. For example, the affinity of ApoE2 to the LDL receptors is markedly reduced compared to ApoE3 and E4.\(^5\) Carriers of the APOE e4 allele have higher total cholesterol levels as compared to those without the APOE e4 allele.\(^4,5\) In addition, the presence of the APOE e4 allele has been suggested to augment pathophysiological states, such as oxidative stress and neuroinflammation.

Neurodegeneration in AD is linked to the accumulation of soluble oligomeric amyloid-β (Aβ) in the brain. In particular, Aβ peptides of 42 amino acids in length (Aβ42) are considered the key mediators of synaptotoxicity and neurotoxicity in AD.\(^7\) According to the prevailing amyloid-cascade hypothesis, Aβ deposition in the brain triggers the hyperphosphorylation of tau and its accumulation as neurofibrillary tangles, activation of inflammatory cells and pathways, initiation of oxidative stress, and decline in synaptic and neuronal health and finally neurodegeneration.\(^8\) This view recently gained further support from the identification of a protective APP variant (A673T), which was later shown to reduce Aβ levels and protect against cognitive decline.\(^9,10\) Physiologic functions of Aβ are not well-established, but significant lines of evidence suggest that Aβ may have potential protective properties under certain conditions. Aβ42 has been shown to have antioxidant effects and decrease the oxidation of lipoproteins in the cerebrospinal fluid (CSF) and blood plasma.\(^11,12\) A study utilizing different experimental autoimmune encephalomyelitis models, demonstrated that the peripherally augmented levels of Aβ40 and Aβ42 effectively suppressed inflammation in different organs, thus attenuating autoimmune inflammation targeting the central nervous system (CNS).\(^13\) Aβ42 was recently shown to confer protection against microbial infections in various in vitro and in vivo AD models, suggesting a potential dual defensive/injurious role for Aβ42.\(^14\) Accordingly, Aβ42 is upregulated upon injury, inflammation and stress conditions in the brain, suggesting that especially Aβ42 may exert either protective or anti-inflammatory effects depending on the prevailing conditions.\(^13,15,16\) Here, we have assessed the association of the different APOE genotypes and plasma Aβ levels with a large number of cardiovascular, metabolic health, and inflammation-related parameters in large population-based cohorts from Finland.

Subjects and Methods

Subjects

The study included a subset of 4913 nondiabetic men from the large population-based Metabolic Syndrome in Men (METSIM) cohort consisting of 10,197 men in total. The participants have been randomly selected from the population register of Kuopio, Eastern Finland, and were aged 45–70 years at baseline. Participants of the study have been extensively characterized for risk factors of metabolic syndrome, type 2 diabetes and cardiovascular disease. Table 1 presents clinical characteristics and laboratory characteristics of the study cohort. The study design has been described in detail previously.\(^17\) Individuals diagnosed at the baseline with AD (n = 3) were excluded from this study. There were no diabetic individuals in the METSIM cohort from which the APOE genotype information was available (Table 1). The METSIM study was approved by the Ethics Committee of the University of Kuopio and Kuopio University Hospital and was conducted in accordance with the Helsinki Declaration. All individuals provided written informed consent.

Participants in the Finnish Geriatric Intervention study to prevent cognitive impairment and disability (FINGER) were used as a replication cohort in the study. Data from the screening/baseline impairment and disability (FINGER) visit, that is, before the start of the intervention were used for the analyses. The FINGER recruitment process has been previously described in detail.\(^18,19\) In brief, the 1260 participants were selected from previous population-based noninterventional surveys. Eligibility criteria were: age 60–77 years; CAIDE (Cardiovascular Risk Factors, Aging and Dementia) Dementia Risk Score of at least six points\(^20\) (Table 2); and cognitive performance at mean level or slightly lower than expected for age according to Finnish population norms. Individuals with dementia, substantial cognitive impairment, and conditions affecting cooperation or safe engagement in the intervention were excluded.\(^18,19\) FINGER was approved by the coordinating ethics committee of the Hospital District of Helsinki and

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Table 1. Clinical and laboratory characteristics of the METSIM Study participants.

| genotype | number of individuals | age (years) | BMI (kg/m²) | systolic blood pressure (mmHg) | diastolic blood pressure (mmHg) | glucose metabolism parameters | inflammatory parameters |
|----------|-----------------------|-------------|-------------|-------------------------------|-------------------------------|-----------------------------|------------------------|
| e2e2     | 317 (6.5%)            | 57.1 ± 7.1  | 26.9 ± 3.5  | 136.7 ± 14.9                  | 87.4 ± 8.8                    | 5.7 ± 0.5                   | 212.9 ± 149.2          |
| e2e3     | 69 (1.4%)             | 58.1 ± 7.0  | 27.5 ± 4.4  | 137.6 ± 14.9                  | 86.8 ± 8.8                    | 5.7 ± 0.5                   | 203.9 ± 149.2          |
| e3e3     | 2921 (59.5%)          | 56.9 ± 6.8  | 26.9 ± 3.8  | 136.7 ± 16.3                  | 87.3 ± 9.4                    | 5.7 ± 0.5                   | 212.9 ± 149.2          |
| e3e4     | 1427 (29.0%)          | 56.8 ± 6.9  | 26.9 ± 3.7  | 137.1 ± 16.3                  | 87.2 ± 9.4                    | 5.7 ± 0.5                   | 212.9 ± 149.2          |
| e4e4     | 179 (3.6%)            | 57.1 ± 7.0  | 26.1 ± 3.6  | 138.3 ± 16.6                  | 87.7 ± 9.8                    | 5.7 ± 0.5                   | 212.9 ± 149.2          |
| P        | 0.56                  | 0.05        | 0.72        | 0.97                          | 0.06                          | 0.77                        | 0.67                   |

All parameters are presented as mean ± standard deviation. For hs-CRP the median and interquartile range (*) are reported due to its skewed distribution. All P-values except age are adjusted for age as covariate.

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Matsuda ISI, Matsuda insulin sensitivity index; OGTT, oral glucose tolerance test.

Uusimaa. Participants gave written informed consent at screening and baseline visits.

The neuropathological human postmortem brain cohort has been previously comprehensively described.21–23 In brief, temporal cortical samples were obtained from 71 individuals (18 males and 53 females; mean age, 81.0 ± 8.8 years) examined in Kuopio University Hospital (KUH). The extent of AD-related neurofibrillar pathology was evaluated after autopsy with AT8-immunostaining, detecting hyperphosphorylated tau.24 The samples were divided into three groups based on the degree of the neurofibrillary pathology according to Braak staging24: Mild (Braak 0-I, RNA n = 18/10 and protein n = 8/4 in APOE e4/e4, respectively), moderate (Braak III–IV, RNA n = 4/9 and protein n = 3/3 in APOE e4/e4, respectively), and severe (Braak V–VI, RNA n = 2/17 and protein n = 2/16 in APOE e4/e4, respectively). The study was approved by the Ethics Committee of the Kuopio University Hospital, University of Eastern Finland, the Finnish National Supervisory Authority, and the Finnish Ministry of Social Affairs and Health.

Genotyping

APOE genotypes based on the SNPs rs7412 and rs429358 were extracted from exome sequencing data of METSIM (Table 1). Individuals with APOE e2e2 and e2e3 genotypes were pooled together for the analyses. In FINGER, DNA was extracted and APOE genotypes were determined (Table 2) as described previously.25 DNA extraction from postmortem inferior temporal cortex and APOE genotyping were performed as described previously.22, 23 Temporal cortex was selected as it is the brain region known to be markedly affected in AD.24

Microarray-based expression analysis and liquid chromatography mass spectrometry-based proteomics assay

RNA extraction from 60 human postmortem temporal cortex samples was performed as described previously.22, 23 Agilent One-Color Microarray-Based Exon Analysis was performed at the Finnish Microarray and Sequencing Centre in Turku and have been previously described in,21 identifying 19,367 unique transcripts. For transcript expression analysis, 3′ untranslated region probe signal was used as a measure of the respective transcript’s global expression level. In case of multiple probes mapping to a single transcript, the probe with the most interquartile-range variation across the samples, was retained in the analysis. Protein and phosphopeptide levels were assessed from 36 protein samples from the temporal cortex using SysQuant liquid chromatography tandem mass
Table 2. Clinical and laboratory characteristics of the FINGER Study participants.

|                   | ε2ε2 + ε2ε3 | ε2ε4 | ε3ε3 | ε3ε4 | ε4ε4 | P       |
|-------------------|-------------|------|------|------|------|---------|
| Number of individuals | 99 (8.4%)   | 29 (2.5%) | 687 (58.5%) | 320 (27.2%) | 40 (3.4%) |         |
| Age (years)       | 69.5 ± 4.8  | 68.7 ± 4.4 | 69.5 ± 4.7 | 69.2 ± 4.6 | 67.5 ± 4.0 | 0.08    |
| Women             | 48 (48.5%)  | 16 (55.2%) | 312 (45.4%) | 158 (49.4%) | 15 (37.5%) | 0.45    |
| BMI (kg/m²)       | 28.0 ± 4.5  | 27.4 ± 4.1 | 28.4 ± 4.6 | 28.2 ± 4.8 | 26.3 ± 3.7 | 0.03    |
| Blood pressure parameters |          |      |      |      |      |         |
| Systolic blood pressure (mmHg) | 137.6 ± 15.5 | 142.5 ± 11.3 | 139.7 ± 15.7 | 140.5 ± 17.9 | 141.2 ± 16.9 | 0.46    |
| Diastolic blood pressure (mmHg) | 79.3 ± 8.3  | 80.4 ± 6.9  | 80.5 ± 9.5  | 80.0 ± 10.0 | 81.4 ± 10.5 | 0.67    |
| Lipid and lipoprotein parameters |          |      |      |      |      |         |
| Total cholesterol (mmol/L) | 5.06 ± 0.94 | 5.35 ± 0.96 | 5.10 ± 1.00 | 5.26 ± 1.08 | 5.46 ± 0.90 | 0.05    |
| LDL cholesterol (mmol/L) | 2.99 ± 0.81 | 3.14 ± 0.84 | 3.04 ± 0.84 | 3.22 ± 0.96 | 3.32 ± 0.81 | 0.03    |
| HDL cholesterol (mmol/L) | 1.44 ± 0.37 | 1.58 ± 0.43 | 1.44 ± 0.37 | 1.43 ± 0.38 | 1.53 ± 0.47 | 0.18    |
| Total triglycerides (mmol/L) | 1.41 ± 1.64 | 1.38 ± 0.73 | 1.35 ± 0.62 | 1.37 ± 0.61 | 1.34 ± 0.56 | 0.93    |
| ApoB (g/L)        | 0.83 ± 0.19 | 0.89 ± 0.17 | 0.87 ± 0.20 | 0.92 ± 0.22 | 0.95 ± 0.21 | 6.00 × 10⁻⁴ |
| Glucose metabolism parameters |          |      |      |      |      |         |
| Fasting plasma glucose (mmol/L) | 6.1 ± 1.0  | 6.0 ± 0.7  | 6.1 ± 1.0  | 6.1 ± 0.8  | 6.0 ± 0.6  | 0.86    |
| 2 h OGTT plasma glucose (mmol/L) | 7.0 ± 2.2  | 6.9 ± 1.7  | 7.0 ± 2.2  | 7.0 ± 2.2  | 6.6 ± 2.0  | 0.92    |
| Inflammatory parameters |          |      |      |      |      |         |
| hs-CRP (mg/L)     | 2.27 ± 5.58 | 1.16 (1.36)* | 2.02 ± 2.50 | 1.05 (1.95)* | 2.93 ± 8.62 | 1.23 (2.13)* | 2.37 ± 5.04 | 0.97 (1.67)* | 1.11 ± 1.54 | 0.60 (0.71)* | 1.80 × 10⁻⁵ |  

All parameters except the number of individuals and gender distribution are presented as mean ± standard deviation. For hs-CRP the median and interquartile range (*) are reported due to its skewed distribution. All P-values except age and gender are adjusted for age as covariate.

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test.
spectrometry (LC-MS/MS) global proteomics (Proteome Sciences) as described.26,27 LC-MS/MS-based global proteomics identified 146,396 peptides mapping to 7516 unique proteins. The average of all peptide values mapping to a single protein were averaged to represent protein expression.

**Clinical measurements**

Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. BMI was calculated as weight (kg) divided by height (m) squared. The genotype groups did not significantly differ from each other in age or body mass index (BMI) (Table 1). In FINGER participants, current use of anti-inflammatory drugs was verified at the screening/baseline visit (ATC codes M01A, M01B, B01AC06, H02A, and H02B for nonsteroids, corticosteroids, and their combinations). History of chronic inflammatory conditions was assessed based on self-report of rheumatoid arthritis diagnosed by a physician, and ICD-10 codes from the Finnish Hospital Discharge Register (K50-51, L40, M05-06, M10, M32-35, and M45).

**Laboratory measurements**

In METSIM cohort, plasma glucose was measured by enzymatic hexokinase photometric assay (Konelab Systems reagents, Thermo Fisher Scientific; Vantaa, Finland). Total cholesterol, low-density cholesterol (LDL-C), high-density cholesterol (HDL-C) and total triglycerides were measured by enzymatic colorimetric tests (Konelab Systems Reagents). Apolipoprotein B (ApoB) was determined by immunoturbidimetry (Konelab Systems Reagents). Plasma concentrations of high-sensitivity C-reactive protein (hs-CRP) were assayed by kinetic immunoturbidimetry (NIPIA, Immage Immunochemistry System, Beckman Coulter, Fullerton, CA). Plasma interleukin-1 receptor antagonist (IL-1Ra) and interleukin-1 β (IL-1β) were measured by immunoassay ELISA (R&D Systems Inc., Minneapolis, MN). All the above assays were performed on samples drawn after a 12-h fasting. ApoB40 and ApoB42 in plasma were quantified with a fluorometric bead-based immunoassay (INNO-BIA plasma Aβ forms, Fujirebio Europe NV, Gent, Belgium) and analyzed on a Bio-Plex 200 instrument (Bio-Rad, California). Plasma samples used for Aβ measurement were 2 h samples from oral glucose tolerance test (OGTT).

In FINGER cohort, blood samples were drawn after overnight fasting (at least 10 h) at baseline visit. The separated serum and plasma samples were frozen immediately in −20°C and mailed monthly to the laboratory of the National Institute for Health and Welfare, where they were stored in −70°C. Serum cholesterol (total, LDL and HDL cholesterol), total triglycerides and plasma glucose levels were determined enzymatically using commercial reagents from Abbott Laboratories on a clinical chemistry analyzer Architect c8000 (Abbott Laboratories, Abbott Park, IL). ApoB was determined with immunoturbidimetric method on a clinical chemistry analyzer Architect c8000 (Abbott Laboratories). Circulating CRP levels were quantified from serum samples using a latex immunoassay (Sentinel Diagnostics, Milan, Italy) with Architect c8000 clinical chemistry analyzer (Abbott Laboratories). In the human postmortem brain samples, soluble Aβ42 levels were determined as described.22

**Statistical analysis**

Due to skewed distributions, logarithmic transformation was used for all variables except for age and total and LDL cholesterol to achieve approximate normal distributions. Variables were compared across the APOE genotype groups by ANOVA or after adjustment for age (ANCOVA). Two-way ANOVA was used to assess the main effects of the extent of AD-related neurofibrillary pathology and APOE genotype on dependent variables. Fisher’s least significant difference (LSD) posthoc analysis was performed for pairwise comparison. APOE ε3ε3 genotype group was considered as a reference group in pairwise comparisons. Spearman’s rank correlation was used to assess correlations between the variables. Partial correlation controlling for age was used to assess the effect of age on the relation between plasma Aβ42 or ApoB42 and hs-CRP levels. All statistical analyses were conducted with SPSS 21.0 software (SPSS, Chicago, IL). P < 0.05 was considered as statistically significant except in the initial analysis with METSIM cohort, in which the threshold of statistical significance was set as P < 0.0002 (P < 0.05/248) given 248 cardiovascular health and metabolic parameters compared (Table S1).17,28,29

**Results**

A total of 248 cardiovascular health- and metabolism-related parameters available in the METSIM cohort (Table S1)17,28,29 were analyzed in relation to APOE genotypes. As expected, all determined lipid and lipoprotein parameters differed significantly among the carriers of the different APOE genotypes (Table 1). Especially, LDL cholesterol in serum and ApoB in plasma differed significantly among the different APOE groups. The highest levels were observed among the APOE ε3ε4 and ε4ε4 individuals (Table 1). No significant differences were found in blood pressure or blood glucose parameters. The most significant difference was detected in hs-CRP levels between the APOE genotypes (Table 1). In pairwise
comparisons with APOE ε3ε3 genotype as the reference group, hs-CRP levels were significantly lower in ε3ε4 (P = 4.93 x 10^{-23}) and ε4ε4 (P = 4.15 x 10^{-14}) group, but not in ε2ε2/ε2ε3 (P = 1.00) or ε2ε4 (P = 1.00) groups. APOE genotypes did not associate with the other inflammation-related parameters available in the METSIM cohort, such as IL1-RA or IL-1β (Table 1). However, hs-CRP levels significantly correlated with IL1-RA (Spearman’s rho = 0.285, P < 0.0001). The significant association between APOE genotype and hs-CRP persisted when individuals with likely ongoing acute infections (hs-CRP > 10 mg/L) were excluded from analyses (n = 4805, 98% of the cohort, P = 2.17 x 10^{-31} pairwise comparison with APOE ε3ε3 genotype as the reference group: ε2ε2/ε2ε3 (P = 1.00), ε2ε4 (P = 1.00), ε3ε4 (P = 4.41 x 10^{-22}), and ε4ε4 (P = 3.94 x 10^{-14})). Since the levels of plasma Aβ40 and Aβ42 were previously determined in a subset of METSIM individuals,^{10} the plasma levels of these Aβ species were compared between individuals with APOE ε4 (n = 66) or without APOE ε4 (n = 149). However, there were no statistically significant differences between APOE groups (APOE ε4+ vs. APOE ε4−: Aβ40 P = 0.58 and Aβ42 P = 0.27). Correlation analyses of the plasma Aβ40 and Aβ42 levels with cardiovascular health- and metabolism-related parameters available in the METSIM cohort revealed a positive correlation of Aβ40 (rho = 0.282, P = 2.7 x 10^{-5}, n = 215), but not Aβ42 (rho = 0.076, P = 0.27, n = 214) with increased age (Fig. 1A and B). Moreover, the levels of plasma Aβ42 (rho = -0.148, P < 0.05, n = 214), but not Aβ40 (rho = -0.059, P = 0.39, n = 215), negatively correlated with the levels of hs-CRP (Figure 1C and D). Controlling for age in the Aβ and hs-CRP correlation analyses revealed that age did not affect the observed outcome measures between plasma Aβ42 or Aβ40 and hs-CRP levels (Aβ42: rho = -0.147, P < 0.05, n = 214; Aβ40 rho = -0.051, P = 0.45, n = 215). Neither Aβ40 nor Aβ42 showed association with other inflammatory, lipid or lipoprotein parameters, blood pressure, or blood glucose metabolism parameters available.

To further study, the key findings observed in the METSIM cohort, lipid and inflammatory parameters were assessed among different APOE groups in the population-based FINGER cohort consisting of both sexes (Table 2). Similar to the results in the METSIM, the levels of hs-CRP, total cholesterol, LDL and ApoB differed between APOE groups and the levels being the highest in the individuals with APOE ε3ε4 and ε4ε4 genotypes (Table 2). Supporting the findings in the METSIM cohort, in pairwise comparisons with APOE ε3ε3 genotype as reference group, serum hs-CRP levels were significantly lower in ε3ε4 (P = 0.017) and ε4ε4 (P = 1.00 x 10^{-6}) groups, but not in ε2ε2/ε2ε3 (P = 0.49) or ε2ε4 (P = 0.33) groups. The result remained the same after adjustment for BMI and the use of anti-inflammatory drugs, as well as after excluding the individuals with hs-CRP >10 mg/L (individuals with likely ongoing acute infection) and individuals with a history of chronic inflammatory conditions (total n = 1068, P < 0.05 for ε3ε4, P < 0.001 for ε4ε4, and statistically nonsignificant for ε2ε2/ε2ε3 or ε2ε4 groups). No significant differences were found in blood glucose parameters (fasting glucose or 2 h oral glucose tolerance test (OGTT)) between the APOE genotype groups.

Finally, a neuropathologically well-characterized post-mortem brain sample set\textsuperscript{21–23} was used to assess the local mRNA and/or protein levels of CRP and ApoB in the inferior temporal cortex in relation AD-related neurofibrillary pathology (Braak staging)\textsuperscript{24} and APOE status. Two-way ANOVA was conducted to compare the main effects of Braak and APOE status and the interaction effect between AD-related neurofibrillary pathology and APOE status on mRNA and/or protein levels of CRP and ApoB. No statistically significant associations between CRP mRNA expression levels and neurofibrillary pathology (P = 0.78) or APOE genotype (P = 0.34) were observed. In addition, there was no significant association between AD-related neurofibrillary pathology and APOE status (P = 0.40) (Fig. 2A). A significant association between protein levels of ApoB, but not mRNA levels, and AD-related neurofibrillary pathology (RNA P = 0.13; Protein P = 0.02) was observed, irrespective of APOE genotype (AD-related neurofibrillary pathology×APOE status interaction RNA P = 0.48; protein P = 0.54). No significant association between APOE genotype and APOB mRNA (P = 0.74) or protein levels (P = 0.13) was observed. Posthoc analysis revealed a significant increase in ApoB protein levels from mild to moderate group in the temporal cortex (P = 0.005) (Fig. 2B). Correlation analyses of CRP (CRP RNA rho = -0.089, P = 0.52, n = 55) and ApoB (ApoB protein rho = 0.124, P = 0.48, n = 36; ApoB RNA rho = -0.018, P = 0.899, n = 55) with the levels of soluble Aβ42 in the temporal cortex were not statistically significant.

**Discussion**

The APOE ε4 allele is the strongest genetic risk factor for AD.\textsuperscript{26} It is also associated with the risk of cardiovascular disease.\textsuperscript{1, 6} Here, we determined the association of different cardiovascular-, metabolic health-, and inflammation-related parameters in relation to the different APOE genotypes in a large population-based cohort (METSIM), consisting of nearly 5000 men from Eastern Finland. As expected, the highest lipid and lipoprotein levels, including those of total cholesterol, LDL and ApoB, were observed among the carriers of APOE ε4 allele. Three
ApoE isoforms, encoded by ε2, ε3, and ε4 alleles in the APOE gene, differentially modulate plasma lipid and lipoprotein levels, and individuals carrying the APOE ε4 allele display higher total cholesterol levels in comparison to individuals with other isoforms. Since approximately 90% of ApoB in plasma is normally bound to LDL, it is not surprising that the highest ApoB levels were detected among APOE ε4 allele carriers. Interestingly though, increased levels of serum ApoB have been shown to correlate with Aβ42 levels in the brain and increased serum ApoB levels have been reported among AD patients. Accordingly, previous studies utilizing transgenic AD mouse models have shown that the overexpression of human ApoB promotes memory decline and increases lipid peroxidation, Aβ load, neurodegeneration, and astrogliosis in the brain. Here, no data regarding Aβ levels in the brain or CSF were available from the participants of the METSIM study. Instead, we utilized a neuropathologically well-established postmortem brain sample set to address ApoB-related changes in the brain. A significant increase in ApoB at the protein, but not at the mRNA level, was detected in relation to advancing AD-related neurofibrillary pathology, suggesting that ApoB is post-translationally dysregulated or aberrantly transferred into the brain during the progression of AD-type pathology. This finding was independent of the APOE status and no association between the levels of ApoB and Aβ42 in the brain was found. Nevertheless, data from our present study and others suggest that ApoB levels are altered in AD both in the brain and periphery, which warrants further mechanistic studies to elucidate the role of ApoB in cellular pathways involved in AD pathogenesis.

The most significant association in the METSIM cohort was found between the risk-conferring APOE ε4 allele and lower levels of plasma hs-CRP as compared to the other
Figure 2. Alterations of CRP and ApoB expression in the postmortem brain tissue with respect to neurofibrillary pathology. Brain CRP mRNA levels (A) and ApoB protein levels (B) in APOE ε4 carriers (APOE ε4+) and noncarriers (APOE ε4-) in the postmortem human temporal cortex with respect to AD-related neurofibrillary pathology. The mean levels ± SD are indicated. Significant differences are denoted as: ** P ≤ 0.01.
APOE alleles. Supporting the initial finding in the METSIM cohort, similar relationships between APOE genotypes and plasma hs-CRP and ApoB levels was observed in the FINGER cohort consisting of approximately 1200 men and women. In general, the distribution of APOE genotypes was similar in both METSIM and FINGER cohorts as previously shown in the Finnish population. Importantly, the allele frequency of APOE ε4 was higher in these Finnish cohorts as compared to cohorts originating from the central Europe. This is consistent with the north-south gradient, which has demonstrated that the APOE ε4 allele frequency is the highest in Finland and Sweden. Although the link between the APOE ε4 allele and low levels of plasma hs-CRP is corroborated by several studies, most of the previous reports derive from considerably smaller cohorts, lacking convincing statistical power. To our knowledge, only one study has so far shown the link between APOE genotypes and hs-CRP levels in a large population cohort of the same scale. Hence, our study provides important support for the evidence that APOE ε4 allele significantly associates with lower levels of plasma hs-CRP levels. CRP is an important regulator of immune responses and it is commonly used as a biomarker for systemic inflammation. The presence of the APOE ε4 allele has been suggested to promote a pro-inflammatory state in comparison to other APOE alleles. Thus, it is surprising that lower hs-CRP levels, indicating lower levels of inflammation, are repeatedly detected among the individuals with the risk-conferring APOE ε4 allele. Here, the observed association between APOE and hs-CRP remained significant even after adjustments for possibly ongoing infection (hs-CRP ≥10 mg/L), history of chronic inflammatory conditions, and ongoing anti-inflammatory drug treatment. This suggests that the association is not explained by other concomitant inflammatory conditions or anti-inflammatory drug treatment. Aside from hs-CRP, APOE genotypes had no effect on the other inflammatory parameters available in the METSIM cohort. On the other hand, plasma hs-CRP levels strongly correlated with the levels of IL1-RA. As anti-inflammatory effects of CRP are partially mediated through induction of IL1-RA, this suggests that low hs-CRP levels are linked with defective anti-inflammatory responses.

Increased level of plasma hs-CRP is a risk factor for cardiovascular disease, stroke, and diabetes, all of which are well-established risk factors for AD. Also, a direct link between elevated plasma hs-CRP in midlife and a risk of AD and dementia has been described. Inflammation is centrally involved in AD pathogenesis, as denoted by the presence of activated microglia and astrocytes as well as the increased levels of inflammatory molecules in the brain. Aβ42 is the key neurotoxic and pro-inflammatory component in AD, but recent studies have also pointed toward a potential anti-inflammatory role for Aβ42 under certain conditions. Since ApoE is the major mediator of Aβ clearance from the brain, we wanted to address potential changes in the plasma concentration of Aβ among different APOE groups. While plasma Aβ40 and Aβ42 levels remained unaffected between the different APOE groups, we observed a significant negative association between plasma Aβ42 and hs-CRP levels even after age adjustment, suggesting that Aβ42 may confer protective effect(s) against peripheral inflammation. In this context, however, it should be noted that correlation between plasma Aβ42 and hs-CRP was not particularly strong, indicating that significantly larger number of samples are still needed to comprehensively validate this relationship. Nevertheless, this finding is utmost importance, considering that Aβ42 has recently been suggested to participate in the defense response against pathogens, stress, and inflammatory conditions, in both the CNS and periphery. Accordingly, decreased plasma hs-CRP levels have been repeatedly reported among AD patients. However, the relationship between the Aβ levels in plasma and CNS should be taken into consideration before further interpretations are made. Here, we were not able to address the observed plasma-derived relationships, such as Aβ and hs-CRP, in the CNS of METSIM and FINGER individuals, owing to lack of systematic Aβ pathology assessments. CRP has been demonstrated to localize to Aβ plaques in the brain of AD patients, but its role in the CNS is not well known. In this study, we did not observe any alterations in the brain CRP mRNA levels at different stages of AD-related neurofibrillary pathology among individuals with or without an APOE ε4 allele. Also, we did not find correlation between brain Aβ42 and CRP levels. It should also be emphasized that the overall mRNA expression of CRP in the temporal cortex was extremely low, suggesting that CRP does not have as a central role in CNS as in the periphery.

Taken together, we report here in exceptionally large population-based cohorts a strong association between APOE ε4 allele and the levels of certain lipid, lipoprotein, and inflammatory parameters, which all may contribute to the risk of AD. The underlying cause and biological role of altered ApoB and hs-CRP levels among APOE ε4 carriers and noncarriers in the etiology of AD and other relevant common diseases need to be addressed further in future mechanistic studies. In the light of recent studies demonstrating anti-inflammatory potential for Aβ42, the observed association between peripheral Aβ42 levels and suppressed levels of plasma CRP is intriguing. Further investigations are required to understand the significance of this finding in the context of immune defense and AD.
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Author Contributions
ML and MH designed the study. HM, AleS, AS, MM, TN, SKH, AH, HS, JK, and ML contributed to collection of the data. HM, MT, AleS, AS, MM analyzed the data. HM, MT, ML, AH, and MH wrote the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of Interest
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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Cardiovascular health and metabolic parameters studied in METSIM cohort at the baseline.